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The edible sea urchin *Paracentrotus lividus* (Lamarck, 1816): a valuable resource for fisheries and aquaculture purposes in Sardinia (Central western Mediterranean)

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Chapter 3

Laboratory rearing of *Paracentrotus lividus* juvenile specimens

Abstract

In this study, several research approaches were used to increase the knowledge on the Mediterranean sea urchin *Paracentrotus lividus*. Morphometric characters and gonad indexes of sea urchin populations were monitored during one year to examine possible differences among seasons in 3 locations of Alghero Bay (NW Sardinia).

Moreover, 2 experiments to test somatic growth, and gonad and alimentary indexes of laboratory-reared specimens fed an artificial diet were also carried out. The experiments lasted 16 and 10 weeks in Scotland and in Sardinia, respectively. In the latter, proximate composition and acidic profile of the sea urchins gonads were also assessed. Samples from natural populations were used as a control. Significant differences between reared and wild specimens were found, both regarding proximate composition (except for total lipids) and fatty acid profile. The results obtained confirm the effectiveness of the diet and rearing method used in enhancing sea urchins' growth.

Finally, an economical analysis on profitability and sustainability of *P. lividus* fisheries in Sardinia was performed. Firms operating with different harvesting methods were analysed, obtaining an overview of the current situation in terms of strengths and weaknesses of restrictions on the harvesting of this species. This information can be used by policy makers to implement important changes to the current fishing policy, by guaranteeing profitability for fishermen and conservation of wild stocks.

1. General introduction

1.1 Introduction

Although water occupies more than 70% of the whole Earth crust, aquatic products satisfy just 1-2% of the humanity food requirement. Recent data by FAO (2012*)* shows an increase in fish products consumption that could bring into question the future of the aquatic resources which are continuously overexploited all over the world. The growth of fish production and the improvement of distribution channels in the past decades has increased the demand in consumption of fish food at an estimated growth rate of 3.2% per year from 1961 to 2009 (Sartori et al., 2015). According to what has been declared in such FAO report, human consumption of fish products is arrived at a level never reached before (about 17 kg *per capita*). Practically, in 2010, about 3 billions of people have satisfied at least 15% of their average protein needs just consuming aquatic foods. However, production resulting from seas, oceans and inland waters exploitation can't be further increased from today's levels, despite the demand for fish products is constantly greater than supplied and also continues its rise. Considering that the global population is estimated to grow until it reaches 9 billion people between 2040 and 2050 (UN, 2015), the collapse of natural resources, caused by the pressure on fish stocks, is a real problem (Sartori et al., 2015). In this scenario, aquaculture may have a dual and important role: on the one hand to ensure productions to meets the market demand (although still far from the required amounts), and on the other hand to allow a reduction of fishing pressure in strongly overexploited areas so as to promote a recovery of aquatic ecosystems, even if this will be a long and partial process.

One of the factors contributing to the increasing demand for fish products is linked to the awareness of consumers about its excellent quality. Indeed, it is a food rich in proteins, vitamins and minerals in highly digestible form. Furthermore, thanks to the large amount of essential fatty acids, it is particularly suitable for the human diet.

According to the last available statistics (FAO, 2014), the global aquaculture production in 2012 was of about 90 million tons, which means an estimate value of about 126 billion €. The whole of the fish and invertebrates production (almost 67 million tons) were destined entirely for human consumption, while the remaining (almost 23 million tons), mainly constituted by aquatic plants, was also used by several industries (i.e., chemicals, pharmaceuticals, cosmetics, food, etc.). Currently, 500 different aquatic species (both animals and plants) are farmed, but less than 10% are of a considerable economic value. Conversely, although some species play a leading role in some markets due to their high commercial value, the production of many of these (especially invertebrates) still shows a marginal and limited development. An example is provided by certain Echinoderms, in particular the sea urchins: due to the delicacies they represent in many countries around the world (Lawrence, 2007), also their farming practices have shown a continuous increase over time. However, the current demand for sea urchins (linked to the consumption of their gonads), is almost completely satisfied by harvesting activities from natural populations that, in various areas of the world, are often overexploited (as many other species) because inadequately managed and/or protected by effective regulation rules. Therefore, despite the trade of these Echinoids constitutes an extremely profitable business, the amount currently derived by aquaculture activities represents less than 1% of the total, compared to about 80,000 tons as a whole of sea urchins sold every year worldwide (Carboni et al., 2012). Globally, many species of sea urchins are harvested and consumed: *Strongylocentrotus droebachiensis* in the North Atlantic states, *Cidaris tribuloides* in West Indies, *Evechinus chloroticus* in New Zealand, *Strongylocentrotus Franciscanus* and *S. purpuratus* in the Pacific coasts of the United States, and *S. intermedius* and *S. nudus* in the Pacific coasts of Japan. At present, just over 20 countries are exporters of this fish

product: for first Chile, followed by United States, Canada, North Korea, Mexico, Australia and China. However, on the basis of annual import of about 20,000 tons (which is in a phase of general increase over the past few decades) and with a local production of about 15,000 tons, Japan, with its 130 million inhabitants, is considered to be the most important market (Grosjean, 2001) and the main consumer of sea urchin gonads (0.27 kg/person on annual basis; Carboni et al., 2012). Recent statistic by FAO (2012) revealed that, in this country, the 64% of the world production of sea urchins is consumed. In this market, sea urchins gonads (both male and female, called *uni* in japanese) are marketed under different forms: fresh (65%), but also dried, salted, frozen or cooked (35%; Saito, 1992; Hagen, 1996). The average price on this market ranges from 18.6 €/kg (for fresh local products, considered as top quality) to 7.9 €/kg (for imported products; Hagen, 1996). The main global producer of sea urchins is considered to be Chile, with over 55,000 tons harvested from more than 78,000 km of the Pacific coasts (Carboni et al., 2012). Global fishery production, however, has dramatically declined over the last 15 years from 115,000 to about 82,000 tons per year. On the other hand, along the European Atlantic and Mediterranean coasts the sea urchin market is quite different if compared with those already described. The main species destined for human consumption is represented by *Paracentrotus lividus* (Lamarck, 1816) (FAO, 2013). It is a macroalgivore regular Echinoid commonly found along the North Atlantic coasts (from southern Morocco and Canary Islands up to Ireland and the southern West coast of Scotland) and throughout the Mediterranean Sea (Bayed et al., 2005; Boudouresque & Verlaque, 2007), where it is the most abundant Echinoid species (Fernandez & Boudouresque, 2000). Its harvest and consumption are carried out only in a few countries, in particular in the South of Italy and in some region of Spain, Portugal, France, Greece and Turkey (Boudouresque & Verlaque, 2007; Fabbrocini & D'Adamo,

2010; Ceccherelli et al., 2011; Fernàndez-Boàn et al., 2013). Furthermore, in France, which is considered the most important European market for sea urchins, in addition to *Paracentrotus lividus* also other species like *Psammechinus miliaris* (Gmelin) and *Sphaerechinus granularis* (Lamarck) are sold (Grosjean, 2001). In other European countries, such as Ireland, Portugal and Croatia, sea urchins harvesting is almost entirely practiced for export (Barnes & Crook, 2001a; Boudouresque & Verlaque, 2007; Bertocci et al., 2012).

Nowadays, echiniculture activities are of great interest, showing a continuous and constant growth, especially for the high economic value that gonads (commonly named as roe) of these Echinoderms has in many countries (Lawrence, 2007). In fact, it is considered as one of the most valuable seafood in the world (Grosjean et al., 1998). However, the current market demand is almost completely satisfied by fishing activities from natural populations, which are often overexploited (Ledireac'h, 1987; Conand & Sloan, 1989; Le Gall, 1990; Hagen, 1996; Grosjean et al., 1998; Spirlet et al., 2000; Andrew et al., 2002).

Although artificial reproduction of many sea urchins species under controlled conditions is extremely easy to perform and already well described by many authors (Fabbrocini & D'Adamo, 2010; Toscano & Cirino, 2010), the main limiting factor for their farming activities is represented by a very slow growth rate. In natural conditions, they can take a few years before gonadal maturation occurs. Also in the case of *P. lividus*, growth rate is rather slow. Their maximum term of life is of about 10-12 years and they reach the commercial size (40-50 mm in test diameter) in 4-5 years (Allain, 1978; Turon et al., 1995; Grosjean, 1998; Sellem et al., 2000; Gago et al., 2001; Grosjean et al., 1996, 2003; Boudouresque & Verlaque, 2007; Sartori et al., 2015). This peculiarity makes that harvesting activities are often indiscriminate, especially on

juvenile forms, thus causing a progressive depletion of natural stocks. Therefore, sea urchin fishing in an unexploited area can be very profitable during the first 5-10 years, but it tends to decline gradually in the following ones (Allain, 1975; Le Gall, 1990). This can be explained by the high efficiency and selectivity of fishing techniques: most exploitable natural stocks are easily manually picked at low tide, or at least using simple equipment at shallow depths (Le Gall, 1987). As focused by Grosjean (2001), probably the biggest problem leading to the overexploitation of the resource is that these Echinoids are harvested before they reach the full maturation of the gonads, so as they have no opportunity to spawn. A lack of recruitment and, consequently, a rapid decline of the standing stock results from intense fishing (Allain, 1971; Campbell & Harbo, 1991). In addition, removing most adults from a site probably has a negative impact on the survival of the remaining juveniles (Grosjean, 2001).

Throughout the last 10-15 years, the interest in finding a way to contrast overexploitation and cope with the increasing market demand has increased in a number of countries such as: Japan, Australia, Canada, Chile, China, New Zealand, Norway, Ireland, Italy and Scotland. Japan was the first to handle this problem and initiated a stock enhancement approach very early (Saito, 1992) by seeding hatchery-raised juveniles into the wild. In fact, hatcheries may be a good solution to ensure recruitment where extreme harvesting eliminates adults before they spawn, but appropriate natural habitats are required in order to ensure adequate protection to the juveniles released. In other countries, research was focused on roe enhancement and several trials on this topic and on the development of larval and juvenile forms were carried out. In the opinion of many authors, an adequate artificial diet could increase gonadal size (de Jong-Westman, 1995; Spirlet, 1999; Spirlet et al., 2000), particularly diets rich in proteins (Klinger et al., 1997; Spirlet et al., 2001). If for the major part gonads

enhancement could be a possibility, in Canada it is a necessity because sea urchins are at the right stage of maturity during winter, when the sea is frozen and sea urchins harvesting under the ice by SCUBA divers is a gruelling and dangerous activity (Grosjean, 2001). Consequently, the solution adopted is to fish these invertebrates during autumn, store them in tanks and feeding them with an adequate diet before their commercialisation (Motnikar et al., 1997). An alternative, as already done in Scotland, could be the use of cages, both in mono and in poly cultures (Keats et al., 1983; Kelly et al., 1998). The research efforts are moreover on designing and promoting integrated multitrophic aquaculture systems (IMA), featuring sea urchins and/or seaweed alongside Atlantic salmon culture activities. In the latter case, as for many mariculture activities, the degradation of the cages by waves storms is a major problem, and site location is critical (Grosjean, 2001). Furthermore, because of their grazing activity sea urchins tend to erode the cage nets and are also a direct cause of depredation which increases maintenance costs (Kelly et al., 1998).

The last step to achieve is the control of the whole life cycle in culture, from spawning to gonads enhancement. Aquaculture techniques currently used for sea urchins rearing aim to the satisfaction, partially at least, of the growing market demand although at present they have to face with many problems. Among these, the most important are the colour variations, the differences in taste and/or in roe texture, the limited shelf-life and a series of intrinsic difficulties related to the safety of the product. In fact, the quality of the gonads is the main factor that influences price (Whitaker et al., 1997), and colour and texture are the principal factors which determine the roe quality (McCarron et al., 2010). Depending on the local market preferences, roe colour can range from a light yellow to a dark orange or almost red. Lighter, pale coloured or dark brown gonads are not as desirable to the market (Robinson et al., 2002). The yellowish-orange colour of

the gonads is principally due to the pigment echinone (Griffiths & Perrot, 1976), which is synthesised from beta-carotene by the sea urchin. The high importance and price put on the desired colour by the sea urchin market emphasises the point that the diet of the sea urchin is directly related to its market quality (Robinson et al., 2002). On the other hand, several studies on various sea urchins species have highlighted the poor colour of the gonads caused by some of the currently manufactured foods for these Echinoids (Barker et al., 1998; Grosjean et al., 1998; Walker et al., 1998; Watts et al., 1998).

1.2 Description of the species

In the Mediterranean Sea, the edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) is the most appreciated species and its harvesting is mainly carried-out in several coastal areas of Spain, France, Greece, Turkey and southern Italy (including Sicily and Sardinia; Boudouresque & Verlaque, 2007 and references therein; Fabbrocini & D'Adamo, 2010; Ceccherelli et al., 2011). This species is a Regular Echinoid, included in the Order Camarodonta and in the Family Parechinidae (Table 1.1).

The body of regular sea urchins (and in general of all the Regular Echinoids) shows a radial pentameric structure, with a soft body held in a pseudo-circular shell (commonly named as test; Figs. 1.1, 1.2). This latter is covered by an epithelium and surrounded by spines, tube feet and pedicellariae. These appendages, which can be moved by the animal, have different functions. The spines are the first defensive system against natural predators, but they can also be used as aid in locomotion and to improve the adhesion to the substrate (Guidetti & Mori, 2005). They may present venomous glandular tissue (Tortonese, 1965) and have different appearance, size and thickness, depending on the species.

In particular, *P. lividus* have movable spines different in length and, above all, in colour

which may be purple, green, brown, black or reddish (Fig. 1.3).

Table 1.1. Systematic framework of *Paracentrotus lividus* (Lamarck, 1816).

Taxon	Name	Author
Phylum	Echinodermata	(Bruguière, 1790)
Class	Echinoidea	(Leske, 1778)
Subclass	Euechinoidea	(Bronn, 1860)
Infraclass	Carinacea	(Kroh & Smith, 2010)
Superorder	Echinacea	(Claus, 1876)
Order	Camarodonta	(Jackson, 1912)
Infraorder	Echinidea	(Kroh & Smith, 2010)
Family	Parechinidae	(Mortensen, 1903)
Genus	*Paracentrotus*	(Mortensen, 1903)
Species	*Paracentrotus lividus*	(Lamarck, 1816)

Among the spines, there are tube feet and pedicellariae. The former are highly movable structures formed by a peduncle and a head that extend from the oral region to the apical end in a perfect pentameric symmetry. The peduncle is thin and contains a calcareous stem as a support, articulated on a small tubercle of the test. The stem does not reach up to the head and, therefore, the upper part of the peduncle remains extremely flexible. Sea urchin is able to elongate and retract its tube feet, and their sucker-shaped tip can glue or unglue it to the substratum (Flammang, 1996; Flammang et al., 1998). On the other hand, the pedicellariae are small pincer-shaped appendages scattered sparsely over the entire surface of the sea urchin test.

Tube feet and pedicellariae have various functions, such as locomotion (aided by spines on hard substrate, as described by Tortonese, 1965), removing debris from the test surface and covering the test with a number of objects (e.g., shells, pebbles, parts of aquatic plants, etc.) for protecting from light. This latter behaviour is linked to the characteristic shade-loving of the sea urchins (sciaphilic species) already focused by

several authors (Millot, 1954, 1975; Lawrence, 1976; Verling et al., 2002).

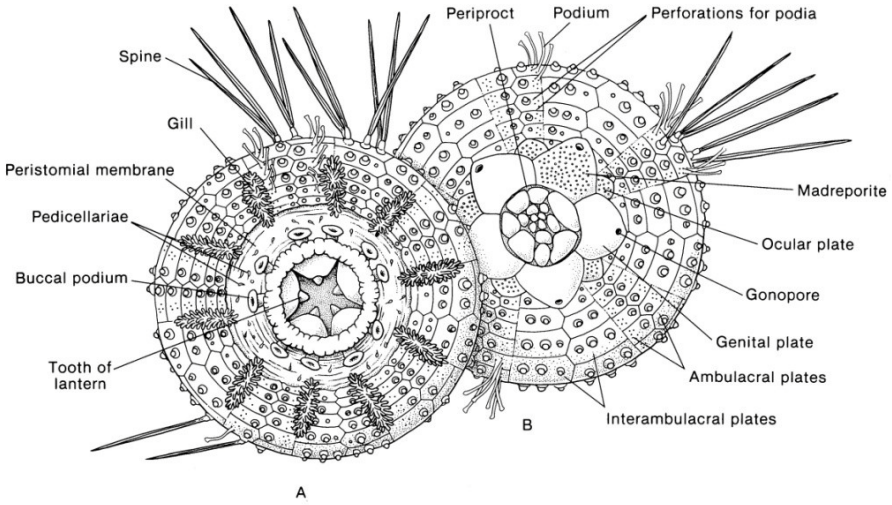

Fig. 1.1. External anatomy of a regular sea urchin. **A.** oral view. **B.** aboral view. (Image from Grosjean, 2001. Originally in: Ruppert & Barnes, 1994).

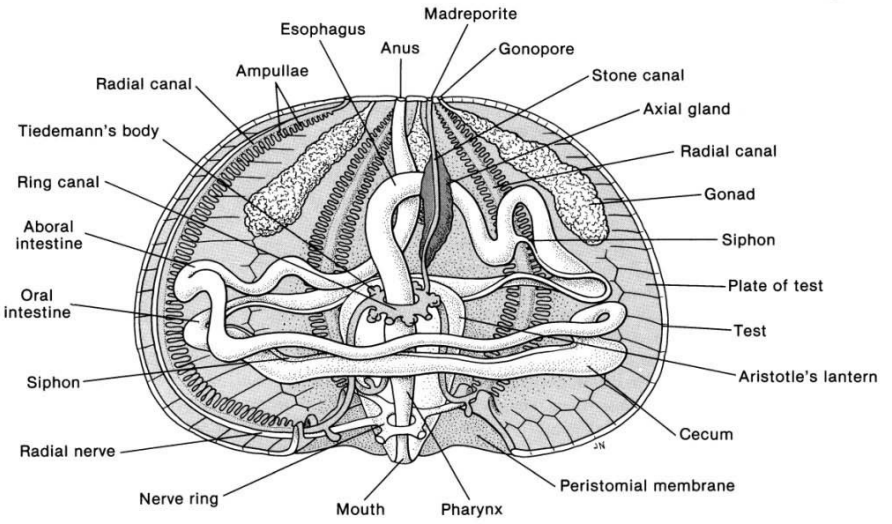

Fig. 1.2. Interior anatomy of a regular sea urchin. Side view. (Image from Grosjean, 2001. Originally in: Ruppert & Barnes, 1994).

Spines and pedicellariae could be regenerated in case of loss due to predatory attacks or unfavourable natural conditions (Tortonese, 1965) such as, for instance, a pH decreasing.

A common feature of all the Regular Echinoids, and of a few of the Irregular, is the typical “lantern of Aristotle” associated with the mouth (Barnes, 1987). This structure is composed by 5 mobile plates, interconnected by more than 40 ossicles. Inside each plate there is a tooth which can be pull out and back and that increase continuously (Tortonese, 1965), up to 1.5 mm per week (Barnes, 1987; Devin, 2002).

Sea urchins belonging to populations which live on shallow hard substrata or in intertidal pools use their teeth to carve holes in rocks to hide from predators or to resist dislodgement by waves. Consequently, in case of high densities of population, the substratum can be completely honeycombed (Boudouresque & Verlaque, 2007). The lantern’s movements are regulated by a complex of muscles, among which the most important are the protractor muscles. Near the buccal opening, there are 5 pairs of short pedicles named buccal pedicles or oral tentacles, usually without suckers and probably with a chemoreceptive function.

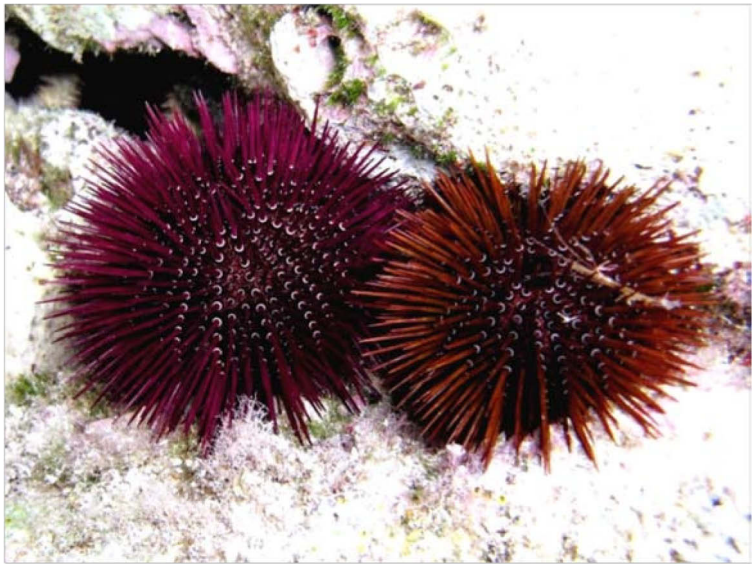

Fig. 1.3. *Paracentrotus lividus* (Lamarck, 1816) specimens.

The test is composed by 20 rows of plates linked together. There are 2 types of

alternated plates, named ambulacral and interambulacral plate, respectively. The latter hosts the spines, whereas one pair of podia emerge from each ambulacral plate (Carboni, 2013).

On the opposite side of the oral region (where the Aristotle's lantern is located), there is a membrane named periproct. The periproct surrounds the anus, which in turn is surrounded by 5 gonopores (the holes from which sperms and eggs are released in the water), one for each gonad, and hosts the madreporite or madreporic plate as described by Tortonese (1965).

The internal cavity, which is named celom, is very wide. It does not include the Aristotle's lantern (because it has an envelope) which is contained in its own cavity. The digestive system is a simple pipe, attached to the inner wall of the body by means of the mesentery. At first, it passes through the Aristotle's lantern, proceeds in its centre among the pyramids and, after folding several times, it ends with the anus.

Another characteristic of all Echinoderms is the aquifer apparatus (also named ambulacral apparatus), which allows locomotion by a local variation of the pressure of endolymph in the pedicels. One more channel, in dorsal-ventral direction, links the circular channel to the madreporic plate and allows exchanges between the internal fluids and seawater.

Finally, the reproductive apparatus is composed by 5 gonads connected to genitals pores (1 for each gonad). Also the gonads are joined by mesenteric filaments to the inner surface of the test. When ripe, they appear voluminous, bright yellow, orange or red coloured (more or less intense) and widespread from the aboral pole almost to the Aristotle's lantern. Towards the aboral pole, each gonad become thinner giving rise to a short gonoduct which flows outside through the genital plate's gonopore (gametes emission area).

1.3 Biology and habitats

Paracentrotus lividus is distributed throughout the Mediterranean Sea and along the North eastern coast of the Atlantic, from Scotland and Ireland to southern Morocco, in the Canary Islands and the Azores (Bayed et al., 2005; Boudouresque & Verlaque, 2007 and references therein). It is a common species in regions where the seawater temperature is between 10 and 15°C during the winter and between 18 and 25°C in the summer (Boudouresque & Verlaque, 2007). This is a subtidal species, spread by surface waters (including cliff pools) up to a depth of about 10-20 meters, although some isolated individuals have been found to a depth of about 80 meters (Tortonese, 1965). Due to several physical and biological factors such as, for example, heterogeneity of habitats, wave motion, slope of the bottom, light, nutrition, mortality, predation and larval survival, the distribution and the density of this species are extremely variables in space and time (Guidetti et al., 2003). In the Mediterranean, a sea with a low tidal range, when the water level reaches the minimum heights *P. lividus* specimens can remain exposed to air and die fairly quickly (Boudouresque & Verlaque, 2007). This Echinoid is one of the most important herbivores of rocky sub-littoral zones and, with its grazing activity, it can play a major role in the sea bottom transformation (Bulleri et al., 1999). At deeper depths, *P. lividus* is distributed mainly on solid rocks, boulders and seagrass beds (such as *Posidonia oceanica* and *Zostera marina*). In contrast, specimens living in shallow waters and/or in cliff pools find refuge in the substrate's irregularities, using them as shelters against the wave action, especially during the winter (Fig. 1.4). This behaviour may also provide an effective protection from predators (Boudouresque & Verlaque, 2007). Small individuals (less than 1-2 cm in test diameter), particularly vulnerable to predation, usually live in shelters, crevices, under pebbles and stones, inside the "matte" of the seagrass *P. oceanica*, and, occasionally, at the bottom of a

dense algal cover. Larger individuals, however, after eating can or cannot return to their shelters depending on the size and abundance of predators (Sala, 1997; Palacín et al., 1997). Their ability to dig holes in rocks is greater in the Atlantic than in the Mediterranean Sea. This fact could be explained by the stronger hydrodynamism which characterises the former if compared with a closed sea, although very extended, such as the Mediterranean. Finally, it is possible to find populations of *P. lividus* also in brackish coastal lagoons, where the individuals can live on muddy and/or sandy substrates (Fernandez & Boudouresque, 2007).

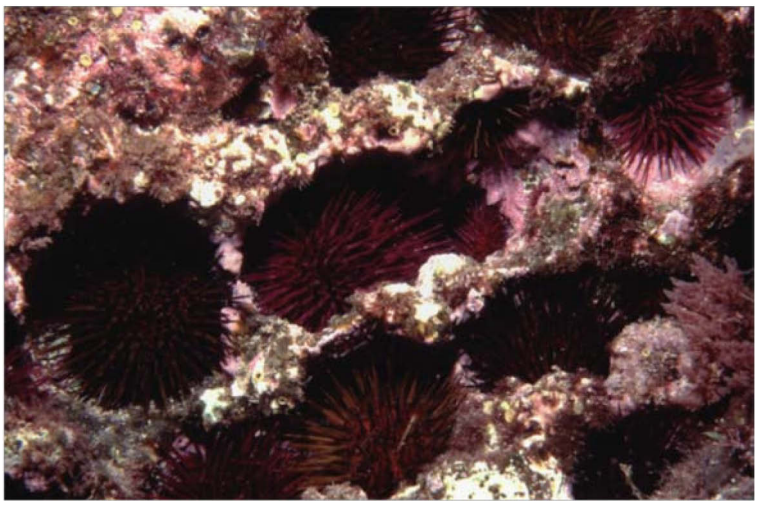

Fig. 1.4. *Paracentrotus lividus* specimens sheltered in substrate's niches.

This species is sensitive to sudden changes in salinity and dies if exposed for long time to extreme regimes, with values between 15-20 and 39-40 PSU (Allain, 1975; Le Gall et al., 1989). As far as size is concerned, there is a significant difference between the open sea resident populations and the ones living in coastal and lagoon environments: in the latter ecosystems, in fact, specimens are characterised by smaller sizes. This is probably due to critical environmental conditions that characterise several coastal lagoons, such as strong changes of temperature and salinity (Le Gall et al., 1989; Fernandez &

Boudouresque, 1997). Moreover, it has been recently demonstrated that massive edible sea urchin mortality occurs when water temperatures crossed 30.5°C in the eastern Mediterranean (Yeruham et al., 2015). On the other hand, there is no doubt about the fact that harvesting is the main cause of absence or reduction of large sea urchins in several regions of the world.

As already mentioned, *P. lividus* is a sciaphilic Echinoid: in order to shelter from light and hide from predators, it is frequently covered with small stones, empty mollusc shells, bits of seaweeds, etc., which are retained by means of pedicels and spines (Millot, 1975; Lawrence, 1976; Verling et al., 2002).

As reported by McCarron et al. (2010), this behavioural trait, known as "covering behaviour", has been documented for several Echinoids such as *Evechinus chloroticus*, *P. lividus* and *Strongylocentrotus droebachiensis* (Dix, 1970; Crook et al., 1999, 2000; Adams, 2001). Furthermore, many studies have reported that *P. lividus* exhibits patterns of nocturnal behaviour to avoid diurnal predators (Dance, 1987). In contrast, studies conducted in Lough Hyne (Ireland) have demonstrated a diurnal activity of this species (Barnes & Crook, 2001a). Boudouresque & Verlaque (2007) showed that in the Mediterranean it forages mainly during the night, especially on algae and on *Posidonia oceanica* leaves. In fact, it is well known that a good percentage of sea urchins diet is composed of macroalgal debris and fragments of aquatic plants transported by currents (Rodriguez & Fariña, 2001) which some species, including *P. lividus*, are able to retain and subsequently consume (Kelly et al., 2012). In addition, when food availability decreases adults can become detritivore (Boudouresque & Verlaque, 2007).

It has been recently demonstrated that at shallow depths (then exposed to a strong hydrodynamic action), the probability of coming into contact with this source of food markedly increases, promoting higher growth and, as a consequence, a higher gonadal

development (Livore & Connell, 2012). However, this result is in contrast with those reported by Guettaf et al. (2000) and Gianguzza et al. (2013). In fact, these latter authors found that the gonadal index turns out to be higher in populations of *P. lividus* living in low hydrodynamic conditions.

This discrepancy highlights the highly localised nature of food supply and, consequently, somatic and gonadal growth are also reflected in the variability of breeding seasons through the geographical distribution of *P. lividus*.

1.4 Density

Paracentrotus lividus exhibits a gregarious behaviour and lives in aggregates in which smaller individuals tend to stay under larger ones (including inside holes for populations living in rocky shores; Grosjean, 2001). This behaviour was interpreted as a defence mechanism against predators (Tegner & Dayton, 1977).

Over its entire geographical distribution and in all depth intervals, densities of *P. lividus* ranging from a few specimens up to a dozen per m^2 are quite common. The highest densities (50 to 100 specimens per m^2) are usually observed in shallow environments, on gently inclined rocks, on pebbles or boulders, in rock pools and in polluted environments. Higher densities correspond to localised aggregations (more than 1,600 specimens per m^2), although the causes of this phenomenon are still unknown. These aggregations may be, also, a defensive tactic against predators, or a food strategy or, even, an ethological expression of reproductive activity (Boudouresque & Verlaque, 2007). However, in order to explain this phenomenon many other hypotheses have been proposed as, for example, the organic pollution (Harmelin et al., 1981) and the overfishing of natural predators which, consequently, increases recruitment of juveniles (Sala & Zabala, 1996; Sala et al., 1998).

High densities, both in the field and in the laboratory, seems to lead sea urchins to cannibalism events, even if reports of cannibalism in this species are not as prevalent in the literature as are, for instance, those observed for sea stars. The process of cannibalism begins by all of the spines being consumed (usually on the aboral surface), then the test is perforated and the guts and gonad are consumed (Richardson, 2010). Despite a few reports, it seems that the cannibalistic process is similar in *Lytechinus variegatus* (Richardson & Watts, 2008; Richardson et al., 2011), *Strongylocentrotus droebachiensis* (Himmelman & Steele, 1971) and *Diadema antillarium* (Levitan, 1989). Often coupled with density, starvation is important in the rate of cannibalism (Richardson et al., 2011). As observed by some authors, this behaviour in sea urchins is probably a response to food limitation (McPherson, 1968; Himmelman & Steele, 1971; Levitan, 1989).

Finally, it was noted that newly metamorphosed *L. variegatus* juveniles show a significant rate of cannibalism in the laboratory (Richardson & Watts, 2008), and that cannibalism decrease as the urchins increase in size (Richardson, personal observation). Populations of *P. lividus* can be relatively stable for several years, although this phenomenon is not common. Conversely, it is possible to observe a rapid change in terms of density in larger-sized individuals. Apparent changes in terms of density can be misleading, both because of the daily and seasonal specimens' behaviour and because of the census method.

For example, in Lough Hyne (Ireland) the apparent size of the population (visible specimens) differed between day and night (respectively 15.8 and 6.7 units per m^2; Barnes & Crook, 2001a). Moreover, this daytime activity is inversely related to the abundance of the main nocturnal predators, including crabs and the starfish *Marthasterias glacialis* (Ebling et al., 1966; Kitching & Thain, 1983; Crook et al.,

2000; Barnes & Crook, 2001b). It is assumed, therefore, that *P. lividus* "learns" both through the perception of the preyed sea urchins fluids and by linking the light intensity to the predation levels, thus adopting an activity pattern opposite to that of the predators.

1.5 Reproductive cycle

Paracentrotus lividus is a gonochoric species, although cases of hermaphroditism have sometimes been observed (Drzewina & Bohn, 1924; Neefs, 1938; Byrne, 1990). As in other species of Echinoidea, the fertilisation takes place in the environment after the emission of gametes, which occurs directly in the water (McEdward & Benjamin, 2001). Eggs and sperm are able to survive for about 24 hours (at least in experimental conditions), but the likelihood of fertilization dramatically decreases after a few hours. The population *sex-ratio* seems to change throughout the year, as well as from year to year.

Gonad maturation occurs *in vitro* in specimens with a test diameter between 13 and 20 mm, and at least at the age of 5 months (Cellario & Fenaux, 1990). In the natural environment, sexual maturity occurs after a longer period of time, probably due to food availability and quality. In addition, adverse conditions and limited food resources could also lead to an increase of the size of the first sexual maturity. In the Mediterranean Sea, the reproductive cycle of *P. lividus* shows 1 or 2 seasonal gonad growth peaks which, however, may differ significantly between near locations (Guettaf & San Martin, 1995; Lozano et al., 1995; Spirlet et al., 1998; Guettaf et al., 2000; Sánchez-España et al., 2004).

Several *in vitro* studies have shown that the somatic and gonadal growth occurs particularly when food availability is high (Lawrence et al., 1992; Gago et al., 2001)

and the amount of organic matter ingested by *P. lividus* is remarkable (Frantzis & Grémare, 1992). However, other physical parameters (e.g., light regime, temperature) can positively affect gonadal growth (Sartori et al., 2015). Reproduction can also be enhanced by redirecting resources at the expense of somatic growth. In conditions of unlimited availability of food, Spirlet et al. (2000, 2001) observed that the temperature is the most important factor affecting sea urchins gonad enhancement. An examination of the effect of temperature on gonad growth in *Paracentrotus lividus* revealed that a combination of 24°C and 9 hours of daylight can improve it, unlike treatments with lower water temperature and longer photoperiod (Spirlet et al., 2000). In contrast, other authors showed that gonadal growth is affected by water temperature only when it exceeds 26°C (Shpigel et al., 2004). Field observations have revelead conflicting results. Larger gonads were observed in subtidal populations with abundant amounts of available food (both offshore and in lagoons). In addition, with constant availability of food, gonadal index was higher at low density population. Generally, adult males and females spawn simultaneously for a few hours. These emission episodes, however, do not affect all individuals of the same population at the same time. This could be a strategy adopted in order to extend the emission activity and to preserve as much eggs and sperm as possible against predators. The gamete suspension, however, can activate a process of sexual products release in others mature male and female specimens. Also this behaviour can be explained as a defensive strategy adopted in case of sudden environmental changes or high stress, with the aim of releasing the gametes of the whole population to prevent extinction. It was found that in *P. lividus* the gametes emission occurs only once or twice per year. As noticed by Walker et al. (1998, 2001), gonads maturation is unrelated to the water temperature, while release of gametes (spawning) would be activated only in response to a minimum temperature threshold. A

water temperature between 13 and 16°C may also provide a rough indication of the starting time of this process. Therefore, when 2 reproductive periods occur they can take place when temperature firstly increases up to a critical value and, afterwards, when it decreases to the same value (Fenaux, 1968; Byrne, 1990; Pedrotti, 1993). In relation to what has been said, the first emission event could be triggered by the length of the day (about 15 hours of light) rather than by the temperature, while the end of spawning period appears to be particularly influenced by the latter (Spirlet et al., 1998). Considering the extreme variability among locations, habitat, individuals and years (and compared to the number of annual peaks), the emission of gametes can take place throughout the year, although often at very low levels. This could be a strategy to ensure that the risk of loss of planktonic stage larvae is distributed over time. The larva that develops after fertilisation (named echinopluteus) is present in the plankton throughout the year and it can live from 20 up to 40 days before settling on the substrate. Subsequently, it metamorphoses into a juvenile sea urchin with a shift from bilateral to pentaradial symmetry (McEdward & Benjamin, 2001). Sexual maturity seems to be reached about in the third year of life (Boudouresque & Verlaque, 2007) with a test diameter of 20-25 mm (Grosjean, 2001).

1.6 Growth

In the wild, *Paracentrotus lividus*' somatic growth appears to be influenced by water temperature, quantity and quality of available food resources and gonadal development (Fernandez, 1996). Seasonal variations in the growth rate seems to be related to the first of the above mentioned factors. In the Mediterranean, the highest growth rate of this species is observed at temperatures between 12 and 18°C in spring and, only sometimes, in autumn. Dwarfism phenomenon, usually observed in coastal lagoons, seems to be

attributable to a premature mortality of individuals rather than to their lower growth rate. In exposed areas, which suffer from low quantity and poor quality of available food, the maximal growth rates at an earlier age than those recorded at deeper depths and without any food limitation were observed (Turon et al., 1995). A study from a Corsican coastal lagoon (where a significant amount of food resources is available), reported the thickening of the sea urchins test, a morphological characteristic that ensures a defence against predation by a large number of fish species (Fernandez & Boudouresque, 1997). Conversely, a further developed Aristotle's lantern was observed in individuals living at depths characterised by the presence of small pebbles and limited amounts of food. This feature, probably, allows a greater efficiency in the grazing activity. Specimens with a test diameter of about 2 cm are generally considered to be 2 years old, while reach the size of 4 cm in 4-5 years with a growth rate of about 1 cm per year (Turon et al., 1995; Fernandez, 1996; Sellem et al., 2000; Gago et al., 2001; Grosjean et al., 1996, 2003; Sartori et al., 2015).

By the analysis of the growth bands in the skeleton, Allain (1978) reported that wild sea urchins reach the size of 40-50 mm in 4 years. Grosjean et al. (1998), instead, in a long term rearing experiment (7 years) observed that laboratory cultured *P. lividus* specimens reached commercial size in a period between 2 and 3.5 years. This difference could be explained by water temperature, which was heated during winter and, above all, by *ad libitum* provision of food throughout the year, thus allowing the growth of sea urchins in optimal conditions. In a cohort of homogeneous *P. lividus* juvenile specimens kept in aquarium, a multimodal distribution of sizes was noted with individuals which grew rapidly or gradually. This variability in growth rate is the consequence of the intra-specific competition. Even in the wild, this inhibition of growth could be very efficient for maintaining population, with many small individuals with high potential growth

rate, however unexpressed due to the density of the larger ones. Nevertheless, a decrease in density of the latter can put an end to the growth block exerted on younger specimens (Grosjean et al., 1996).

1.7 Recruitment

In the North western Mediterranean Sea, *Paracentrotus lividus* larvae are present in the plankton throughout the year. However, 1 or 2 peaks are usually observed: the first between May and June and the second, if present, from September to November. Lozano et al. (1995) suggested that the autumn peak can be due to a prolonged survival of larvae resulting from reproductive events of the spring-summer season, which took place in adverse environmental conditions. The *in situ* lifespan of planktonic larvae was estimated in 23-29 days, but it may reduce to 14-19 days *in vitro* with unlimited food availability. In general, the larvae produced by well-fed individuals survive better, their growth is faster and the metamorphosis occurs before those which are obtained from poorly fed specimens. The test diameter of newly metamorphosed individuals is approximately 0.4 mm. In the Mediterranean Sea, the settlement of benthic forms occurs once or twice during the whole year and the main recruitment occurs in spring, although some new recruits are found throughout the year.

At present, little or nothing is known about the influence of benthic communities (and their species composition) on the settlement and survival of *P. lividus* post-metamorphic stages. Some benthic species can hinder the settlement and/or the survival of larval forms. The survival of low number of recruits that settle on the substrate is extremely low, as well as their subsequent survival and the achievement of sexual maturity size. In fact, during early stages of benthic life mortality rates are highly dependent on density. The interannual variation of recruitment is also considerably high. These differences are

usually typical of the populations' demographic structure, which can shows over-represented, under-represented or even absent cohorts. Substantial changes in abundance and size composition of populations can significantly change the impact of this species on the environment.

1.8 Fishing and commercial value of the sea urchin in Sardinia

The edible sea urchin *Paracentrotus lividus* is a valuable seafood product in several Italian coastal areas (Tortonese, 1965), particularly in Sardinia, where the harvesting of this species is an ancient practice. A homemade harpoon (the so-called *manu de ferru*) was the tool used in ancient times in Sant'Antioco Island (South western Sardinia) for the fishing of this Echinoid. The device in question was equipped with 6 points and was curved in order to hold the sea urchin and easily detach it from the rocks. But the best-known and most primitive method for fishing sea urchins was, at least until a few decades ago, by means of an ordinary reed (*Arundo donax*) of several meters in length, longitudinally cut in the end to about 10 centimeters in order to form a cross, with a piece of cork or a stone wedged into it and fixed with a ligature.

The harvesting of this species is intensively practised in order to obtain roe (male and female gonads; Fig. 1.5) principally near Cagliari (southern coast), in the area of Alghero-Sassari (North western coast) and in the neighbourhood of Oristano (central western coast). In contrast, along the eastern coast of Sardinia (Baronie-Dorgali and Orosei-Ogliastra) fishing effort for sea urchins is minimal or absent.

This product is usually consumed fresh and raw, but it is used for the preparation of many typical dishes (i.e., pasta, pizza, croutons, etc.; Fig. 1.6), either at the restaurant or at home. As a consequence, sea urchin market is an important economic sector in Sardinia, primarily for fishermen, for dealers and traders and also for consumers. In the

last few decades, the demand for this product has significantly increased and its commercialization has become widespread in the region and distributed all over the year.

Fig. 1.5. *Paracentrotus lividus* roe.

For this reason, local fishermen abandoned the above mentioned traditional fishing techniques and commercial harvesting activities are now carried out by divers (mainly SCUBA divers and much less free divers without breathing apparatus).

Intensive commercial fishing began in Sardinia at the end of 1980s, and in 1994 the Regional Department for Environmental Protection introduced regulatory measures to reduce the risk of overfishing. During the same period of time, a number of sea urchin fisheries were set up and have steadily increased their production up to 2009, with 160 fishing licenses issued (Addis et al., 2009).

Nowadays, there are strict regulations for the harvesting, transportation, storage and processing of this species (the last is the 2423/DecA/49 of October 15, 2015). From an environmental point of view, the current constraints have established a better management system of this resource but do not allow commercial sea urchin harvesting

to be profitable enough.

A number of studies examined the effect of fishing restrictions (i.e., through the comparison between marine protected areas and exploited areas) on *P. lividus* populations in the Mediterranean Sea (Bertocci et al., 2014). However, contrasting results in terms of density, abundance of large-sized individuals, and total biomass were found (Sala & Zabala, 1996; Sala et al., 1998; Guidetti et al., 2005; Gianguzza et al., 2006; Ceccherelli et al., 2009, 2011; O'Sullivan & Emmerson, 2011; Pais et al., 2007, 2012). In contrast, there are only a few studies from the European Atlantic coasts (Bertocci et al., 2012, 2014).

Fig. 1.6. Several typical dishes prepared with sea urchins roe.

Due to the absence of consistent historical data, it is impossible to quantify the quantity of sea urchins landed annually in recent years. According to recent estimates, however, approximately 30 million sea urchins (approximately 1,800 t) are consumed in Sardinia every year, accounting for an income of more than 10 millions € (Carboni et al., 2012). Starting from this state of affairs, Sardinia's annual *per capita* consumption is 1.1 kg, about 4 times the Japanese consumption (0.27 kg/person). However, these estimates might be more accurate when considering a broader spectrum of consumers (i.e., not

only the locals but also the tourists). In fact, tourism roe market demand has caused an increasing interest and dramatic changes in patterns of consumption of *P. lividus* in Sardinia (Pais et al., 2011, 2012).

1.9 Interest for aquaculture

At present, Chile is considered the major producer of sea urchin roe and Japan and Europe the principal consumers. In particular, the first is the world's largest importer of this product (Carboni et al., 2012). Considering what has been said, it is not surprising that there is a high level of interest in aquacultural activities due to growing demand for this seafood. In fact, aquaculture has the potential for production of sea urchins for human consumption (Lawrence et al., 2001).

Over the last 10-15 years, the interest in commercial breeding of Echinoids has increased in many countries, including Japan, Australia, Canada, Chile, China, New Zealand, Norway, Ireland, Italy and Scotland (Carboni et al., 2012). In the light of this fact, in the last few decades several edible sea urchin species were investigated (*Anthocidaris crassispina*, *Lytechinus variegatus*, *Paracentrotus lividus*, *Psammechinus miliaris*, *Strongylocentrotus droebachiensis*, and *S. nudus*), and numerous scientific studies on aquaculture of these edible Echinoids have been conducted since the 1980s (Lawrence, 2013).

In particular, many studies aimed at optimizing sea urchin *P. lividus* rearing have been reported in the literature (Boudouresque et al., 1996; Fernandez, 1997; Basuyaux & Blin, 1998; Grosjean et al., 1998; Fernandez & Pergent, 1998; Fernandez & Boudouresque, 2000; Spirlet et al., 2000; Jacquin et al., 2006; Cook & Kelly, 2007a; McCarron et al., 2009; Fabbrocini & D'Adamo, 2010; Fabbrocini et al., 2012). Furthermore, scientific research has led to remarkable advances in the assessment of

effects of different diets on the colour, quantity and quality of the gonads of this sea urchin species (Spirlet et al., 2000; Shpigel et al., 2005, 2006; Carboni et al., 2013; Vizzini et al., 2015).

Unlike in many other countries, some Scottish scientists have also focused their research efforts on the development of new breeding techniques involving the co-culture sea urchins, algae and Atlantic salmons (but also other species, such as the Atlantic mussel *Mytilus edulis*), in order to achieve economic and environmental benefits (the so-called Integrated Multitrophic Aquaculture) and to reduce pollution in marine environments (Cook & Kelly, 2007b, 2009).

1.10 Aim of the thesis

The main purpose of this thesis was to increase the knowledge of the edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) as a resource in Sardinia. To pursue this objective, several research approaches have been employed. 1) Morphometric characters and annual gonad index of sea urchin populations were monitored throughout a year in a coastal district of North eastern Sardinia. In fact, for sea urchin breeding purposes, it is necessary to have a basic knowledge of the main characters of this species (in particular gonad development and maturity stages). 2) Two experiments to test the response of this Echinoid to an artificial diet were carried out in a closed-circuit rearing system. Special attention was focused on the somatic and gonadal growth in order to enhance the development of sustainable and profitable aquaculture practices. 3) An analysis of the profitability and sustainability of edible sea urchin fishery in Sardinia. This latter could represent an important issue for policy makers in order to change the current sea urchin fishing policy and, therefore, increasing profitability for the Sardinian fishery firms without compromising the natural wild stocks of this important resource.

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2. Morphometric relationships and annual gonad index of *Paracentrotus lividus* from a coastal district of North western Sardinia

2.1 Introduction

Given the high commercial value of its gonads, the edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) is commonly harvested along the Mediterranean and North eastern Atlantic coasts (Boudouresque & Verlaque, 2001 and references therein). In Sardinia (central western Mediterranean), although the fishing of this species is strictly regulated by a regional decree (D.A. n. 270 dated 03.03.1994 and subsequent amendments), the populations of *P. lividus* are heavily exploited by both authorised and unauthorised fishermen, poachers, and occasional collectors throughout the year. In the last years, numerous studies on this species have been carried out in Sardinia (Ceccherelli et al., 2009, 2011; Addis et al., 2009, 2012; Pais et al., 2007, 2011, 2012; Pinna et al., 2009, 2012, 2013) but there is only limited information about the main morphological features of this sea urchin from this Italian region (Pais et al., 2006; Addis et al., 2015).

In natural conditions, the somatic growth of this species is related to seawater temperature, food quantity and quality, and gonadal maturation (Fernandez, 1996 and references therein). As far as this latter is concerned, Byrne (1990) reported maximum values of the gonad index in May and June from the West coast of Ireland (North eastern Atlantic) during several years of observations, although this value varied from year to year. On the other hand, Bayed et al. (2005) noted an increase in gonad index of *P. lividus* between January and March in the northern Moroccan Atlantic coast, coinciding with the beginning of a period of algal reproduction, concurrent with an increase of seawater temperatures and food consumption by the sea urchins.

In the Mediterranean Sea, Fenaux (1968) indicated that at Villefranche-sur-Mer (South eastern France) sea urchins attained the maximum gonadal index values during the spring. In contrast, Lozano et al. (1995) detected that the gonadal maturation of this

Echinoid occurred for the major part during the winter on the Catalan coast (North eastern Spain), and that the main spawning events took place in spring or early summer, suggesting that phytoplankton abundance has a positive effect on the onset reproductive cycle.

In any case, it is clear that the gonadal maturity of *P. lividus* can greatly vary due to geographic, demographic or edaphic factors, but it can also be strongly influenced by habitat characteristics and predator pressure. Thus, due to its economic importance, the present part of this thesis was aimed at providing baseline data on morphometric relationships and annual gonad index of adult specimens of *P. lividus* in a coastal district of North western Sardinia (Alghero) where the sea urchin fishery is a traditional activity.

2.2 Materials and methods

Adult sized specimens of *Paracentrotus lividus* were sampled monthly in 3 rocky locations (Calabona, Punta Negra and Porto Agra, hereafter CB, PN and PA, respectively; Fig. 2.1) near the town of Alghero from November 2011 to October 2012. These locations were randomly selected from a larger number of sites strongly impacted by both professional fishermen and amateur divers and, in particular, the third one (Porto Agra) was exactly located just outside the boundaries of the Capo Caccia-Isola Piana Marine Protected Area. At all the considered locations, the calcareous substrate was mainly colonized by erect algae such as Chlorophyta, Phaeophyceae and Rhodophyta and several other encrusting calcareous red algae.

Fifty sea urchin specimens were monthly collected by 2 free divers during the same sampling day in each shallow rocky site (i.e., between 2 and 4 m depth). To improve accuracy, all the field work activities were always performed by the same 2

experimenters between 10:00 a.m. and 13:00 p.m. The harvested samples were immediately transported to the laboratory where they were measured and dissected as described as follows.

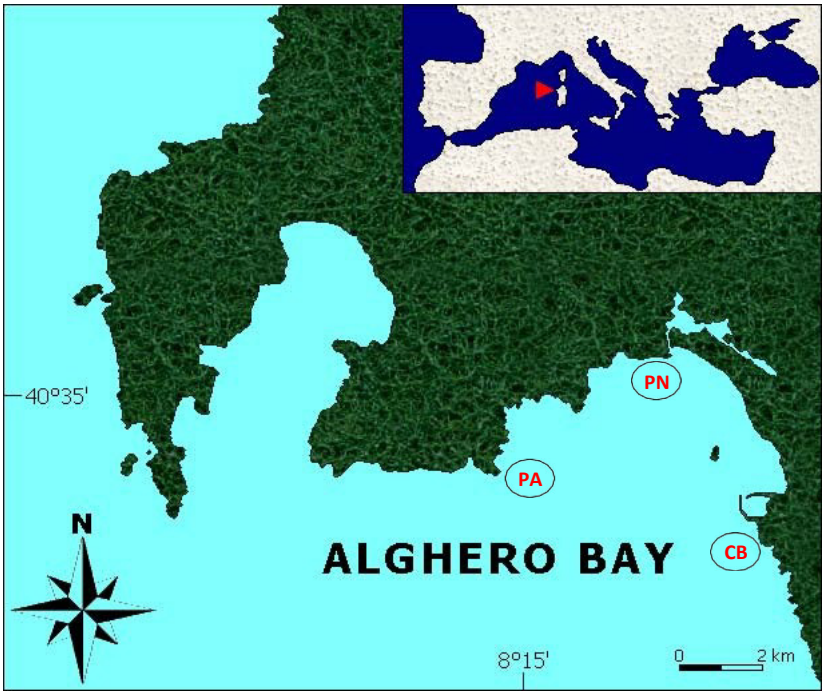

Fig. 2.1. Study sites in Alghero Bay: Calabona (CB), Punta Negra (PN), Porto Agra (PA).

The most important external morphometric characters such as diameter (D, perpendicular to the oral-aboral axis) and height (H, oral-aboral axis) of the test without spines of each sea urchin were measured to the nearest 0.1 mm using a digital calliper. All the above-mentioned measurements were always carried out by the same experimenter to improve accuracy. The total wet body weight (BW) of each specimen was then recorded to the nearest 0.1 g by means of an electronic balance after draining residual water on absorbent paper for 10 minutes. Subsequently, the sea urchins were dissected (Fig. 2.2) and gonad wet weight (GW) was measured by a precision balance to the nearest 0.01 g to estimate the gonad index (GI) as described by Lawrence et al. (1965):

$$GI = \frac{GW}{BW} \times 100$$

One-way Analysis of Variance (ANOVA) was used to test the null hypothesis that there were no differences in test diameter (D), test height (H) and total body weight (W) of the sea urchins at the 3 locations studied. Two-way ANOVA was performed to test for differences among the GI monthly values of *P. lividus* at the 3 locations.

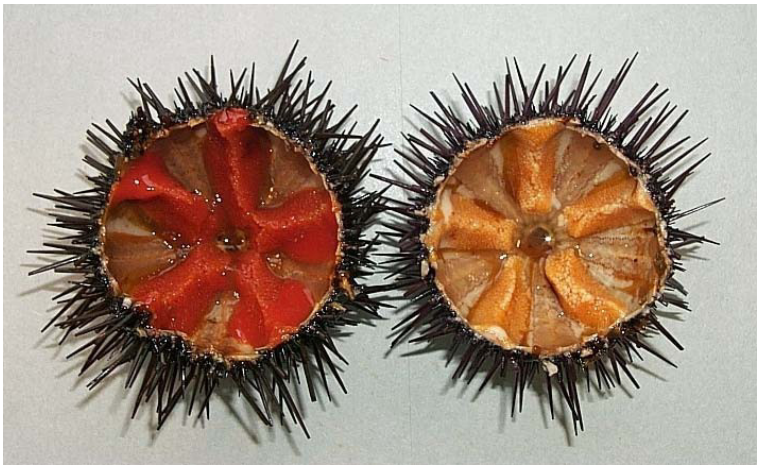

Fig. 2.2. *Paracentrotus lividus* female (left) and male (right) gonads (roe).

Before the analysis, Cochran's C test was used to check the assumption of the homogeneity of variances and, whenever necessary, data were appropriately transformed. Where data transformation did not correct violations in the assumption of homogeneous variances, an alpha-level adjustment to 0.01 was used to compensate for increased type I errors (Underwood, 1997). Finally, Tukey's test was used for *post hoc* comparisons.

Furthermore, in order to find a correlation between GI and seawater temperature, the monthly average values (November 2011 - October 2012) of surface water temperature in Alghero Bay were achieved by consulting http://www.seatemperature.org.

All the values of each morphometric variable examined in the sea urchins collected in

the 3 locations were pooled and the allometric relationships between test diameter and total weight, test diameter and test height, and total weight and test height were estimated by applying the regression analysis using the power function $Y=aX^b$ (i.e., $W=aD^b$, $W=aH^b$, and $H=aD^b$, respectively).

After linearisation, they were then compared using ANCOVA (Sokal & Rohlf, 1995) to detect putative differences among sea urchins from the 3 locations.

All the statistical analyses were always performed using the STATISTICA® software package and a significance level of 5% ($p<0.05$) was used.

2.3 Results

In total, 1,800 adult sized specimens of *Paracentrotus lividus* were examined during the study period (600 in each of the 3 locations). The average test diameter of all the sea urchins sampled (ranging from 32.5 to 67.2 mm) was equal to 49.7±4.9 mm (48.5±3.6 mm at CB, 48.9±4.2 mm at PN and 51.7±5.0 mm at PA, respectively; Figs. 2.3, 2.4 and 2.5), while the average test height (ranging from 16.9 to 38.2 mm) was 24.9±3.0 mm (24.4±2.5 mm at CB, 24.6±3.0 mm at PN and 25.8±3.2 mm at PA, respectively). The average body weight of all the specimens collected (ranging from 19.2 to 133.9 g) was equal to 53.7±14.0 g (50.4±10.5 g at CB, 50.4±12.6 g at PN and 60.4±15.9 g at PA, respectively).

One-way ANOVA detected significant differences for the above-mentioned morphometric variables of the sea urchins collected at the 3 locations. In particular, specimens sampled at PA (located at the boundaries of the Capo Caccia-Isola Piana Marine Protected Area) where larger than those from CB and PN, revealing significant higher values of all the biometric features analysed. No significant differences between sea urchins from CB and PN were instead detected.

Two-way ANOVA showed significant differences in GI monthly values (Fig. 2.6) both for the factors “location” and “month” as well as for their interaction (Table 2.1).

Table 2.1. Two-way ANOVA results on GI of sea urchins in the 3 locations.

Source	df	MS	F	p
Location	2	77.60	16.97	<0.001
Month	11	293.29	64.13	<0.001
Location × Month	22	71.16	15.56	<0.001
Residual	1,764	4.57		

In particular, the *post hoc* Tukey's test showed that the GI monthly value was significantly higher (4.5) in the sea urchins from Porto Agra (PA) than in those from the 2 other locations [Punta Negra (PA) and Calabona (CB), 4.0 and 3.8, respectively].

Furthermore, the *a posteriori* comparison evidenced that the average GI was highest in February and April (6.8 and 6.4, respectively), followed by March and January (5.3 and 4.8, respectively), and then by November (4.2). The average GI was quite similar during all the other months of the year, reaching a minimum value in June (2.7) and a maximum in July (3.5).

Monthly surface seawater temperatures in Alghero Bay varied greatly throughout the year during the study period (Fig. 2.6), with values ranging from a minimum of 13.4°C recorded in February to a maximum of 24.1°C in August. Nevertheless, the temperatures were always over 21°C from July to October. The Pearson correlation coefficient showed a significant inverse correlation between the average GI monthly value and the seawater surface temperature for the sea urchins from CB ($r=-0.916$, $p<0.001$) and PA ($r=-0.624$, $p=0.030$), whereas no significant correlation ($r=-0.359$, $p=0.251$) was found for *P. lividus* specimens sampled at PN (Fig. 2.6).

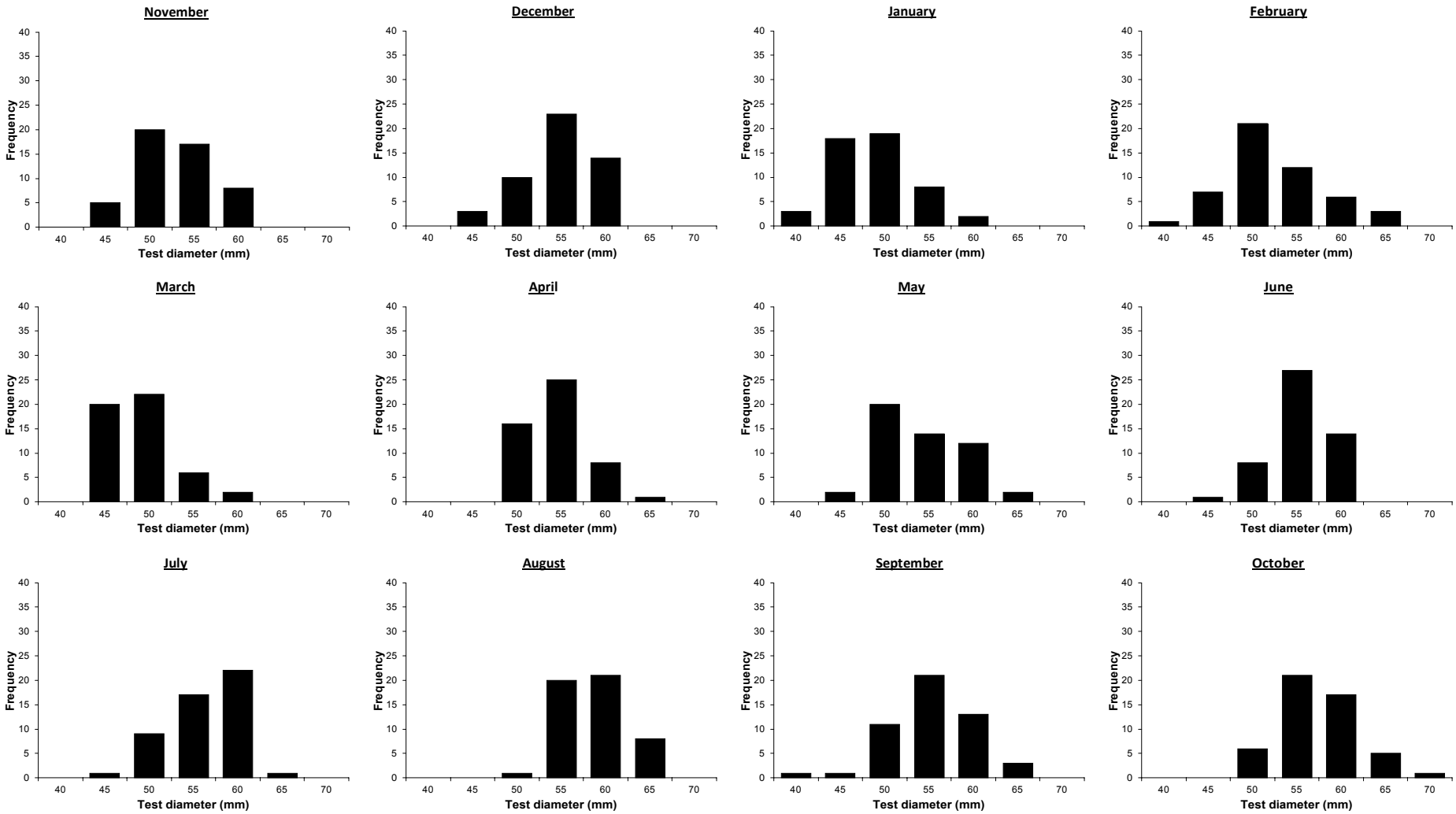

Fig. 2.3. Mean test diameter of sea urchins sampled at Calabona.

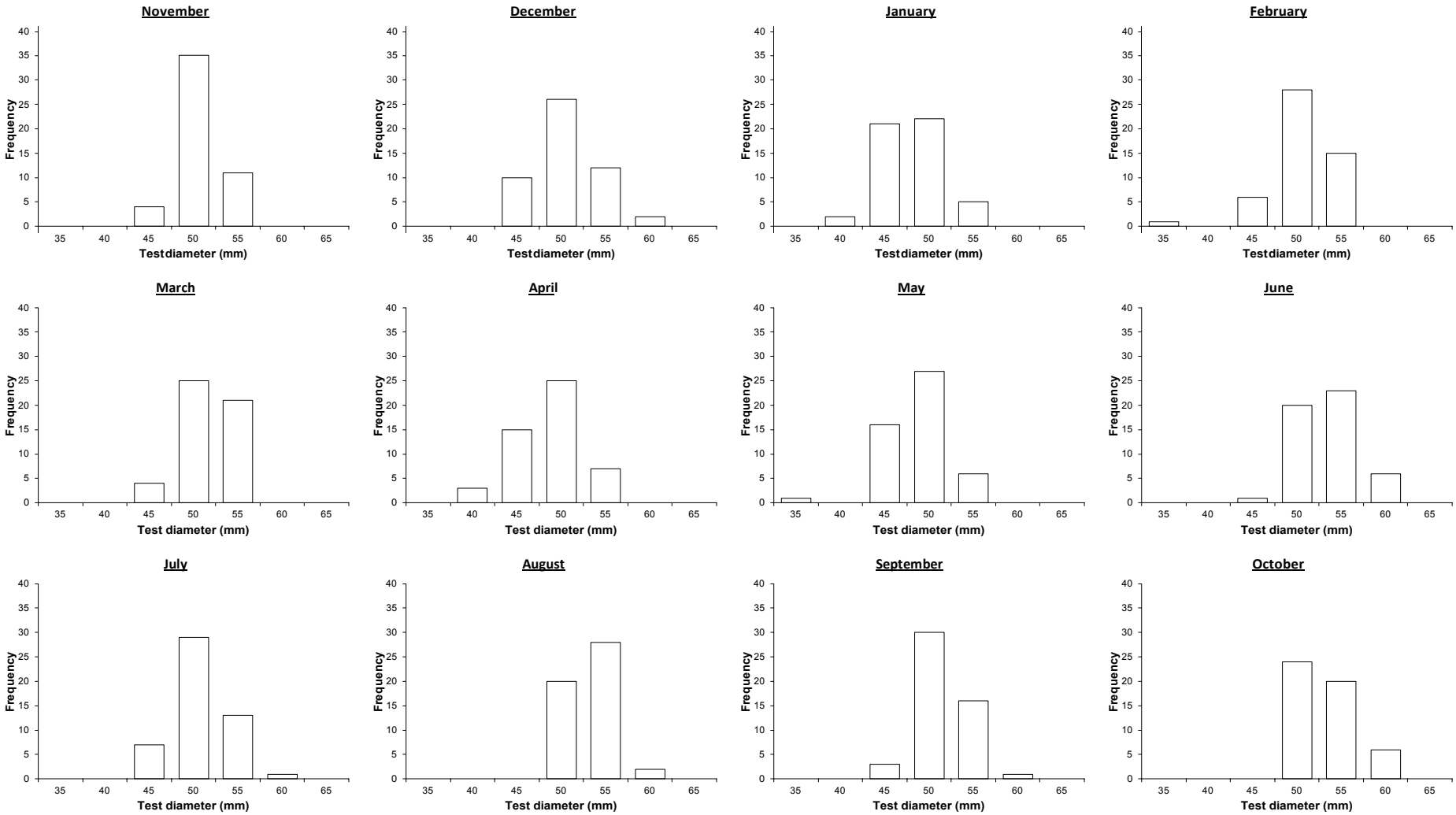

Fig. 2.4. Mean test diameter of sea urchins sampled at Punta Negra.

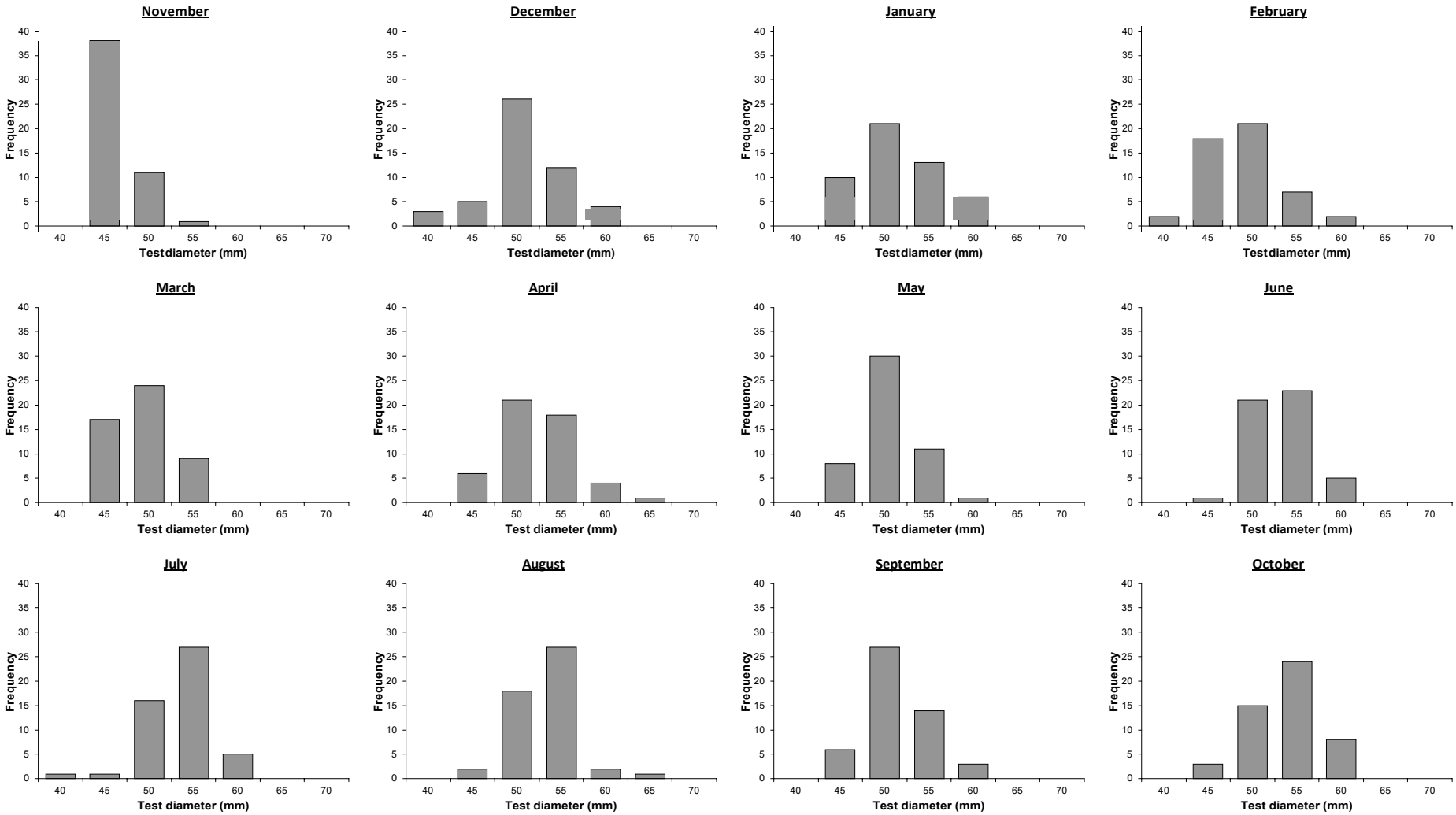

Fig. 2.5. Mean test diameter of sea urchins sampled at Porto Agra.

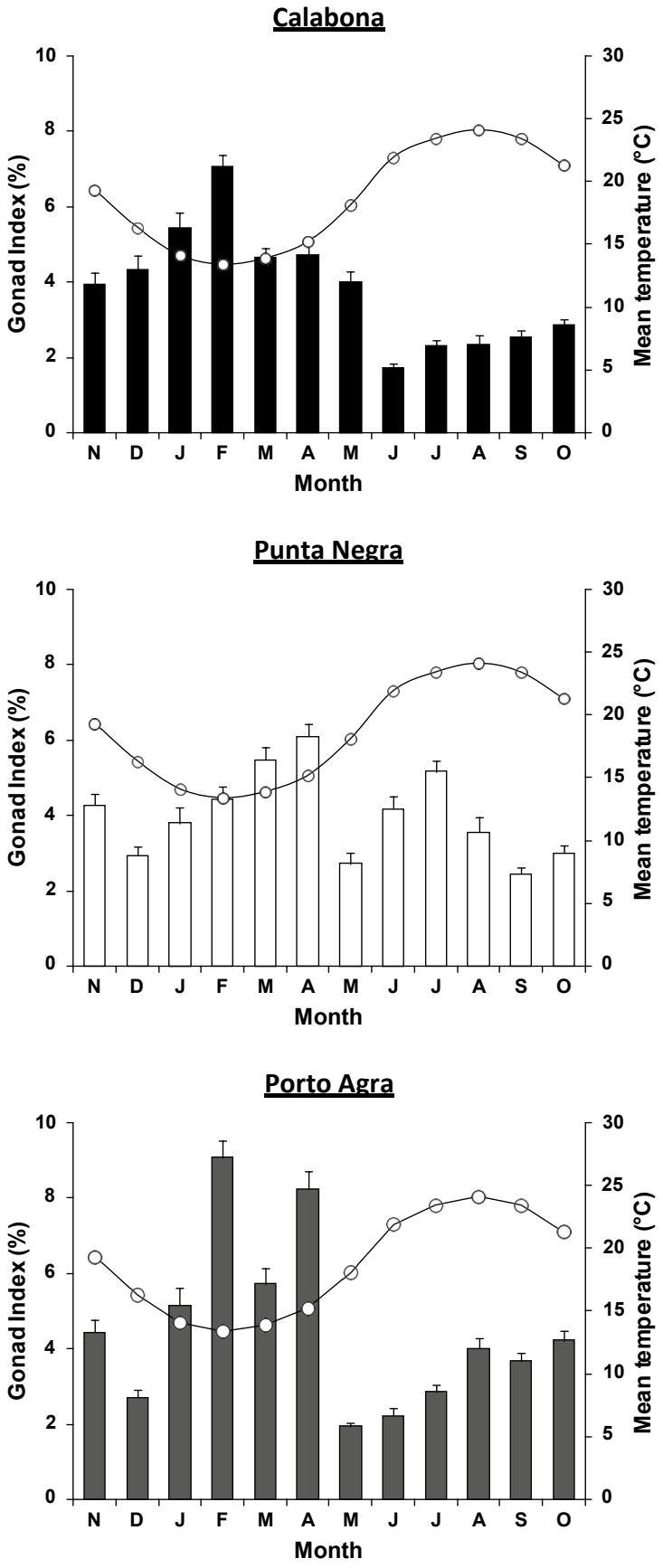

Fig. 2.6. Mean monthly GI values (+SE) and seawater temperatures in the 3 locations.

With regard to regression results on the morphometric variables considered, since ANCOVA did not detect any significant differences among sea urchins from the 3 locations for all the computed regressions, the following equations obtained by pooling all data (Fig. 2.7) measured for test diameter (D), test height (H) and body weight (W) were validated:

$$W = 0.0017\ D^{2.6514}\ (r = 0.93)$$

$$W = 0.1366\ H^{1.8512}\ (r = 0.85)$$

$$H = 0.4347\ D^{1.0371}\ (r = 0.79)$$

Lastly, these equations were compared to those reported in other studies both from other countries and in Sardinia, as illustrated in Table 2.2.

Table 2.2. Regression analysis values for *Paracentrotus lividus* in different study areas.

Weight/Diameter	Weight/Height	Height/Diameter	Reference
$D=1.323\ W^{0.340}$ r=0.93	H=0.436 W+0.433 r=0.82	H=0.578 D-0.347 r=0.71	Ballesteros (1981)
$W=0.0032\ D^{2.479}$ r=0.92	$W=0.5396\ H^{1.417}$ r=0.77	$H=0.4252\ D^{1.035}$ r=0.72	Pais et al. (2006)
$W=0.0062\ D^{2.48}$ r=0.92	$W=0.0803\ H^{1.95}$ r=0.83	$H=0.85\ D^{0.87}$ r=0.83	Antoniadou & Vafidis (2009)
W=2.926 D-0.366 r=0.96	W=2.125 H+0.765 r=0.84	H=0.526 D-0.916 r=0.87	Küçükdermenci & Lök (2014)
$W=0.0017\ D^{2.651}$ r=0.93	$W=0.1366H^{1.8512}$ r=0.85	$H=0.4347D^{1.037}$ r=0.79	This study

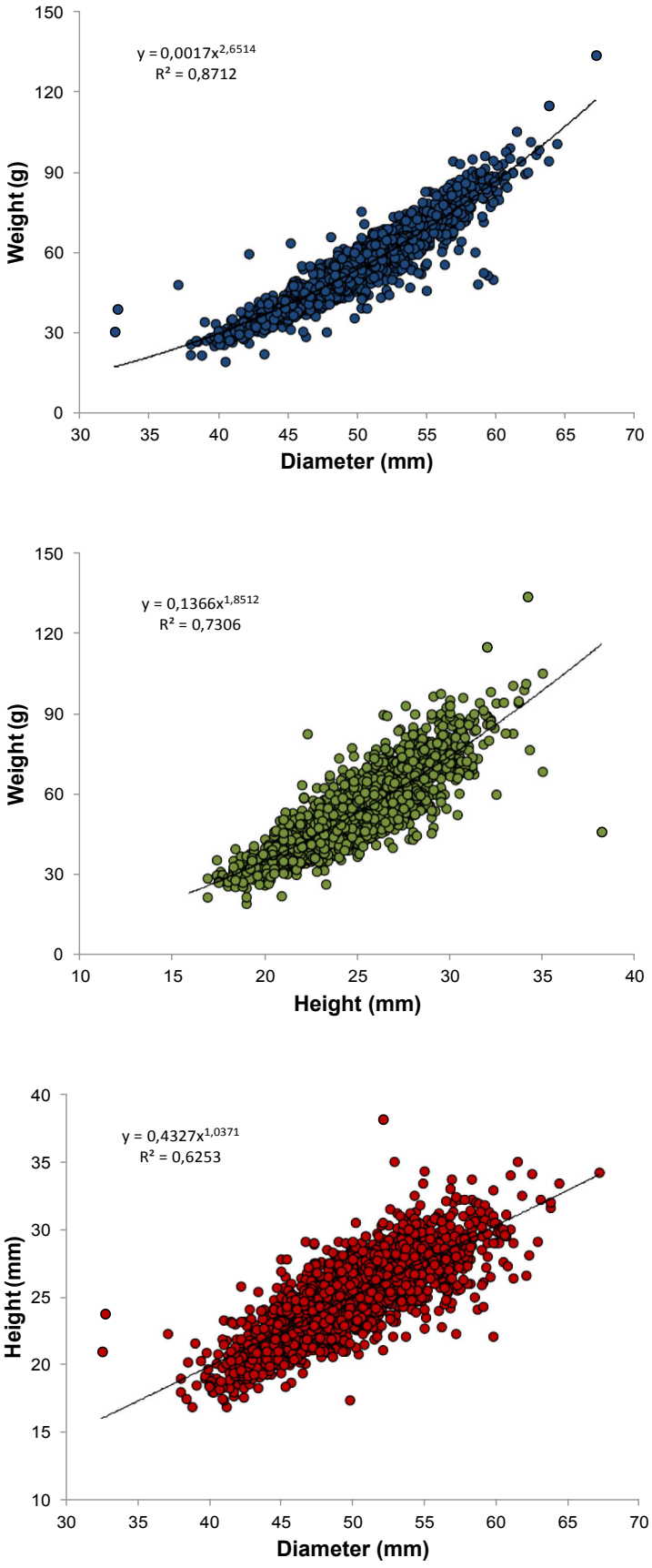

Fig. 2.7. Regression plots of the morphometric variables examined in sea urchins.

2.4 Discussion

The study of the morphometric features is a powerful tool for the characterisation of the populations of several sea urchin species in different regions of the world. The quantification of specific characteristics of an individual, or group of individuals can reveal the degree of speciation induced by both biotic and abiotic conditions, and contribute to the definition of different stocks (Ebert, 1982). This is particularly important for commercial species and for the marine ecosystem as a whole. Consequently, the knowledge of morphometric features of a target species like the edible sea urchin *Paracentrotus lividus* is an essential step to design more efficient management strategies for both the conservation and sustainable exploitation of this important seafood resource.

Furthermore, when a sea urchin population has a restricted range of similar size classes its reproductive cycle can be described through the gonad index (Gonor, 1972; Ebert et al., 2011). In the present study, this index was considered adequate to infer the gonadal maturation of *P. lividus* in a district of North western Sardinia where sea urchin harvesting is considered a traditional practice.

The main spawning activity of this Echinoid takes place in the coastal waters of the Mediterranean during spring to early summer (Lozano et al., 1995; Sellem & Guillou, 2007). Some studies also reported that secondary reproductive events occur in autumn and winter, along with a prolonged spawning period in its southern distributional range (Guettaf & San Martin, 1995; López at al., 1998; Guettaf et al., 2000; Sánchez-España et al., 2004; Tomas et al., 2004).

On the other hand, some authors showed that the use of gonad index (GI) to determine the sexual cycle and spawning period of *P. lividus* is not sufficient (Soualili & Guillou, 2009). In fact, they observed numerous increases and decreases of the GI peak that did

not reflect spawning events as they were not followed by observations of spawned stages. As a matter of fact, gonads usually store biochemical components as an energy source for subsequent gametogenesis but also can use them as reserves for metabolism following any disturbance in the environment (storms, temperature decrease, etc.) as reported by Byrne (1990).

As far as the annual GI is concerned, the results achieved were consistent with those reported by Fenaux (1968) from Villefranche-sur-Mer (South eastern France), while only partially in agreement with the observations made at the same latitude by Lozano et al. (1995) from the Catalonian coast (North eastern Spain). Furthermore, the GI peak periods evidenced in this study are to some extent similar to those recently reported by Küçükdermenci & Lök (2014) from Foca (South Aegean Sea, Turkey).

Nevertheless, it has been frequently noted that *P. lividus* GI values may fluctuate from one year to another (as reported by Byrne, 1990), and also that they can differ conspicuously between localities (Boudouresque & Verlaque, 2001; Sánchez-España et al., 2004). Further research for longer periods (and covering a wider sampling area) is therefore needed to confirm and refine these preliminary results for the coast of Alghero Bay.

Morphometric relationships are important for comparative growth studies, because they are analysed to estimate seasonal and temporal variability of the physiological status, reproduction and growth of a species. During growth, in general, height (H) changes related to diameter (D). In species that are almost spherical in shape (such as the sea urchins), relationship between diameter and height is reversed, and improvement of diameter increases volume as well more than improvement of height (Ebert, 1982).

The morphometric relationships observed for *P. lividus* were quite different from those reported at about the same latitude (Catalonia, Spain) by Ballesteros (1981). However, it

is worth noting that to calculate the regression functions this author examined only a very small amount of large sea urchins (only 100 specimens with D=58.6±4.8 mm) in comparison to the 1,800 adult sized individuals (D=49.7±4.9 mm) investigated during the present study.

The regression results obtained for the relationships weight-diameter and height diameter are very similar to those previously reported by Pais et al. (2006) for the same geographic area. However, the regression coefficients for the relationship between weight-height is quite different to that found by these authors. In the same way, the relationships weight-diameter reported here are in agreement to those reported by Antoniadou & Vafidis (2009) from the South Aegean Sea (South eastern Greece), whereas the relationships between height-diameter and weight-height were quite different. Lastly, all the morphometric relationships considered in this study are very dissimilar to those reported by Küçükdermenci & Lök (2014) from the western Turkish coast (eastern Mediterranean).

Although this investigation was not primarily intended as a study of the population biology of *P. lividus*, several conclusions may be drawn on the assemblages of this species in the locations examined. In fact, for various reasons many habitats are marginal for the survival of sea urchins after settlement, and the probability of annual survival approaches zero, regardless of investment in maintenance (Ebert, 1982). Considering the outcomes of this study, and in particular the morphometric relationships detected in the 1,800 specimens examined, it is possible to confirm the good health status of the sea urchin populations in the 3 locations studied (i.e., Calabona, Punta Negra and Porto Agra).

In particular, the sea urchins sampled in the latter (i.e., just outside the boundaries of the Capo Caccia-Isola Piana Marine Protected Area) exhibited the best morphological traits

and the highest condition index in comparison with those collected from the 2 other locations, where this species is heavily harvested by both professional and recreational fishermen.

About this fact, it is worth emphasising the important role of marine protected reserves in enhancing the demographic size structure and vital rates of a target species (such as the edible sea urchin) and, consequently, in supporting nearby fisheries in the surrounding areas (Guidetti et al., 2008; Sala et al., 2012). Therefore, also *P. lividus* can benefit of the so-called "reserve effect" (i.e., the improvement of populations in terms of abundance and size) in the neighbouring areas outside marine parks and protected reserves.

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3. Laboratory rearing of *Paracentrotus lividus* juvenile specimens

3.1 Introduction

The edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) is an Echinoderm widely distribute in the Mediterranean Sea and along the North eastern coasts of the Atlantic, from Morocco up to, thanks to the effect of the Gulf Stream influencing water temperature, the Irish and Scottish coasts (Grosjean et al., 1998; Boudouresque & Verlaque, 2007). It is one of the only 16 species worldwide harvested as a food source (Keesing & Hall, 1998) among the about 1,000 indentified sea urchin species (Yokota, 2002). In fact, sea urchins are highly valued as a gourmet food or, conversely, a completely inedible food depending on the gastronomic cultures (Grosjean et al., 1998). Their consumption depend on people's food habits but also by the available sea urchin species in their own region. Globally, the main captured species are *Loxechinus albus*, *Strongylocentrotus intermedius*, *S. nudus*, *S. franciscanus* and *S. droebachiensis* (Hagen, 1996; Keesing & Hall, 1998; Andrew et al., 2002; Agatsuma, 2007a, 2007b). These species are usually common along the coasts of the principal producers, that is Chile, Japan and USA. As already said, *P. lividus* is the most widespread sea urchin species in the Mediterranean Basin, where it is largely consumed (Fernandez & Boudouresque, 2000). It is a particularly appreciated seafood for its roe (that is the gonads, also called "*uni*" in Japan), which is considered as a delicacy. Its quality is very important influencing the price of the product (Whitaker et al., 1997), and the main key-factors in determining roe quality are colour and texture (Robinson et al., 2002; McCarron et al., 2010). Depending on the local market preferences, the gonad colour can range from a light yellow to a dark orange or almost red, while lighter, pale and dark gonads are not appreciated by the consumers (Grosjean et al., 1998).

The main European market for roe is France, which is the world's second largest consumer after Japan (Hagen, 1996; Carboni et al., 2012; FAO, 2013). In Italy, the

harvesting of *P. lividus* is a widespread activity mainly exerted in southern regions, greater islands included (Guidetti et al, 2004; Gianguzza et al., 2006; Pais et al., 2007). However, sea urchin is considered as an appreciated resource in the whole Mediterranean Basin (Régis et al., 1986) and, more recently, in other European non-Mediterranean areas (Byrne, 1990; Barnes & Crook, 2001). It is a seafood eating since ages, so much that evidence of sea urchin consumption in the Mediterranean area since prehistory have been found (Pantazis, 2009; Gutierrez-Zugasti, 2011).

Such historical consumption has shown a significant increasing trend over the past decades, leading to the overexploitation of the natural resource to meet the market demand all over the world (Conand & Sloan, 1989; Grosjean et al., 1998; Boudouresque & Verlaque, 2007). In this respect, Echinoderms' natural stocks have and still suffer a marked reduction in production volumes throughout the years (Andrew et al., 2002; Williams, 2002). In such a context, the worldwide supply of high quality sea urchin roe will be unable to meet market demand, unless commercial sea urchin aquaculture develops to partially replace the steady decrease in natural captures (Grosjean et al., 1998). For this reason, over the last decades a marked interest in finding a viable way to contrast overexploitation of the wild stocks, and to cope with the increasing market demand, has been widely developed. Indeed, much research has been done to reproduce the entire life cycle of *P. lividus*, since aquaculture techniques have the potential for production of this species for human consumption (Parisi et al., 2012). Aquacultural practices, in fact, could represent a valid alternative to fishing (Fernandez & Pergent, 1998). Actually, as the possibility of improving cultivated sea urchin roe quality has been demonstrated (Spirlet et al., 2000; Shpigel et al., 2005), local and tourism roe market demand could be partially supported by the industrial applications of such techniques (Parisi et al., 2012). At present, however, the only European operating sea

urchin farm is represented by Dunmanus Seafood, in Ireland, which is involved in reseeding operations. The latter is indeed one of the first explored ways to deal with overexploitation, reseeding natural habitats with cultured juveniles (Agatsuma & Momma, 1988; Gomez et al., 1995).

As sea urchins are appreciated for their gonads, the attention of the researchers is mainly focused on gonadal growth and gonad colour's enhancement (Fernandez, 1997; Basuyaux & Blin, 1998; Fernandez & Pergent, 1998; Fernandez & Boudouresque, 2000; Spirlet et al., 1998, 2001; Pantazis, 2009; Fabbrocini et al., 2012), as well as on culture and development of larval and juvenile forms (Grosjean et al., 1996, 1998; Shpigel et al., 2004; Luis et al., 2005; Carboni et al., 2013, 2014, 2015). Despite these efforts, the way ahead to achieve the goal of ensuring a greater independence from the natural resources is still so long.

In such a context, the aim of the experiments hereafter described was to evaluate the effects of an artificial diet on the survival, somatic and gonadal growth of *P. lividus* juvenile specimens, in order to give additional informations on the development of the sub-adult stages of this Echinoid.

3.2 Materials and methods

3.2.1 Experiment at SAMS

The Scottish Association for Marine Science (SAMS) is an independent marine science organisation located near the small town of Oban, on the central western coast of Scotland (Fig. 3.1). The SAMS is active on different research sectors, and its activities are divided in 4 principal large fields: Ecology, Bio-geochemistry and earth science, Physics and technology, and Microbial and molecular biology. The main research fields of the Ecology Department are: ecosystem processes, deep sea system, marine

renewable energy impacts, aquaculture, fisheries and how this all fits together in larger social-economic-ecological systems. Belonging to it, the centre for aquaculture is specialised in the assessment and understanding of the interaction between aquaculture and the environment from both these perspectives: the impacts from environment to aquaculture activities and aquaculture effects on the environment. Furthermore, the efforts of the researchers are pointed also on the diversification of aquaculture practices (e.g., Integrated Multi-Trophic Aquaculture - IMTA, co-culture of seaweeds and marine invertebrates; Cook & Kelly, 2007a, 2007b, 2009; Kelly & Chamberlain, 2010; Hughes et al., 2012a, 2012b;) and on the socio-economic systems related to aquaculture (Alexander et al., 2013, 2015; Tett et al., 2015).

Fig. 3.1. Aerial view of the SAMS institute.

3.2.1.1 Sea urchins

For this study, a total number of 90 *Paracentrotus lividus* (Lamarck, 1816) specimens was used, divided in 4 classes: Small 1 year old, Large 1 year old, Small 2 years old and Large 2 years old. All these sea urchins were produced at SAMS following the culture methods described by Kelly et al. (2000). The average test diameters (TD, excluding

spines) of the sea urchins used in this experiment were:

- Small 1 year old specimens (S1): 15-20 mm TD
- Large 1 year old specimens (L1): 20-30 mm TD
- Small 2 years old specimens (S2): 20-30 mm TD
- Large 2 years old specimens (L2): 30-40 mm TD

To improve the significance of the experimental design, each treatment was replicate 3 times. Before the beginning of the experiment, the sea urchins were placed in an acclimatization tank with a water temperature of 14.6±0.2°C (Fig. 3.2). Over the following 4 weeks, the temperature was raised roughly of about 1°C per week until reaching 18°C. Throughout the acclimatization period, the sea urchins were starved in order to standardise their nutritional status and to allow any critically injured animals to be eliminated from the experiment, as suggested by several authors (Spirlet et al., 2000; Pearce et al., 2002; Robinson et al., 2002). Just a little quantity of the brown seaweed *Alaria esculenta* was supplied during the middle of the acclimatization period in order to prevent starvation to death.

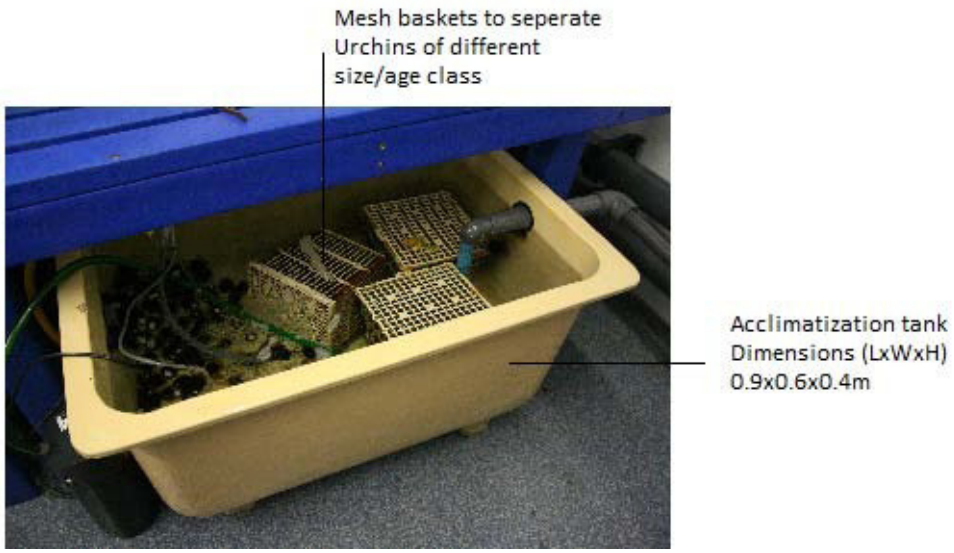

Fig. 3.2. Acclimatization tank used at SAMS laboratory.

The specimens were then distributed in 12 replicate trays (5 specimens per tray, 3 replicates for each class; Fig. 3.3), each one in an hydroponic pot with a volume of 186 cm^3. The different classes were randomly assigned to each tray. Starting from February 2014, each sea urchin was fed every 2/3 days for the following 4 months. The diet was distributed after cleaning the tray, by siphoning uneaten food and faeces, providing a sufficient amount of pellet per each specimen.

Fig. 3.3. Trays used for the experiment at SAMS.

3.2.1.2 Biometrics and dissections

In order to determine the initial size and weight of the sea urchins, at the beginning of the experiment the morphometric measurement of the test diameter and of the weight were taken. The test diameter (TD) was measured manually by means of a dial calliper, being careful to accurately measure the maximum diameter (Fig. 3.4). This procedure was executed 3 times for each specimen, and the spines were excluded from the measurement.

Sea urchins were also weighed using a precision balance (2 decimal places) after 1 minute drying on absorbent paper. The same procedure was repeated every 30 days

during the experiment.

Fig. 3.4. Measuring the test diameter (TD) of a specimen.

Biometrics were made on the 90 sea urchins used in the experiment, plus 6 sea urchins for each of the above mentioned size class (24 sea urchins more, so 114 specimens as a whole). The latters, after the measurement, were dissected using a pair of scissors. After dissection, test, gonads and gut of the specimen were divided by a pair of tweezers, and measured separately using a precision balance. The so obtained gonad wet weight and gut wet weight were used to calculate the gonad index (GI) and the alimentary index (AI), respectively, expressed as a percentage. The gonad index was calculated as follows:

$$\mathrm{GI} = \frac{\mathrm{Wgn}}{\mathrm{Wb}} \times 100$$

where GI is the gonad index expressed as a percentage, Wgn is the wet weight of the gonads and Wb is the wet body weight of the sea urchin. Similarly, the alimentary index was calculated as:

$$\mathrm{AI} = \frac{\mathrm{Wgt}}{\mathrm{Wb}} \times 100$$

where AI is the alimentary index expressed as a percentage, Wgt is the wet weight of the gut and Wb is the wet body weight of the specimen.

The same procedures were also repeated at the end of the experiment with 24 sea urchins used during the experiment, also in this case 6 sea urchins per each class.

3.2.1.3 Diet

The diet used during this experiment was based on SABS diet, as described by Robinson et al. (2002). Due to the absence on the market (at least during the planning of this experiment) of the original antioxidant ethoxyquin, it was necessarily substituted with Paradigmox^{TM} by Kemin Industries Inc., Des Moines, IA, USA. The pigment source used was Algro Natural® (250 mg β-Carotene kg^{-1} dry weight ingredients) as supplied by Nutra-Kol Pty Ltd, WA, Australia.

Because of the higher temperatures of this experiment, and in order to assure the firmness of the pellet, the gelatin amount was increased to 7.28% (instead of 5.00%). Furthermore, the wheat content was increased from 19.00% to 21.14%. The diet composition is illustrated in Table 3.1.

The ingredients were weighed using a 3 decimal precision balance and, with the exception of the gelatin, manually blended together. Once well amalgamated, the gelatin dissolved in hot water was added and the whole mixture was rapidly mixed to prevent the production of clumps. The mixture was then processed using a simple mincer equipped with a perforated plate (roughly 0.7-0.8 cm in hole diameter). The so obtained food filaments were then manually cut in pellets of 0.5±0.1 grams (Fig. 3.5). After about 24 hours drying in a low temperature oven (about 36°C), the pellets were packed and frozen at -20°C. The needed quantity of pellets was defrosted at room temperature, for at least one hour, before being administered to sea urchins.

Table 3.1. Composition of the administered food.

Ingredient	%
Soybean meal	21.27
Wheat	21.14
Rapeseed meal	21.27
Potato flour	19.84
Gelatine	7.28
Sodium alginate	2.24
Flax Oil	2.24
Lecithin	2.24
Vitamin	0.56
Mineral	0.34
Inositol	0.01
Vit C	0.09
Paradigmox	0.22
Algro natural	1.25

Fig 3.5. Processing of the mixture by a mincer and pellets ready to be administered.

3.2.1.4 Experimental conditions and equipments

During this experiment, each sea urchin was hosted in a plastic pot with a volume of 186 cm^3 contained in a tray (5 pots for each tray; Fig. 3.6). The pots were modified, before the beginning of the test, applying a 2.5 mm plastic mesh on the bottom which was fastened using cable ties, in order to prevent the chance of pellets falling through

the pot uneaten. Each pot was then fastened to an egg crate base to ensure the pots remained submerged. The egg crates were made heavier, by fixing a stone on the bottom, but were not joined to the tray. This solution was chosen both to avoid the buoyancy of the egg crate (which was plastic made) and to permit easiest cleaning operation (the egg crate can be easily removed from the tray).

Fig. 3.6. Superior view of the pots attached to the egg crate.

The interior dimensions of the tray were 27×16.5×11 cm (length×width×height), containing a water volume of 3.53 litres. The desired water level was maintained by an outflow pipe, which height was regulated in order to prevent the possibility of sea urchins escape their pot. The water was heated, using 6 simply aquarium heaters, at the temperature of 18±1°C, and constantly recorded by a HOBO pendant Temp/Light logger randomly assigned to a tank. The water was provided directly by the sea.

The SAMS institute, in fact, is just few metres far from the shoreline, and this proximity has enabled the installation of a system for the water pumping directly by the sea. It is composed of 2 pumps (of which operation is alternated) followed by a series of sand

filters (with grain size gradually smaller) and biological filters. The filtered water was then pumped in the laboratories when it is required. At the laboratory, the water flow, for each tray, was of about 15 litres/hour and was assured by a submerged pump. Water quality was verified on a weekly basis by randomly testing one tray using an API liquid saltwater test kit. The photoperiod used was of 12 hours of light and 12 hours of dark.

3.2.1.5 Data analysis

All the data collected during the trial have been computerized and then subjected to statistical-mathematics analysis. Furthermore, in order to highlight any differences in terms of growth between the analysed groups, the results were processed by ANOVA (General Linear Model - GLM). In case of significant differences between the groups, the Tukey *a posteriori* test was used for *post hoc* comparisons.

3.2.2 Experiment at the University of Sassari

The experiment described below, performed in Sardinia at the University of Sassari (hereafter UNISS), was carried out during 2 different time periods. In fact, the first part was conducted during spring-summer 2014 and the second one exactly one year later. This was because of the scarcity of space in the laboratories, so that it was necessary to split the experiment into 2 different parts. The choice to wait for exactly one year was taken in order to collect the sea urchins during the same period of the year, with similar water temperature and photoperiod, so as to guarantee a physiological condition as close as possible between the specimens used for the 2 experiments.

3.2.2.1 Sampling site and sampling design

The selected site for the sampling was Costa Paradiso, in the northern coast of Sardinia

(41° 05' 06" N, 8° 93' 76" E; Fig. 3.7), characterised by the typical granite sea bottom in a geographical area called Gallura.

Fig. 3.7. Aerial view of Costa Paradiso, the collection site for UNISS experiment.

The specimens were collected by the divers at a maximum depth of about 4 m. This site was selected for the low human pressure that characterises it, for the abundance of sea urchins (also during the period when the harvesting is permitted), for ease of access and for the usually good marine and visibility conditions, thanks to the granitic sea bottom and to the absence of sand, even in case of rough sea. The specimens were gently sampled using an harvest tool and, once outside the water, measured by a precision calliper to exclude those specimens out of the selected size ranges. All the operations regarding collection and movement of the sea urchins were carried out with extreme caution, in order to avoid stress or damages to the animals. The specimens harvested were then placed inside an insulated watertight container, equipped with an aerator, for the transportation. To the purpose of regulating the main water variables of the acclimatization tank at the lab, also temperature and salinity of the water were measured

using a precision pH-meter and a saline refractometer. Afterwards, the sea urchins were moved to the laboratory of Aquaculture of the University of Sassari and here they were transferred to the acclimatization tank.

3.2.2.2 Sea urchins

During this trial, a total number of 144 specimens (72 in 2014 and 72 in 2015) of *Paracentrotus lividus* (Lamarck, 1816), divided in 6 size classes, was used. The specimens were divided in 6 different classes depending on their test diameter (TD) measured excluding spines as follows:

- Class 1 (C1): 15-20 mm TD
- Class 2 (C2): 20-25 mm TD
- Class 3 (C3): 25-30 mm TD
- Class 4 (C4): 30-35 mm TD
- Class 5 (C5): 35-40 mm TD
- Class 6 (C6): 40-45 mm TD

All the sea urchins were collected from natural environment during spring 2014 and spring 2015. The specimens sampled which were inside these size ranges and in good health condition were disposed into a cooler with fresh sea water, to ensure the minimum stress as possible during the transportation. In the laboratory, the sea urchins were put in an acclimatization tank (Fig. 3.8) previously regulated at the same temperature recorded during the sampling operations. Then, water temperature was risen, passing from 14 (as registered during the sampling) to 18±1°C. During the weeks following the collection, sea urchins were starved in order to standardise their nutritional status. Just a little amount of the Mediterranean seaweed *Ulva lactuca* was provided during this period.

At the beginning of the trial, the specimens were distributed in 6 replicate crates positioned inside the experimental tank (12 specimens per crate, 2 crates for each class; Fig. 3.9), each one in a pot with an interior volume of about 217 cm^3.

Fig. 3.8. Acclimatization tank hosting specimens before the beginning of the UNISS experiment.

Fig. 3.9. Model of the experimental tray used for the UNISS trial.

Starting from 12/05/2014 and from 22/05/2015, each sea urchin was fed every 2 or 3

days (on Monday, Wednesday and Friday) for the following 10 weeks. The diet was distributed in abundance after cleaning the tray by siphoning uneaten food and faeces.

3.2.2.3 Biometrics, dissections and diet

During this experiment, the same diet as described for the Scottish trial was used. Also for the biometrics and the dissection exactly the same procedures were adopted. There were just few differences regarding the number of sea urchins dissected (ten specimens per each class instead of six) and the frequency of the biometrics (every fifteen days instead of once a month).

3.2.2.4 Experimental conditions and equipments

During the acclimatization period, sea urchins were hosted inside a plastic crate in the acclimatization tank, already divided per size class. At the beginning of the experiment, test diameter and weight of 24 specimens of each size class were measured: each sample was taken from the crate inside the acclimatization tank and placed on tissue paper to dry. Sea urchin test diameter and weight were measured with a dial calliper and a precision balance, respectively. Sea urchins were then transferred to the experimental tank, where they were singularly fed with pellets provided every 2 or 3 days a week for the following 10 weeks.

For the described experiment, a closed circuit system technically known as RAS (Recirculated Aquaculture System) was used (Fig. 3.10). By means of a temperature control system and a neon lamp (light intensity equal to 1,000 lux) regulated by a timer, water temperature (18±1°C) and photoperiod (12 h light : 12 h dark) were kept constant during the entire period of the trial. This type of aquaculture system was used since it requires a relatively limited water renewal. An external pump (flow rate = 2000 l/h;

power = 35 W) ensured a continuous flow of the water contained in the experimental tank to a bio-mechanical filtration device. The latter is constituted by a container in fiberglass, of rectangular shape (80×68×58 cm) and volume of approximately 0.3 m^3, connected directly to the main structure and equipped with a system that allows the distribution of water (by percolation) on a layer of spherical filling bodies (*bio-balls*).

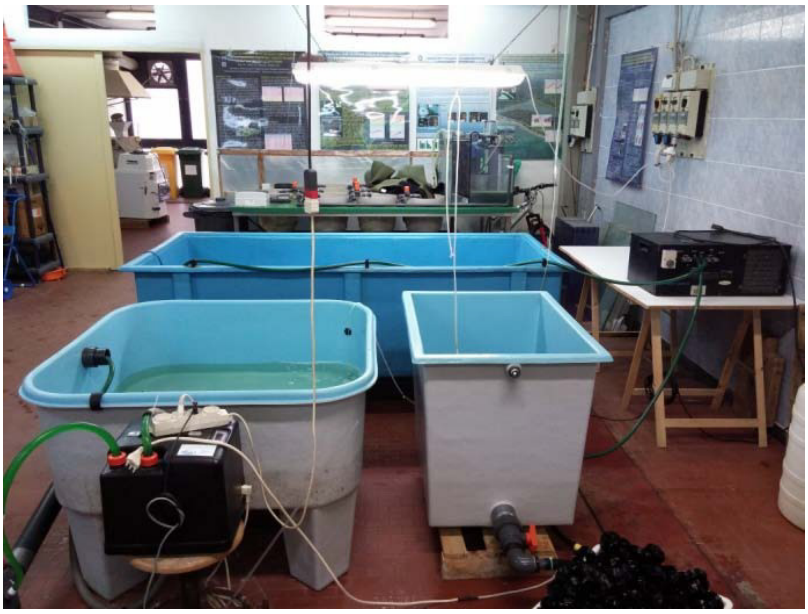

Fig. 3.10. Machinery used for the UNISS trial: acclimatization tank (bottom left); bio-mechanical filtration system (bottom right); experimental tank (top).

Due to their design, the *bio-balls* guarantee a wide area for the development of bacterial populations (*Nitrosomonas* spp. and *Nitrobacter* spp., above all) responsible of the reduction of nitrogen present in the water, thereby optimizing the efficiency of the biological filter. The decomposition, implemented by these microorganisms, causes the transformation of the ammonia (NH_3) presents in the water in a less toxic form for the reared sea urchins. Furthermore, in order to ensure the adequate oxygenation of the whole system, on the corners of the above-mentioned tank 4 PVC pipes (diameter of about 3 cm) were positioned. Inside each pipe a porous stone, in which air is blown by

means of a pump was positioned (the so called *air lift*). The experimental tank was constituted by a tank in fiberglass (of rectangular shape, 220×100×60 cm interior dimensions) with a capacity of about 1.3 m^3, containing sea water having optimal chemical-physical characteristics for the storage of sea urchins. Inside it, 6 independent plastic modules (each one made by 2 fruit boxes, 50×20×30 cm, joined together at the bottom surface) were positioned, to each of which was associated, superiorly, a faucet (installed on a PVC pipe of internal diameter equal to 22 mm) for adjusting the incoming water flow. In each module, 12 plastic pots (height = 6.5 cm; lower diameter = 5.6 cm; upper diameter = 7.6 cm) were fastened by means of cable ties. The water line was regulated in order that the superior part of the pot was not submerged, so as to prevent the possibility of sea urchins escape. Each pot, moreover, was perforated to allow a constant and adequate water flow inside it. Finally, to avoid the loss of the administered pellet, on the lower surface of each plastic module under the pots a fine mesh was applied.

Throughout the period of the trial, the main variables of the water contained in the rearing system were monitored and, in case of deviations from optimal values, the necessary corrections have been carried out. Specifically, daily checks concerned temperature (18±1°C), salinity (38±1 PSU), pH (8.0±0.2), nitrites and nitrates (low concentration). To maintain minimum values of ammonia, moreover, it was decided to frequently replace an amount of water of about 1/5 of the entire volume contained within the system (approximately 750 l). The water was usually collected in plastic bins filled by means of a pump directly from the sea, along the coast near the location of Punta Tramontana (northern Sardinia, 40° 86' 97" N, 8° 62' 69" E) and then filtered with 60 μm filter. Conversely, in case of adverse weather condition, the water was prepared at the laboratory using deionized water and marine salt.

In 2015 experiment, there were few slight differences compared to that of 2014, both concerning the cleaning of the whole system. The first one was the addition of a mechanical filter, placed between the experimental tank and the bio-mechanical filtration system. An external pump (flow rate = 600 l/h; power = 29 W) was also added in order to provide against the bottleneck resulting from the additional filter. The second modification was the arrangement of the modules inside the experimental tank (Fig. 3.11): in the first experiment, in fact, they were linked together causing some complication on the cleaning operations of the experimental tank. For this reason, it was decided to leave the boxes separated. Thus, it was possible to remove each box individually, cleaning the underlying surface and rapidly replace the box, and consequently causing less stress as possible to the sea urchins.

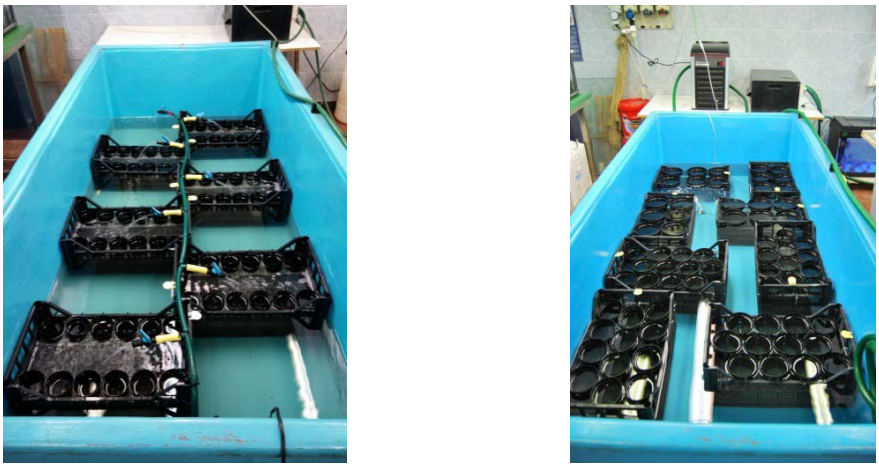

Fig. 3.11. Interior of the experimental tank during the 2014 (on the left) and 2015 (on the right) experiments.

In addition, for the second experiment pots different in size were used. This was because of the unsuitableness of the above mentioned pots for the biggest sea urchins (C6 class, 40-45 mm): simply, they were too big for the pot. Hence it was necessary to replace 24 pots with 24 larger ones (height = 12 cm; lower diameter = 10 cm; upper

diameter = 14 cm). Due to the greater dimensions, it was also necessary to use 2 extra boxes, so, inside the experimental tank, there were 4 boxes with standard pots and 4 boxes equipped with the bigger ones.

3.2.2.5 Proximate composition

The chemical analysis on the proximate composition were carried out in triplicate and concerned both the food given to sea urchins and the edible portions of the same (i.e., the gonads or roe). As regards the latter, it worth noting that for the 2 littlest size classes (C1 = 15-20 and C2 = 20-25 mm, respectively) it was not possible to get enough sample to analyse. Contextually, the same analyses were carried out on a group of wild specimens of analogous size used as a control.

Specimens of *Paracentrotus lividus* were dissected as already described and the edible portion was placed in a Falcon tube. The gonadal tissue was later homogenised for 1 minute at 10,000 rpm by means of Ultra-Turrax T25. The food, instead, was finely ground with an electric grinder FOSS-Knifetec.

3.2.2.6 Moisture and ash

A quantity of approximately 1 g of homogenised gonads (both fed and control specimens) was weighed on precision balance within a porcelain crucible of known weight. The crucibles containing the samples were then placed in an oven, at a temperature of 105°C for 24 hours. Upon cooling in a desiccator with silica gel, the samples were weighed again in order to calculate the dry weight by subtracting from the initial fresh weight (AOAC, 1990). The content in terms of ashes, however, was obtained by placing the same sample (within the same crucible) in a muffle furnace at 550°C for 5 hours (AOAC, 1923; Mortensen & Wallin, 1989). After an hour of cooling,

this was weighed in order to calculate the difference between the weight of the dried sample and the remaining ash content. The same procedure was also followed for the food of which, however, 2 g were approximately weighed.

3.2.2.7 Crude protein

The protein content was determined using the Kjeldahl method (AOAC, 1992). From each sample, about 0.5 g of fresh homogenate of gonads and about 0.8 g of ground food were taken. These were weighed on precision balance inside a paper boat and then stored in a glass test tube. To start the digestion process, 10 ml of 96% sulfuric acid (H_2SO_4) were added to each test tube together with a catalyst tablet (FOSS, DK) composed by 5.5 g of K_2SO_4 and 0.5 g of $CuSO_4 \times 5H_2O$. The test tubes were plugged with a glass bells and positioned (under a fume hood) in a digester at 450°C for 4-5 hours, so as to facilitate the oxidation process, namely the transformation of organic nitrogen into ammonium sulphate. After cooling for about an hour, the samples were distilled and titrated with HCl 0.1 N using a Kjeltec 2300 analyser (FOSS, DK) and the content of crude protein was calculated using the following formula:

$$\mathrm{CP} = \frac{(0.875 \times \text{ml HCl } 0.1\mathrm{N})}{\mathrm{Wws}}$$

Where CP is the Crude Protein and Wws is the wet weight of the sample.

3.2.2.8 Total lipids

For the determination of the total lipids, a modified method of Folch et al. (1957) was used. About 3 g of homogenate sample were placed and weighed inside a 50 ml Pyrex® test tube, in which 20 ml of a dichloromethane/methanol solution in the ratio 2:1, and 0.1% hydroxytoluene butoxide (BHT) were subsequently added. Following a pass in an

ultrasonic bath (Branson 1510) for 5 minutes, the samples were vortexed for 1 minute, centrifuged at 2000 rpm at the temperature of 4°C for 10 minutes (centrifuge Thermo Scientific, Heraeus Megafuge 16R Centrifuge) and, finally, filtered under vacuum through a Whatman n. 541 filter (Whatman International Ltd., Maidstone, England). The filtrate was gently mixed in a separating funnel, with 5 ml of 0.73% NaCl, by inverting the funnel several times for about 3 minutes. Then, the separating funnels containing the sample were left to stand overnight to promote the complete separation of the phases. The lower phase, containing the lipids fraction, was collected by gravitation in a Pyrex® flask previously weighed. It was evaporated in a rotary evaporator "Rotavapor Buchi 461" for 6 minutes. Subsequently, the flasks were placed in a vacuum desiccator and, on the day after, they were weighed to calculate the total amount of lipids present in the sample in order to assess the amount of hexane (1 ml/25 mg of fat) required for the subsequent step of methylation. For the determination of total lipids contained in the food, a Soxhlet apparatus was used, consisting of a Pyrex® ball, a Soxhlet extractor and a coolant fall. An amount of 2 g of the ground sample were weighed and placed inside a Whatman cellulose thimble (30×100 mm; Whatman International Ltd., Maidstone, England). Each thimble was then corked with previously degreased cotton. Afterwards, the 250 ml Pyrex® flasks were weighed. 200 ml of stabilised ethyl ether and the thimble of cellulose containing the solid material to be extracted were added inside the extractor. The solvent was led to boiling point (60-70°C) and its vapors, once reached the refrigerant through the lateral connection, began to drip on the material placed inside thimbles, thus allowing the extraction of the organic substances. When the level of the solvent entered in contact with the sample and beat the top curve of the siphon, it was sucked into the flask and a new cycle of extraction can starts. The thus obtained fat was subsequently collected in the Pyrex®

flask previously weighed. After 5 hours from the start of the process, the ether was removed and the flask, containing now only the fat, was placed in an oven at 105°C. On the next day, the flask was weighed and the amount of fat present in the sample expressed as a percentage. The calculations have been made taking into account the values previously noted and applying the following formula:

$$TL = \frac{Wf}{Ws} \times 100$$

where TL is the total lipids amount expressed as a percentage, Wf is the weight of the extracted fat and Ws is the weight of the sample.

3.2.2.9 Acidic profile

For the determination of fatty acids, a methylation basic modified (FIL-IDF, 1999) was carried out. From each sample of fresh gonads, 1 ml of extract was taken, subsequently transferred into a *vial* container and then exposed to a flow of nitrogen for 15 minutes. For each *vial* thus prepared, 0.5 ml of sodium methoxide 0.5 M were added and the whole was vortexed for 2 minutes, to favour the release of the fatty acids from triglycerides. One additional ml of hexane was added in *vials* and, after a subsequent passage to the vortex mixer for 1 minute, the sample was left to decant for 15 minutes. Finally, from each *vial* 0.5 ml of the hexane phase (supernatant) were collected and then transferred to a new *vial* for the analysis of the fatty acid profile by means of a gas chromatograph Agilent Technologies 7890A GC System. The amount of each fatty acid was expressed as a percentage of total methyl esters (FAME) presents in the sample. For the determination of the fatty acids in the food, however, it has been used the method described by Kramer et al. (1997), which provides an esterification in acid environment. Subsequently, the samples were read to the gas chromatograph.

3.2.2.10 Data analysis

As for biometrics, dissection and diet, also for the data analysis the same procedures as described for SAMS experiment were adopted. For proximate composition and fatty acid profile One-way ANOVA analysis was carried out. Moreover, the Tukey's test was also performed for *post hoc* comparisons. ANOVAs were always performed by using the Minitab software package.

3.3 Results

3.3.1 SAMS experiment

The experiment carried out examined the effects of an artificial diet on the somatic growth of juvenile sea urchins artificially cultured at SAMS. The specimens were divided in 4 classes, considering both size and age: Small 1 year old (S1; 15-20 mm TD), Large 1 year old (L1; 20-30 mm TD), Small 2 years old (S2; 20-30 mm TD) and Large 2 years old (L2; 30-40 mm TD). The experiment lasted 4 months.

3.3.1.1 Survival and growth

The survival rates registered during the experiment (Fig. 3.12) are to be considered good, with a final total value of 90%. The L1 class showed the best results, with a survival rate of 100% until the end of the trial. Conversely, the L2 group exhibited the worst performance, with a final survival rate of 80%. The S1 and S2 classes showed final survival rates of 87% and 93%, respectively.

Regarding the somatic growth, the smallest class (S1) showed the highest increment in test diameter (TD; Fig. 3.13), passing from 17.6±1.3 mm at the beginning to 26.4±2.1 mm at the end of the experiment (+50%). The mid classes showed a similar increase: from 24.5±3.7 and 24.7±1.4 mm to 30.4±2.0 and 29.5±1.6 mm for L1 class (+24%) and

S2 class (+20%), respectively. The lowest increment was shown by the specimens belonging to the L2 class: from 29.3±1.3 mm to 32.8±1.5 mm, corresponding to an increase of only 12%. It is worth noting the difference of 1 year among the L1 and S2 specimens, but also the correspondence of the dimension (20-30 mm in test diameter) and the similarity in the final growth rate (24% and 20%, respectively).

The same trend was observed for total wet weight (TWW; Fig. 3.14): S1 specimens increased from 4.7±0.9 to 11.7±2.6 g (+148%), the L1 and S2 increased from 8.7±1.6 and 8.7±3.8 g to 16.1±2.5 and 15.6±2.2 g, respectively (+84% and +80%). Again, specimens belonging to the L2 class showed the worst result, increasing from 13.5±2.0 to 20.2±2.7 g (+50%). Survival and growth results are reported in Table 3.2.

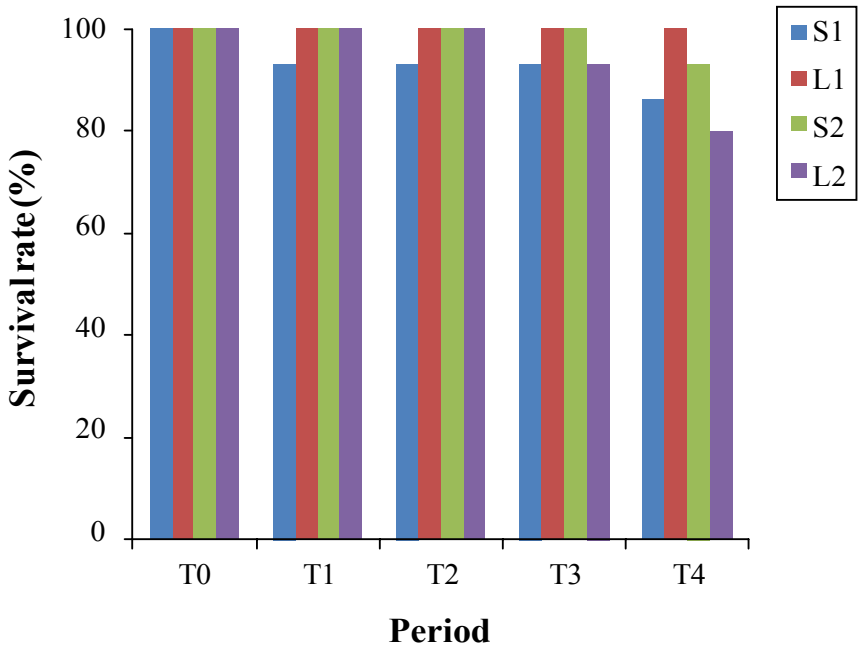

Fig. 3.12. Survival rates of *Paracentrotus lividus* throughout the experimental period.

As far test diameter and total wet weight is concerned, detail of ANOVA results and Tukey's test comparison from the 4 classes examined are reported in Table 3.3. For the

considered variables, significant differences were observed both for "Time" and "Class" factors, and in the case of test diameter also for the interaction "Time×Class". No significant differences were found in the latter interaction for the total wet weight.

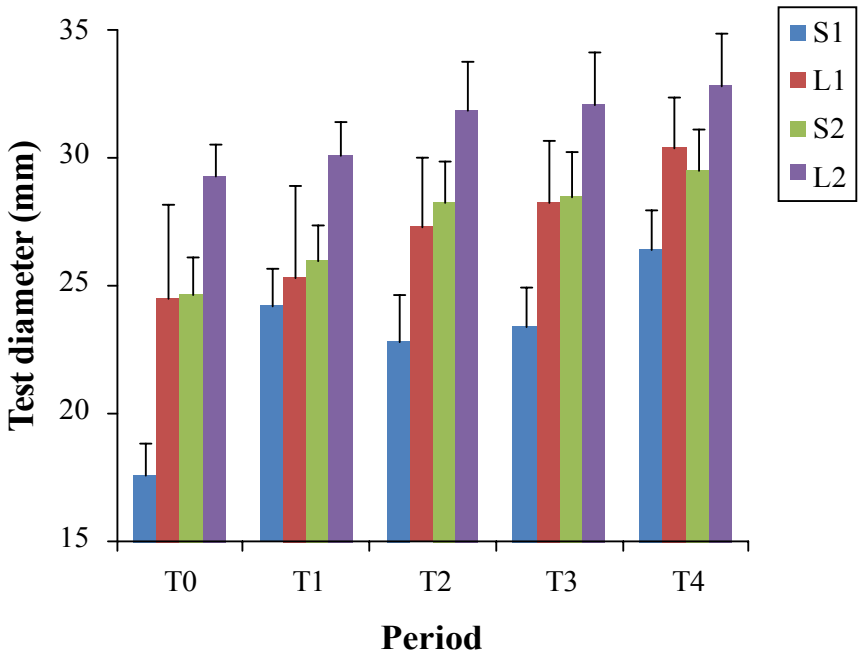

Fig. 3.13. Mean test diameter (mm±SD) of *Paracentrotus lividus* specimens divided per class.

Tab. 3.2. Survival rates registered at the end of the SAMS experiment and Test Diameter (TD; mm±SD) and Total Wet Weight (TWW; g±SD) at the beginning and at the end of the trial.

Class	Final survival rate	Test Diameter		Total Wet Weight	
		Initial	Final	Initial	Final
S1	87%	17.6±1.3	26.4±2.1	4.7±0.9	11.7±2.6
L1	100%	24.5±3.7	30.4±2.0	8.7±1.6	16.1±2.5
S2	93%	24.7±1.4	29.5±1.6	8.7±3.8	15.6±2.2
L2	80%	29.3±1.3	32.8±1.5	13.5±2.0	20.2±2.7

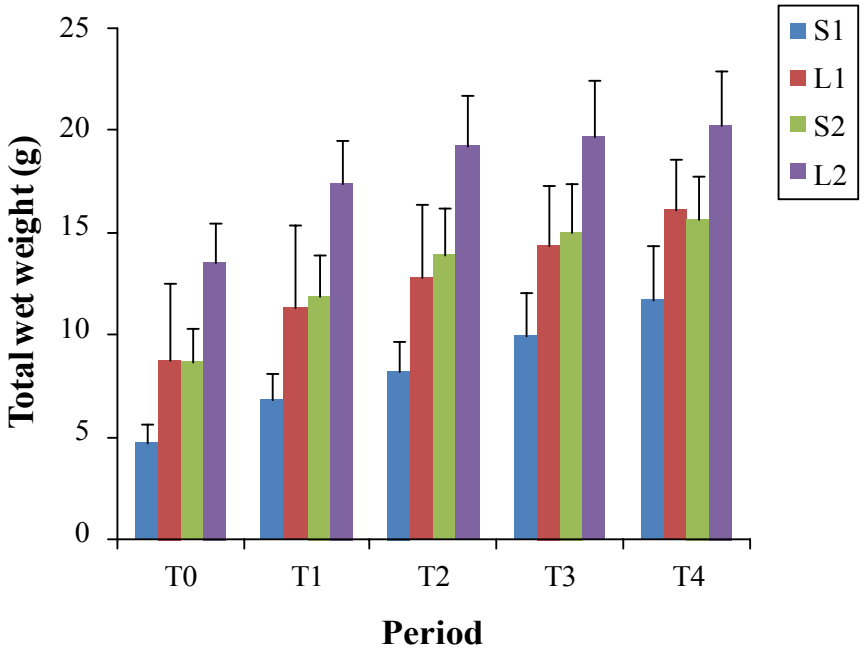

Fig. 3.14. Mean total wet weight (g±SD) of *Paracentrotus lividus* specimens divided per class.

Tab. 3.3. ANOVA results for growth parameters of the 4 classes of *Paracentrotus lividus* specimens examined.

Source of variation	Test Diameter				Total Wet Weight			
	df	MS	F	p	df	MS	F	p
Time	4	300.19	70.54	**0.000**	4	432.25	68.17	**0.000**
Class	3	1,004.56	236.07	**0.000**	3	1,105.98	174.42	**0.000**
Time×Class	12	10.40	2.44	**0.005**	12	3.80	0.60	0.843
Residuals	270	4.26			270	6.34		
Tukey's test								
Time		T5>T4=T3>T2>T1				T5≥T4≥T3>T2>T1		
Class		L2>S2=L1>S1				L2>S2=L1>S1		

S1 = Small 1 year old; L1 = Large 1 year old; S2 = Small 2 years old; L2 = Large 2 years old. Significant differences are marked in bold.

3.3.1.2 Dissections

The dissection of the sea urchins put on evidence a massive growth in weight of all the 3 considered anatomical structures: gonads, gut and test (Figs. 3.15, 3.16, 3.17). The

less increments were observed for the test weight, varying from 2.18±0.47, 5.07±0.58, 4.5±1.21 and 6.8±0.63 g to 8.19±1.54, 11.67±1.34, 10.01±1.07 and 14.02±1.81 g for S1, L1, S2 and L2 classes, respectively. Considering the gut weight, 2 classes (i.e., S2 and L2) have shown a more than doubled values, passing from 0.20±0.08 and 0.33±0.10 g at the beginning of the trial, to 0.55±0.45 and 0.86±0.45 g at the end, respectively. The smallest specimens exhibited considerable growth at the end of the experiment: the L1 class was more than 3 time bigger (from 0.13±0.04 to 0.58±0.28 g) and the S1 grew about 6 times (from 0.06±0.04 to 0.45±0.41 g). However, the largest increase was observed for the gonads weight. In fact, they increased from a minimum of nearly 7 times (L2) to a maximum of almost 70 times (S1). In particular, the initial values were 0.03±0.03, 0.25±0.17, 0.18±0.12 and 0.48±0.14 g for S1, L1, S2 and L2 specimens, respectively, while the final measurements were 2.11±0.80, 2.35±0.91, 2.51±0.50 and 3.76±1.45 g in the same order as before. Dissection's results are shown in Table 3.4.

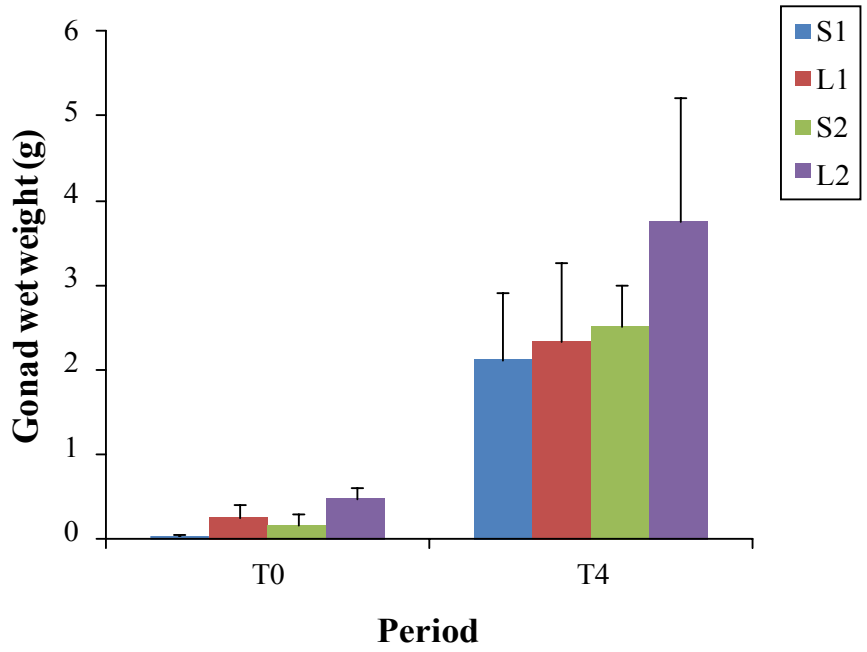

Fig. 3.15. Gonad wet weight (g±SD) of *Paracentrotus lividus* specimens divided per class.

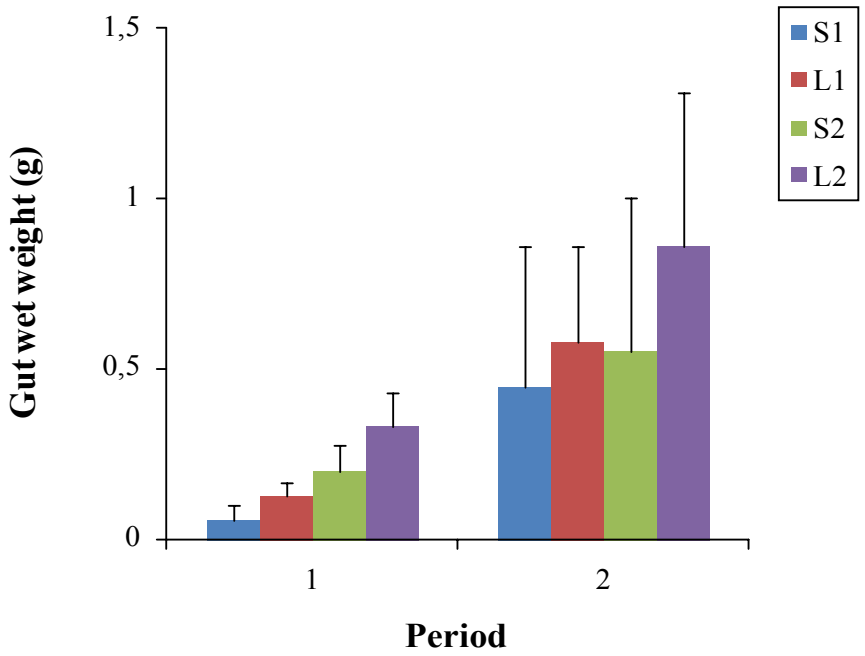

Fig. 3.16. Gut wet weight (g±SD) of *Paracentrotus lividus* specimens divided per class.

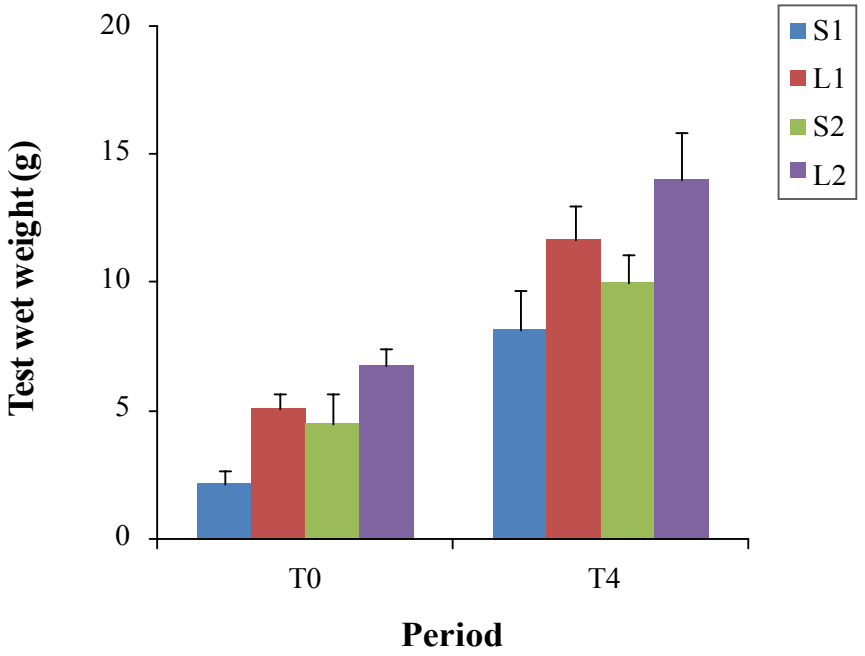

Fig. 3.17. Test wet weight (g±SD) of *Paracentrotus lividus* specimens divided per class.

Tab. 3.4. Gonads, gut and test wet weight (g±SD) at the beginning and at the end of the experiment at SAMS.

Class	Gonad wet weight		Gut wet weight		Test wet weight	
	Initial	Final	Initial	Final	Initial	Final
S1	0.03±0.03	2.11±0.80	0.06±0.04	0.45±0.41	2.18±0.47	8.19±1.54
L1	0.25±0.17	2.35±0.91	0.13±0.04	0.58±0.28	5.07±0.58	11.67±1.34
S2	0.18±0.12	2.51±0.50	0.20±0.08	0.55±0.45	4.5±1.21	10.01±1.07
L2	0.48±0.14	3.76±1.45	0.33±0.10	0.86±0.45	6.8±0.63	14.02±1.81

The gonadal and alimentary indexes shown the same trend just described (Figs. 3.18, 3.19). In fact, the gonad index increased more than the alimentary index. The most grown class was the littlest (S1), passing from 0.9±1.0% at the beginning of the experiment to 17.7±6.2% at the end of the same. The gonad index of the S2 class increased more than 7 times (from 2.3±1.3% to 16.6±2.4%), while the remaining grown of about 4 times (from 3.3±2.2% and 3.9±0.8% to 13.8±4.7% and 17.5±5.7% for L1 and L2, respectively).

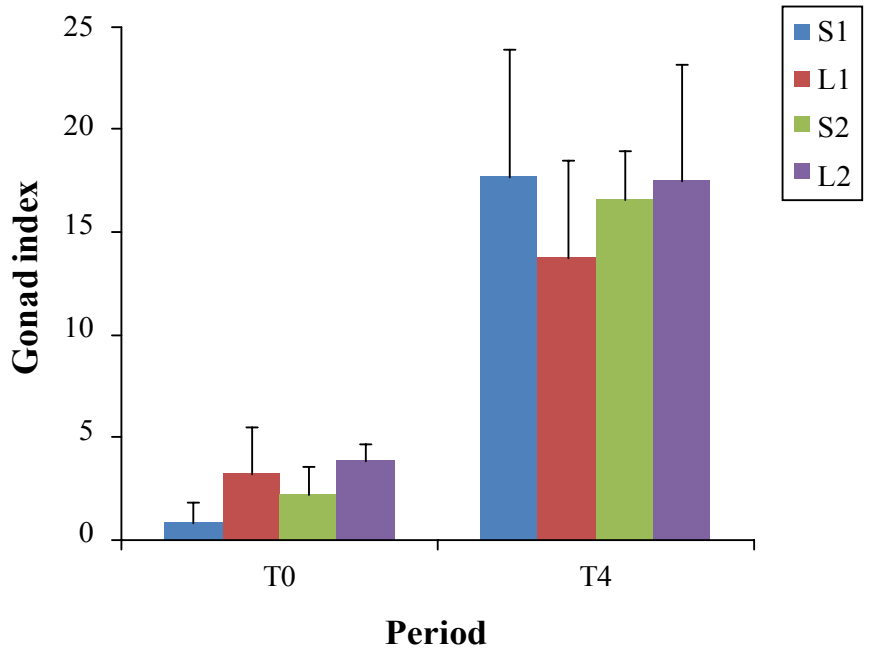

Fig. 3.18. Gonadal index (%±SD) of *Paracentrotus lividus* divided per class.

Also the alimentary index highlighted an increase even if smaller than gonad's one: the more increase was, again, the S1 class, passing from an initial value of 1.8±0.9% to reach 4.0±3.5% at the end, while the lesser increase was observed for S2, with values from 2.8±0.6% to 3.5±2.5%. Finally, the alimentary indexes of L1 and L2 specimens changed from 1.7±0.4% to 3.5±1.8% and from 2.7±0.6% to 4.2±2.4%, respectively. The changes in both gonadal and alimentary indexes are summarised in Table 3.5.

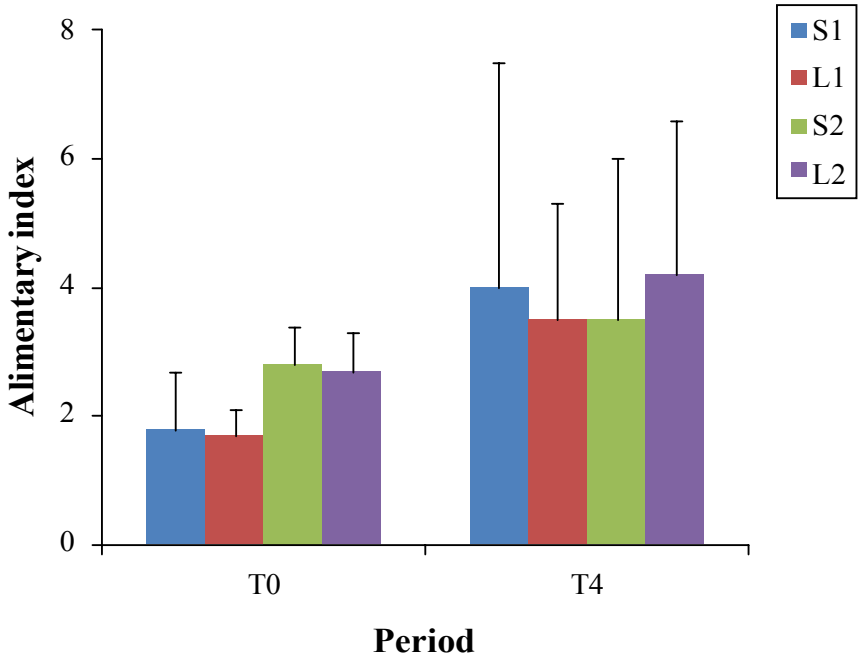

Fig. 3.19. Alimentary index (%±SD) of *Paracentrotus lividus* divided per class.

Tab. 3.5. Gonadal Index and Alimentary Index of the SAMS experiment's specimens.

Class	Gonadal index		Alimentary index	
	Initial	Final	Initial	Final
S1	0.9±1.0	17.7±6.2	1.8±0.9	4.0±3.5
L1	3.3±2.2	13.8±4.7	1.7±0.4	3.5±1.8
S2	2.3±1.3	16.6±2.4	2.8±0.6	3.5±2.5
L2	3.9±0.8	17.5±5.7	2.7±0.6	4.2±2.4

As regards the 3 anatomical structures examined for the dissections (i.e., gonads, gut and test weight), ANOVA detected significant differences among the 4 classes for both "Time" and "Class" factors. No significant difference was highlighted for the interaction between the 2 factors ("Time×Class"). For gonad and alimentary indexes, significant differences were found only for the factor "Time". The detailed ANOVAs results are showed in Tables 3.6 and 3.7 for dissections and indexes, respectively.

Table. 3.6. ANOVA results for dissected compartments of the 4 classes of *Paracentrotus lividus* specimens examined.

Source of variation	**Gonads Weight**				**Gut Weight**				**Test Weight**			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Time	1	71.81	148.35	**0.000**	1	2.21	26.61	**0.000**	1	481.84	349.56	**0.000**
Class	3	2.48	5.14	**0.004**	3	0.23	2.86	**0.049**	3	57.07	41.41	**0.000**
Time×Class	3	0.97	2.00	0.129	3	0.01	0.23	0.874	3	1.62	1.18	0.330
Residuals	40	0.48			40	0.08			40	1.37		
Tukey's test												
Time		T5>T1				T5>T1				T5>T1		
Class		L2>S2=L1=S1				L2≥S2=L1≥S1				L2>L1=S2>S1		

S1 = Small 1 year old; L1 = Large 1 year old; S2 = Small 2 years old; L2 = Large 2 years old. Significant differences are marked in bold.

Table. 3.7. ANOVA results for Gonadal and Alimentary indexes of the 4 classes of *Paracentrotus lividus* specimens examined.

Source of variation	**Gonadal Index**				**Alimentary Index**			
	df	MS	F	p	df	MS	F	p
Time	1	2,278.15	170.68	**0.000**	1	28.56	7.73	**0.008**
Class	3	9.80	0.73	0.538	3	1.52	0.41	0.744
Time×Class	3	20.23	1.52	0.225	3	1.08	0.29	0.829
Residuals	40	13.35			40	3.69		
Tukey's test								
Time		T5>T1				T5>T1		
Class		L2=S2=S1=L1				L2=S2=S1=L1		

S1 = Small 1 year old; L1 = Large 1 year old; S2 = Small 2 years old; L2 = Large 2 years old. Significant differences are marked in bold.

3.3.2 UNISS experiment

The experiment performed at the University of Sassari involved a total number of 144 juvenile sea urchins of wild origin and reared in a RAS system, divided in 6 size classes: C1 (15-20 mm TD), C2 (20-25 mm TD), C3 (25-30 mm TD), C4 (30-35 mm TD), C5 (35-40 mm TD) and, finally, C6 (40-45 mm TD). The experiment lasted 10 weeks.

3.3.2.1 Survival and growth

The survival rate (Table 3.8) of the juvenile *Paracentrotus lividus* specimens examined revealed results overall quite satisfactory, with values of 100% up to 60 days from the start of the experiment (T4) for the 3 smallest classes (C1, C2 and C3), and until the end of the trial (T5, 70 days) for the remaining classes (C4, C5 and C6).

During the final period (T5), in fact, particularly for smaller individuals (C1 and C2), a progressive increase of mortality was observed. In particular, the final survival values of 62.5% for specimens of size classes C1 and C2 and of 87.5% in the C3 class were recorded. In contrast, C4, C5 and C6 classes reached the 100% in survival rate (Fig. 3.20).

As regards the growth rates (Table 3.8), at the end of the trial satisfactory average increases were observed, especially on test diameter. The average values of each class increased from 18.1±1.6, 22.8±1.3, 27.3±1.1, 32.2±1.6, 37.0±1.2 and 42.3±1.3 mm (for C1, C2, C3, C4, C5 and C6, respectively) registered at the initial time (T0) to 18.6±1.8, 24.1±1.8, 28.2±1.2, 33.3±1.6, 37.9±1.2 and 43.2±1.2 mm (for C1, C2, C3, C4, C5 and C6, respectively) at the end of the trial (T5). Expressed as a percentage, the results varied from +5.9% for C2 class to +2.1% for C6 class.

The other percentage variations were +3.4% (C4), +3.2% (C3), +3.0% (C1) and +2.4%

(C5). As for as the total wet weight is concerned, instead, a totally different trend was observed on the specimens belonging to the 3 smallest classes (C1, C2 and C3): at the beginning of the trial (T0), in fact, the specimens of the 3 experimental groups showed values of 3.65±0.98, 7.29±1.69 and 12.21±1.82 g (C1, C2 and C3, respectively).

After 6 weeks (T3), the average weight of all 3 classes size was significantly increased (C1 = 4.73±1.39 g, C2 = 8.79±2.35 g and C3 = 13.89±1.94 g) but, starting from the 8th week onwards (T4), there was a general decrease in the average total wet weight to the final values of 3.81±1.38, 8.16±2.48 and 11.49±2.28 g (C1, C2 and C3, respectively).

Conversely, the 3 biggest size classes (C4, C5 and C6) showed a constant positive trend for the whole duration of the experiment. The initial values were 16.56±2.54, 25.81±2.74 and 36.38±3.63 g for C4, C5 and C6 classes, respectively. Following the same order, the final values registered were 18.67±2.85, 26.52±2.69 and 37.39±3.39 g, respectively.

As a percentage, the worst performance was shown in C3 class, with a loss of 5.8% in total wet weight, while the best was registered for C4, with an increase of 12.8%. The remaining classes showed percentage variations of +4.2% for C1, +12.0% for C2 and +2.8% both for C5 and C6 specimens. The trend of growth rate and total wet weight are reported in Figs. 3.21 and 3.22.

As highlighted for SAMS experiment, also in this case statistical analysis showed significant differences for TD and TWW growth between the examined classes both for "Time" and "Class" factors. Conversely, no significant difference was found for the interaction of the 2 factors ("Time×Class"). The results of ANOVA and Tukey's test are showed in Table 3.9.

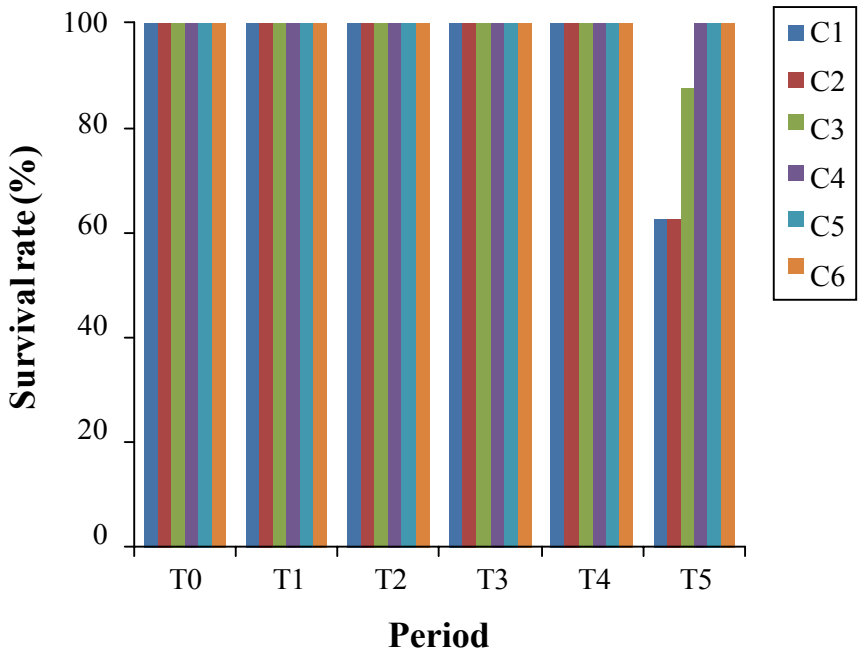

Fig. 3.20. Survival rate of *Paracentrotus lividus* specimens throughout the experimental period.

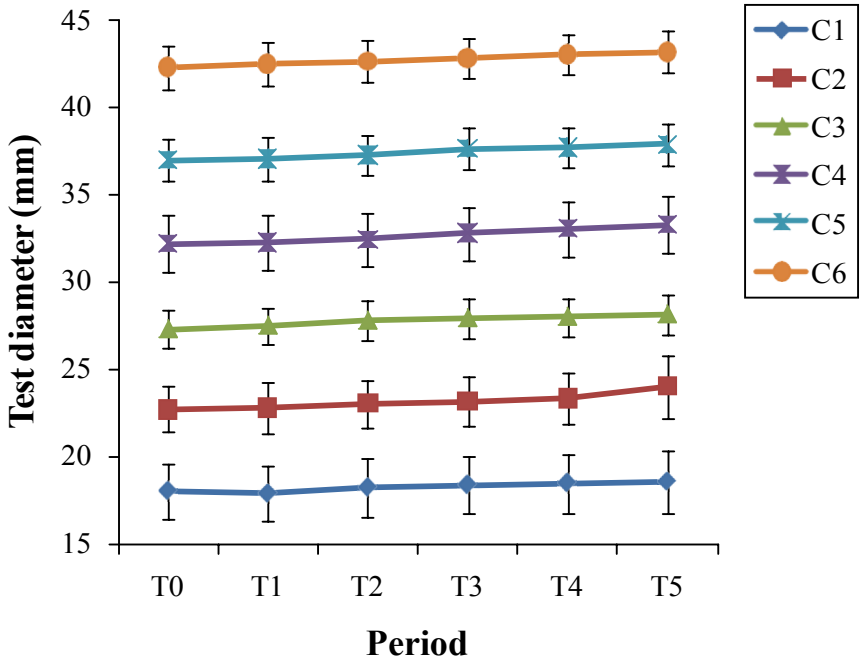

Fig. 3.21. Test diameter (mm±SD) of *Paracentrotus lividus* specimens divided per size class.

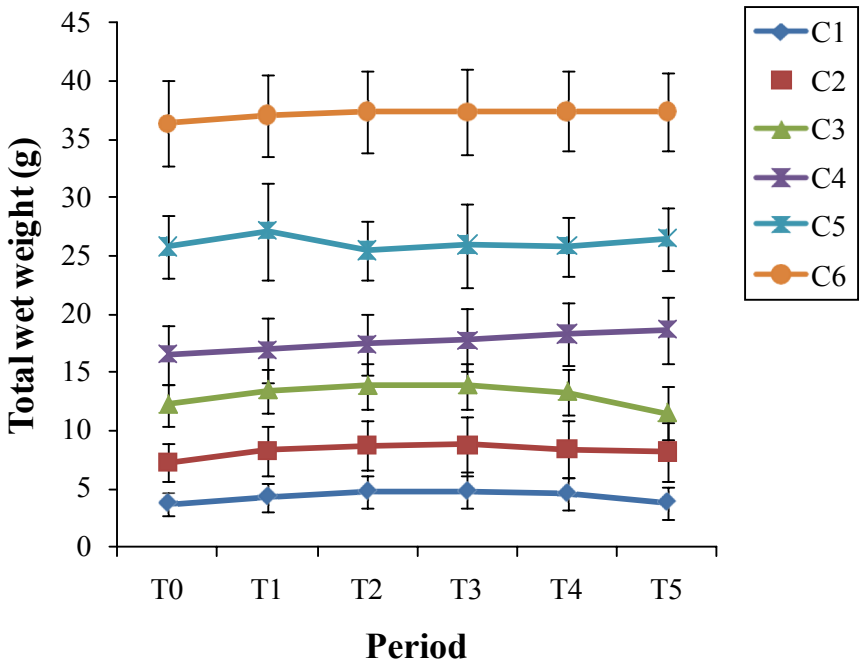

Fig. 3.22. Total wet weight (g±SD) of *Paracentrotus lividus* specimens divided per size class.

Table 3.8. Survival rates registered at the end of the UNISS experiment and Test Diameter (TD; mm±SD) and Total Wet Weight (TWW; g±SD) at the beginning and at the end of the trial.

Class	Final survival rate	Test Diameter		Total Wet Weight	
		Initial	Final	Initial	Final
C1	62.5%	18.1±1.6	18.6±1.8	3.65±0.98	3.81±1.38
C2	62.5%	22.8±1.3	24.1±1.8	7.29±1.69	8.16±2.48
C3	87.5%	27.3±1.1	28.2±1.2	12.21±1.82	11.49±2.28
C4	100%	32.2±1.6	33.3±1.6	16.56±2.54	18.67±2.85
C5	100%	37.0±1.2	37.9±1.2	25.81±2.74	26.52±2.69
C6	100%	42.3±1.3	43.2±1.2	36.38±3.63	37.39±3.39

Table. 3.9. ANOVA results for growth parameters of the 6 classes of *Paracentrotus lividus* specimens examined.

Source of variation	Test Diameter				Total Wet Weight			
	df	MS	F	p	df	MS	F	p
Time	5	17.40	9.28	**0.000**	5	23.30	3.47	**0.004**
Class	5	11,353.90	6,046.63	**0.000**	5	20,563.10	3,059.96	**0.000**
Time×Class	25	0.30	0.16	1.000	25	7.70	1.14	0.290
Residuals	807	1.90			807	6.70		
Tukey's test								
Time		T6≥T5≥T4≥T3≥T2≥T1				T4=T5=T3=T2≥T6≥T1		
Class		C6>C5>C4>C3>C2>C1				C6>C5>C4>C3>C2>C1		

Significant differences are marked in bold.

3.3.2.2 Dissections

On the one hand the dissection of the specimens involved in this experiment has highlighted a dramatic increase of the gonads weight (percentage increases from +251% up to +8041%; Fig. 3.23), on the other hand a contrasting trend in test wet weight (Fig. 3.24). As regards the latter, in fact, 3 classes showed an increase (C1 = from 1.50±0.27 to 2.11±0.97 g, +40.5%; C2 = from 5.50±0.70 to 5.59±0.88 g, +1.8%; C4 = from 9.99±1.38 to 11.58±1.59 g, +15.8%), while the remaining 3 classes showed a decrease (C3 = from 7.67±0.81 to 7.18±0.83 g, -6.4%; C5 = from 16.48±0.79 to 15.70±1.14 g, -4.8%; C6 = from 20.87±1.73 to 20.77±1.49 g, -0.5%). Conversely, the gut wet weight showed a positive trend in all the classes considered (Fig. 3.2.5), in some case with little growth (C3 = from 0.41±0.13 to 0.42±0.10 g, +3.7%), but mostly with an important increase (C2 = from 0.21±0.04 to 0.27±0.08 g, +27%; C5 = from 0.48±0.10 to 0.69±0.19 g, +43%; C6 = from 0.65±0.13 to 0.78±0.19 g, +21%), and in few cases with more than doubled values (C1 = from 0.05±0.02 to 0.15±0.10 g, +188%; C4 = from 0.27±0.05 to 0.56±0.09 g, +107%). Finally, gonads wet weight showed a massive increase in all the size classes investigated.

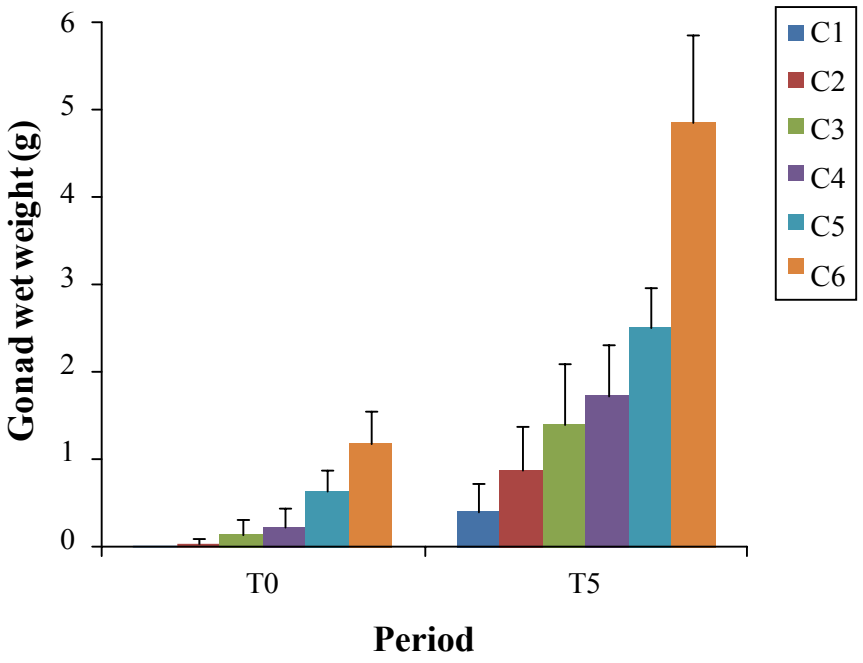

Fig. 3.23. Gonad wet weight (g±SD) of *Paracentrotus lividus* specimens divided per size class.

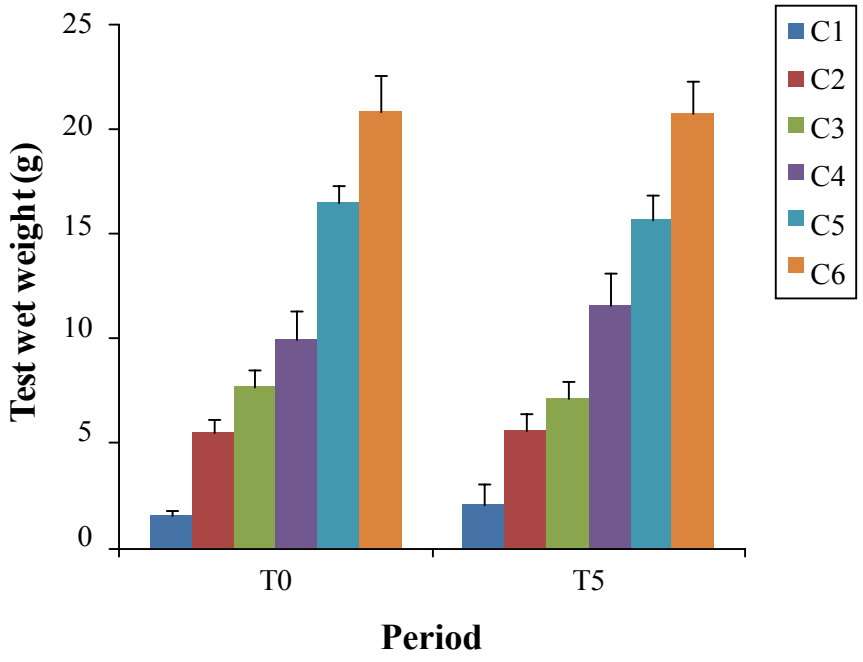

Fig. 3.24. Test wet weight (g±SD) of *Paracentrotus lividus* specimens divided per size class.

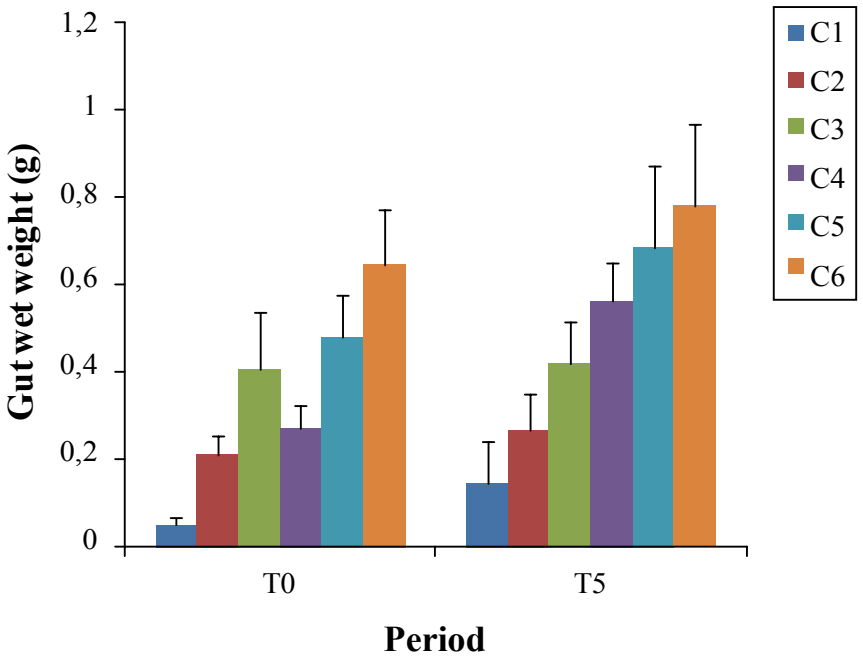

Fig. 3.25. Gut wet weight (g±SD) of *Paracentrotus lividus* specimens divided per size class.

The maximum increase was registered in the smallest class (C1 = from 0.005±0.001 to 0.40±0.32 g), while the minimum in the biggest class (C6 = from 1.19±0.36 to 4.19±1.00 g). The specimens of the remaining classes growth from initial values of 0.05±0.06, 0.14±0.18, 0.24±0.21 and 0.64±0.24 to final measurements of 0.88±0.51, 1.41±0.69, 1.73±0.60 and 2.52±0.45 for C2, C3, C4 and C5, respectively. These results are illustrated in Table 3.10.

Table 3.10. Gonads, gut and test wet weight (g±SD) at the beginning and at the end of the UNISS experiment.

Class	Gonad wet weight		Gut wet weight		Test wet weight	
	Initial	Final	Initial	Final	Initial	Final
C1	0.005±0.001	0.40±0.32	0.05±0.02	0.15±0.10	1.50±0.27	2.11±0.97
C2	0.05±0.06	0.88±0.51	0.21±0.04	0.27±0.08	5.50±0.70	5.59±0.88
C3	0.14±0.18	1.41±0.69	0.41±0.13	0.42±0.10	7.67±0.81	7.18±0.83
C4	0.24±0.21	1.73±0.60	0.27±0.05	0.56±0.09	9.99±1.38	11.58±1.59
C5	0.64±0.24	2.52±0.45	0.48±0.10	0.69±0.19	16.48±0.79	15.70±1.14
C6	1.19±0.36	4.19±1.00	0.65±0.13	0.78±0.19	20.87±1.73	20.77±1.49

The calculation of the gonadal and the alimentary indexes (Table 3.11) have shown the same trend already described for gonads and gut growth. As regards the first (Fig. 3.26), the most increased class was the C1 (from 0.2±0.1% to 8.5±4.8%), followed by C2 (from 0.6±0.8% to 9.2±3.9%) and C3 (from 1.0±1.2% to 11.1±5.1%) classes. The worst performance was registered for the specimens belonging to C6 class, with values of 3.3±1.0% at the beginning of the trial and of 10.7±2.2% at the end. The remaining classes have grown from 1.4±1.3% and 2.4±1.0% up to 8.6±2.9% and 9.2±1.6% for C4 and C5 classes, respectively. About the alimentary index (Fig. 3.27), the best and the worst performances have been showed by C4 class (from 1.5±0.5% to 2.8±0.3%) and C3 class (from 3.1±0.6% to 3.4±0.7%), respectively.

The other classes have increased their AI from 1.8±0.5%, 2.4±0.5%, 1.8±0.4% and 1.8±0.3% to 3.3±1.3%, 3.0±0.5%, 2.5±0.6% and 2.0±0.5% for C1, C2, C5 and C6 class, respectively.

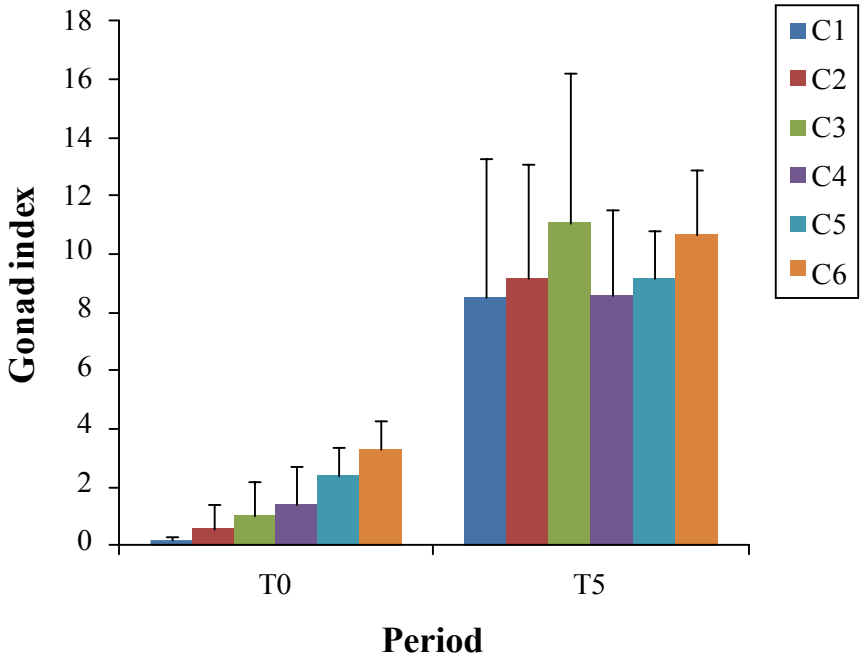

Fig. 3.26. Gonad index (%±SD) of *Paracentrotus lividus* divided per size class.

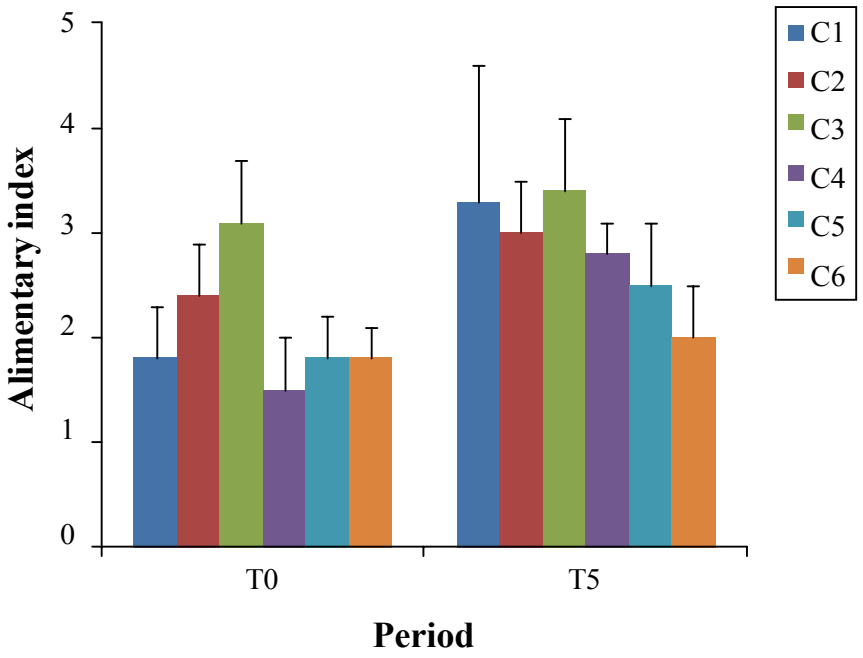

Fig. 3.27. Alimentary index (%±SD) of *Paracentrotus lividus* divided per size class.

Table 3.11. Gonadal index and alimentary index of the UNISS experiment's specimens.

Class	Gonadal index		Alimentary index	
	Initial	Final	Initial	Final
C1	0.2±0.1	8.5±4.8	1.8±0.5	3.3±1.3
C2	0.6±0.8	9.2±3.9	2.4±0.5	3.0±0.5
C3	1.0±1.2	11.1±5.1	3.1±0.6	3.4±0.7
C4	1.4±1.3	8.6±2.9	1.5±0.5	2.8±0.3
C5	2.4±1.0	9.2±1.6	1.8±0.4	2.5±0.6
C6	3.3±1.0	10.7±2.2	1.8±0.3	2.0±0.5

Looking at dissection's results, ANOVA performed on gonads, gut and test weight revealed significant differences for all the three factors considered (i.e., "Time", "Class" and "Time×Class"), with the only exception of the test weight for the "Time" factor (Tab. 3.12). For gonadal and alimentary indexes, significant differences were observed

both for “Time” and “Class” factors, and in the case of AI also for the interaction “Time×Class”. Regards the GI, any significant difference was detected for the interaction of the two factors. The results of the ANOVA are showed in Table 3.13.

Table. 3.12. ANOVA results for dissected compartments of the 6 classes of *Paracentrotus lividus* specimens examined.

Source of variation	Gonads Weight				Gut Weight				Test Weight			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Time	1	64.14	283.52	**0.000**	1	0.53	43.17	**0.000**	1	0.68	0.54	0.464
Class	5	15.98	70.64	**0.000**	5	0.99	79.89	**0.000**	5	989.73	781.39	**0.000**
Time×Class	5	3.95	17.49	**0.000**	5	0.05	4.16	**0.002**	5	3.61	2.85	**0.019**
Residuals	106	0.22			108	0.01			108	1.27		
Tukey's test												
Time	T6>T1				T6>T1				T6>T1			
Class	C6>C5>C4≥C3≥C2≥C1				C6>C5>C4=C3>C2>C1				C6>C5>C4>C3>C2>C1			

Significant differences are marked in bold.

Table. 3.13. ANOVA results for Gonadal and Alimentary indexes of the 6 classes of *Paracentrotus lividus* specimens examined.

Source of variation	Gonadal Index				Alimentary Index			
	df	MS	F	p	df	MS	F	p
Time	1	1,930.98	267.40	**0.000**	1	17.69	47.23	**0.000**
Class	5	17.91	2.48	**0.036**	5	4.59	12.25	**0.000**
Time×Class	5	7.45	1.03	0.403	5	1.42	3.79	**0.003**
Residuals	106	7.22			108	0.37		
Tukey's test								
Time	T6>T1				T6>T1			
Class	C6≥C3=C5=C4=C2≥C1				C3≥C2≥C1≥C4=C5≥C6			

Significant differences are marked in bold.

3.3.2.3 Proximate composition

For the proximate composition, the comparison between reared (CNR) and wild (CNW) sea urchins specimens showed highly significant differences for all the considered

variables, except for the content in total lipids. The average percentage of moisture (Fig. 3.28) found in artificially fed subjects varies from 68.63±0.06% (C4R) to 71.36±0.06% (C6R) and was significantly higher than those found in wild specimens, which varies from 77.39±0.00% for the C5W class, to 81.96±0.01% for the C3W class.

Similarly, the mean ash content (Fig. 3.29) showed analogous results, with a significantly lower value (minimum content: 5.07±0.04% in the C3R class; maximum content: 6.67±0.17% in the C6R class) in reared individuals with respect to those of natural origin (minimum content: 11.35±0.13% in the C5W class; maximum content: 16.54±0.02% in the C3W class). The same analysis, moreover, was performed on food used during the experiment: the obtained results revealed average percentages of moisture and ash equal to 6.31±0.02 and 4.83±0.02%, respectively.

With regards to the content in terms of crude protein (CP; Fig. 3.30), reared Echinoderms were characterised by lower average values, varying from a minimum of 45.09±0.79% (C3R) to a maximum of 50.61±0.14% (C5R), than those observed in the wild ones, from 55.26±1.39% (C3W) to 60.25±0.23% (C6W).

In the food provided during the test, an average of 30.34±0.20% in crude protein content was found. Contrarily to what has been shown, the average percentage of total lipids (TL) exhibited a totally different trend, as shown in Fig. 3.31. The values found, in fact, have put in light a remarkable similarity between the gonads of the fed specimens (min: 19.00±0.81% for the C5R class, max: 20.94±1.34% for the C3R class) and those of wild source (min: 20.12±1.40% for the C6W class, max: 20.93±0.22% for the C5W class).

In the food, however, it has been found an average amount of total lipids equal to 3.82±0.02%. The complete results of proximate composition are illustrated in Table 3.14.

Table 3.14. Proximate composition (%±SD) of reared and wild sea urchins and of the administered food.

Class	Moisture	Ash	Crude Protein	Total Lipids
C3R	69.12±0.23^{G}	5.07±0.04^{G}	45.09±0.79^{E}	20.94±1.34^{A}
C4R	68.63±0.06^{H}	6.14±0.12^{F}	46.08±0.72^{E}	19.88±0.44^{A}
C5R	69.76±0.01^{F}	6.39±0.03^{EF}	50.61±0.14^{D}	19.00±0.81^{A}
C6R	71.36±0.06^{E}	6.67±0.17^{E}	50.39±0.26^{D}	19.53±0.44^{A}
C3W	81.96±0.01^{A}	16.54±0.02^{A}	55.26±1.39^{C}	20.80±0.14^{A}
C4W	79.17±0.35^{B}	15.61±0.22^{B}	57.00±0.30^{BC}	20.63.1.76^{A}
C5W	77.39±0.00^{D}	11.35±0.13^{D}	58.41±0.10^{B}	20.93±0.22^{A}
C6W	77.99±0.01^{C}	11.85±0.24^{C}	60.25±0.23^{A}	20.12±1.40^{A}
Food	6.31±0.02	4.83±0.02	30.34±0.20	3.82±0.02

Data expressed as main value ± standard deviation.
Values with different letters in the superscript are significantly different (A, B, C, D, E, F, G, H = $p < 0.05$).

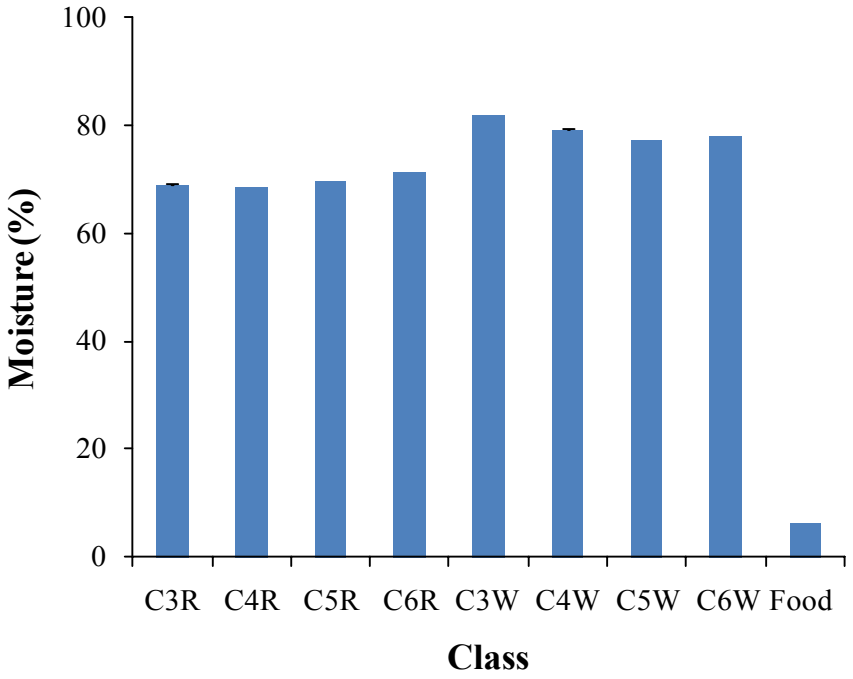

Fig. 3.28. Moisture mean contents (%±SD) of the gonads of reared and wild specimens and of the administered food.

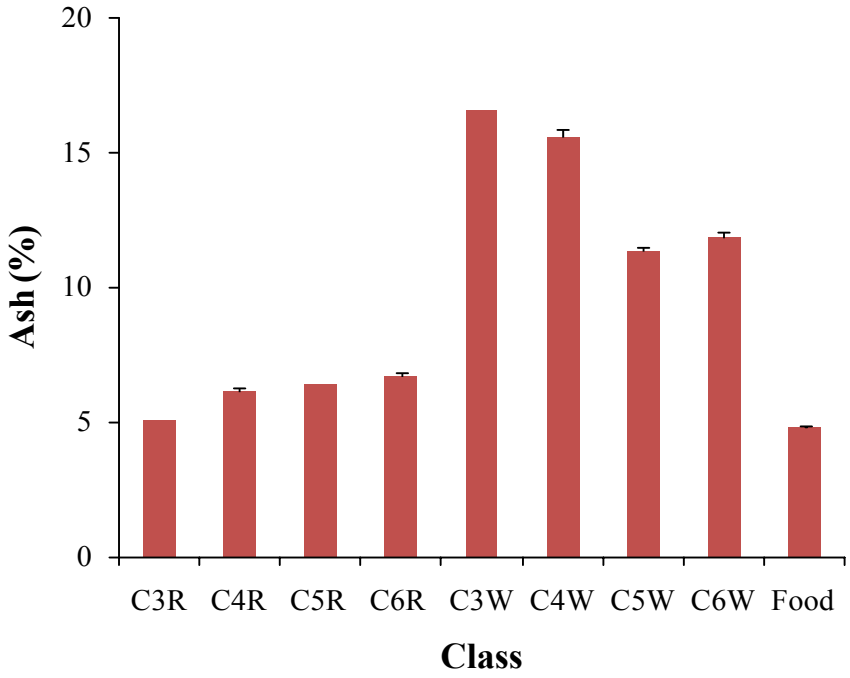

Fig. 3.29. Ashes mean contents (%±SD) of the gonads of reared and wild specimens and of the administered food.

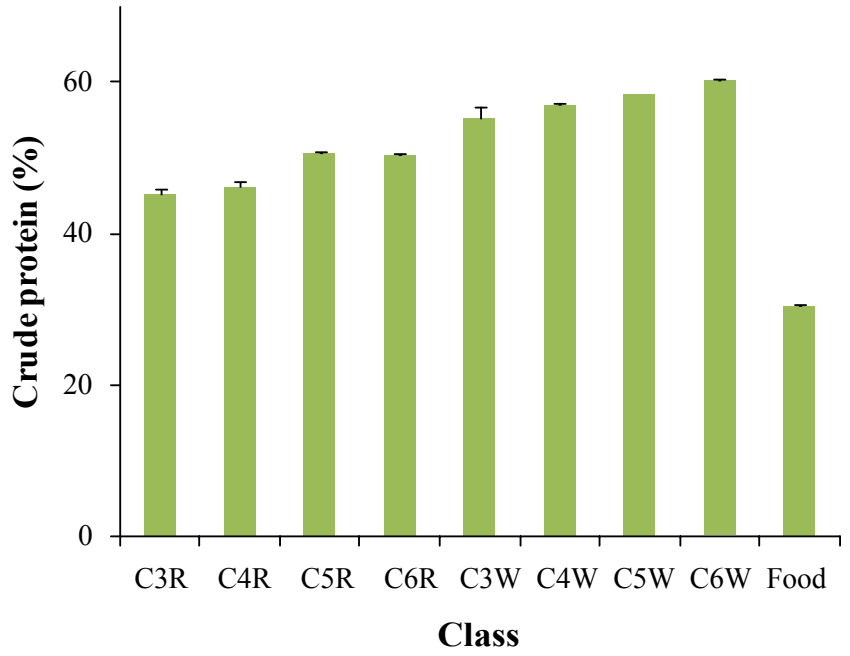

Fig. 3.30. Crude protein mean contents (%±SD) of the gonads of reared and wild specimens and of the administered food.

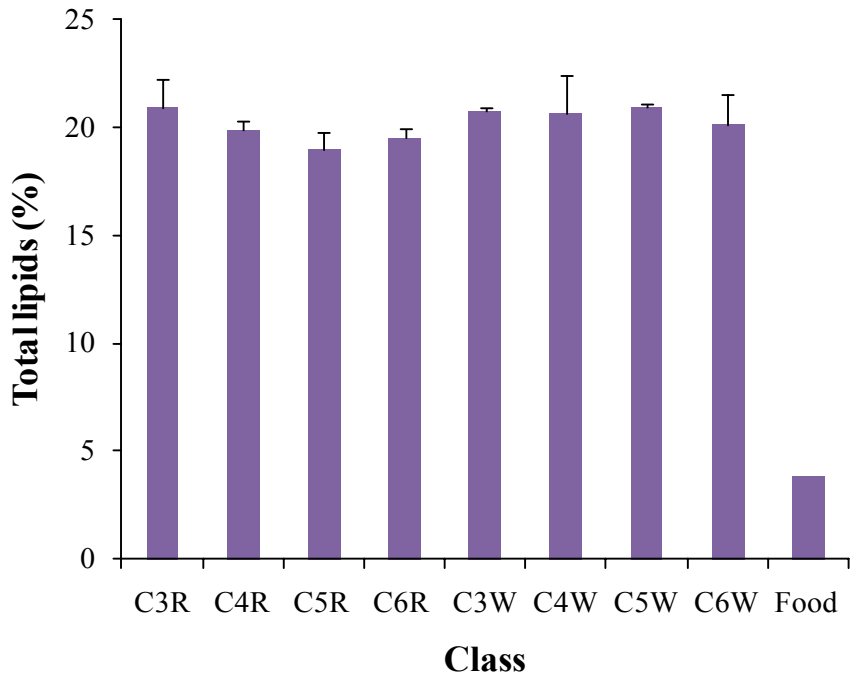

Fig. 3.31. Total lipids mean contents (%±SD) of the gonads of reared and wild specimens and of the administered food.

As regards the above-mentioned variables, ANOVA showed significant differences for moisture, ash and protein, among the 6 different *Paracentrotus lividus* classes. On the other hand, for lipids content no significant differences were found. Tukey's test results are integrated in Table 3.14.

3.3.2.4 Fatty acids

The fatty acid profile of the gonads of reared sea urchins (excluding the C1 and C2 classes because of the shortage of the samples), of the wild specimens of similar size and of the food used during the trial are showed in Table 3.15.

Among the considered fatty acid groups, the monounsaturated (MUFA) were the most underrepresented. Among them, however, similar values were found in the samples of natural origin (from 20.75±0.08% to 21.55±0.28% for C3W and C4W, respectively) and in the reared ones (from 19.98±0.14% for C3R class to 21.92±0.16% for C6R

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specimens). In this fraction, the fatty acid C18:1n9 was the predominant and individuals artificially fed showed higher percentages (from 7.39±0.04% for the C6R class to 9.17±0.12% for the C4R class) compared to the wild ones (from a minimum of 1.56±0.05% registered in the biggest specimens, C6W, to a maximum of 2.14±0.01% for the littlest ones, C3W). Generally between MUFA, class n9 was the most abundant in the reared group, while the n7 was the most represented in the control group. Saturated fatty acids (SFA) showed intermediate percentages. In particular, in samples taken from the wild higher values, ranging from 30.43±0.07% (C4W) to 31.59±0.11% (C5W) compared to those found in sea urchins from the experiment, which varies from 23.21±0.14% (C4R) to 26.40±0.05% (C6R), have been observed.

Among them, the C14:0 (from 4.76±0.03% to 6.37±0.01% and from 7.59±0.24% to 9.19±0.36% for reared and wild specimens, respectively) and the C16:0 (from 13.84±0.02% to 15.40±0.10% and from 16.42±0.10% to 17.06±0.11% for fed and wild specimens, respectively) were those predominant.

However, the group of fatty acids more abundantly represented was certainly that of polyunsaturated (PUFA), with values significantly higher in reared specimens (mean content of 51.23±0.25%) compared to those taken from the wild (mean content of 41.20±0.37%).

Between these, the fatty acids C18:2n6, C18:3n3 and C20:4n6 were particularly different. Specifically, the first two [from 13.94±0.15% (C6R) to 18.93±0.15% (C3R) and from 10.67±0.09% (C6R) to 14.28±0.10% (C3R), respectively] were greater in artificially fed sea urchins (due to the high concentration found in food, corresponding to 27.23%). By contrast, for the C20:4n6 greater values for specimens from the wild were observed (from 6.00±0.40% for C5W, to 11.23±0.05% for C3W specimens).

As part of the PUFA, worthy of special attention are the eicosapentaenoic (EPA) and

docosahexaenoic acid (DHA), which represent two of the most important and representative fatty acids of the n3 series, especially in fish products. In particular, both revealed significantly higher percentages in sea urchins coming from the natural environment (13.05±0.09% and 2.79±0.07% as mean values for EPA and DHA, respectively) rather than in those kept in captivity and artificially fed (mean values of 4.58±0.04% and 0.47±0.01% for EPA and DHA, respectively).

Consequently, the EPA/DHA ratio was higher in fed sea urchins (13.28±0.91% as mean value) compared to wild specimens (5.24±19% as mean value), while their sum (EPA+DHA) was higher in natural sea urchins (range from 12.29±0.16% to 17.13±0.26% for C3W and C4W specimens, respectively) than in the specimens artificially fed (range from 3.02±0.05% to 6.97±0.03% for C3R and C6R classes, respectively).

Finally, the sum of fatty acids belonging to the n3 series has revealed major values in the wild sea urchins than in those artificially fed (from 21.08±0.20% to 25.97±0.28% and from 20.15±0.22% to 21.48±0.04%, respectively). By contrast, the sum of the n6 series acids was significantly higher in reared specimens (from 22.29±0.43% to 27.59±0.08% for C6R and for C3R class, respectively) than in sea urchins found in the natural environment (from 11.25±0.41% to 17.92±0.21% for specimens assigned to C6W and C3W classes, respectively).

As regards the acidic profile (comprised sum and ratios), ANOVA detected significant differences among the 6 classes in all the fatty acid variables considered. The Tukey's test results are highlighted in the table below.

Table 3.15. Fatty acids profile (%±SD) of the gonads of reared sea urchins, of the wild ones of analogous dimension and of food used during the experiment.

Fatty acid	C3R	C4R	C5R	C6R	C3W	C4W	C5W	C6W	Food
C14:0	5.09±0.19^{EF}	4.76±0.04^{F}	5.41±0.03^{E}	6.37±0.01^{D}	9.19±0.36^{A}	7.59±0.24^{C}	8.32±0.20^{B}	8.05±0.26^{BC}	0.09
isoC15:0	0.03±0.00^{D}	0.05±0.00^{D}	0.09±0.00^{C}	0.11±0.00^{C}	0.43±0.02^{A}	0.34±0.01^{B}	0.34±0.01^{B}	0.34±0.01^{B}	0.00
C15:0	0.27±0.00^{F}	0.36±0.01^{E}	0.46±0.01^{D}	0.51±0.01^{D}	1.22±0.01^{A}	1.12±0.02^{B}	1.04±0.01^{C}	1.11±0.05^{B}	0.06
C16:0	15.40±0.10^{D}	13.84±0.02^{F}	14.32±0.07^{E}	15.33±0.04^{D}	16.42±0.10^{C}	16.64±0.17^{BC}	17.06±0.11^{A}	16.95±0.32^{AB}	11.77
C16:1 n10	0.09±0.01^{F}	0.11±0.02^{EF}	0.17±0.00^{DE}	0.21±0.01^{CD}	0.26±0.04^{C}	0.56±0.05^{B}	0.66±0.02^{A}	0.69±0.03^{A}	0.00
isoC17:0	0.06±0.00^{D}	0.08±0.02^{CD}	0.10±0.01^{C}	0.11±0.02^{C}	0.37±0.01^{A}	0.27±0.02^{B}	0.25±0.02^{B}	0.24±0.01^{B}	0.00
C16:1 n9	0.34±0.01^{A}	0.33±0.01^{ABC}	0.30±0.01^{BCD}	0.28±0.00^{D}	0.34±0.03^{AB}	0.28±0.01^{D}	0.30±0.01^{CD}	0.27±0.01^{D}	0.06
C16:1 n7	0.83±0.02^{D}	0.94±0.01^{D}	1.31±0.02^{C}	1.61±0.01^{B}	2.96±0.06^{A}	3.04±0.07^{A}	3.02±0.05^{A}	2.92±0.06^{A}	0.24
C16:1 n5	0.11±0.01^{E}	0.15±0.00^{E}	0.22±0.01^{D}	0.30±0.01^{C}	1.10±0.04^{A}	0.58±0.02^{B}	0.59±0.01^{B}	0.58±0.02^{B}	0.03
C17:0	0.11±0.01^{E}	0.13±0.00^{E}	0.15±0.01^{D}	0.16±0.01^{D}	0.36±0.01^{A}	0.33±0.01^{BC}	0.33±0.00^{C}	0.35±0.01^{AB}	0.08
C18:0	2.95±0.01^{C}	2.94±0.06^{C}	2.91±0.06^{CD}	2.83±0.01^{DE}	2.71±0.03^{E}	3.15±0.01^{B}	3.27±0.03^{AB}	3.34±0.07^{A}	2.95
C18:1t n9	0.31±0.01^{B}	0.36±0.01^{A}	0.37±0.34^{A}	0.34±0.01^{AB}	0.31±0.03^{B}	0.30±0.01^{B}	0.36±0.01^{A}	0.34±0.03^{AB}	0.00
C18:1 n9	8.86±0.02^{B}	9.17±0.12^{A}	8.05±0.02^{C}	7.39±0.04^{D}	2.14±0.01^{E}	1.72±0.10^{FG}	1.75±0.05^{F}	1.56±0.05^{G}	21.65
C18:1c n7	1.84±0.02^{F}	2.04±0.05^{E}	2.22±0.01^{D}	2.42±0.02^{C}	3.49±0.02^{A}	3.07±0.05^{B}	3.15±0.07^{B}	3.16±0.13^{B}	3.09
C18:2 n6	18.93±0.15^{A}	18.87±0.22^{A}	15.84±0.02^{B}	13.94±0.15^{C}	2.10±0.08^{D}	1.67±0.28^{DE}	1.74±0.15^{DE}	1.32±0.08^{E}	31.87
C20:0	0.28±0.01^{D}	0.30±0.01^{D}	0.36±0.04^{C}	0.43±0.01^{B}	0.47±0.03^{B}	0.70±0.01^{A}	0.71±0.02^{A}	0.70±0.01^{A}	0.25
C18:3 n6	0.06±0.00^{D}	0.07±0.00^{D}	0.12±0.01^{C}	0.13±0.00^{C}	0.50±0.02^{A}	0.37±0.02^{B}	0.40±0.01^{B}	0.38±0.02^{B}	0.00
C20:1 n13	1.70±0.02^{G}	1.83±0.02^{F}	2.16±0.01^{E}	2.37±0.02^{D}	3.38±0.08^{C}	4.27±0.07^{A}	3.99±0.04^{B}	4.01±0.02^{B}	0.00
C20:1 n11	0.30±0.00^{F}	0.43±0.01^{E}	0.50±0.01^{D}	0.51±0.01^{D}	0.82±0.02^{C}	1.00±0.03^{A}	0.93±0.01^{B}	0.95±0.02^{B}	0.00
C20:1 n9	4.52±0.06^{A}	4.11±0.04^{B}	3.98±0.06^{B}	4.08±0.06^{B}	3.02±0.06^{CD}	2.91±0.04^{D}	2.98±0.05^{CD}	3.08±0.03^{C}	0.18
C18:3 n3	14.28±0.10^{A}	13.55±0.13^{B}	11.98±0.07^{C}	10.67±0.09^{D}	2.70±0.00^{E}	2.15±0.18^{F}	2.22±0.11^{F}	1.82±0.03^{G}	27.23
C20:1 n7	0.00±0.00^{G}	0.60±0.01^{F}	0.68±0.05^{E}	0.78±0.04^{D}	0.98±0.02^{C}	1.07±0.02^{B}	1.05±0.02^{BC}	1.19±0.04^{A}	0.00
5,13 C20:2	4.73±0.07^{B}	5.05±0.04^{A}	4.76±0.01^{B}	4.41±0.03^{C}	2.74±0.07^{DE}	2.63±0.05^{E}	2.79±0.01^{D}	2.46±0.01^{F}	0.00
7,13 C20:2	0.27±0.04^{D}	0.42±0.07^{C}	0.50±0.02^{C}	0.52±0.02^{C}	0.71±0.06^{B}	0.84±0.02^{A}	0.79±0.03^{AB}	0.82±0.04^{AB}	0.00
C18:4 n3	0.13±0.02^{D}	0.27±0.01^{D}	0.86±0.02^{C}	0.97±0.00^{C}	3.07±0.01^{AB}	3.03±0.11^{B}	3.23±0.08^{A}	3.01±0.07^{B}	0.00
C20:2 n6	3.40±0.06^{A}	3.15±0.03^{AB}	3.27±0.02^{AB}	3.07±0.31^{B}	2.29±0.01^{C}	1.90±0.01^{D}	1.92±0.02^{D}	2.01±0.01^{CD}	0.00
C20:2 n3	1.32±0.00^{A}	0.00±0.00^{D}	0.00±0.00^{D}	0.00±0.00^{D}	0.42±0.01^{B}	0.09±0.02^{C}	0.08±0.01^{C}	0.12±0.03^{C}	0.00
C22:0	0.51±0.02^{B}	0.76±0.03^{A}	0.80±0.02^{A}	0.56±0.01^{B}	0.34±0.02^{C}	0.28±0.01^{C}	0.28±0.02^{C}	0.29±0.05^{C}	0.29
C20:3 n6	1.41±0.02^{A}	0.00±0.00^{C}	0.00±0.00^{C}	0.30±0.53^{BC}	0.56±0.03^{B}	0.49±0.01^{BC}	0.48±0.01^{BC}	0.51±0.04^{BC}	0.00
C22:1 n9	1.08±0.09^{E}	1.42±0.02^{D}	1.54±0.00^{CD}	1.64±0.02^{C}	1.94±0.07^{B}	2.72±0.05^{A}	2.70±0.03^{A}	2.64±0.03^{A}	0.00
C20:3 n3	1.73±0.01^{D}	1.71±0.03^{D}	1.94±0.02^{BC}	1.85±0.08^{C}	1.88±0.03^{BC}	1.96±0.02^{B}	1.94±0.03^{BC}	2.06±0.04^{A}	0.00
C20:4 n6	3.75±0.07^{E}	4.55±0.01^{D}	4.53±0.09^{D}	4.61±0.04^{D}	11.23±0.05^{A}	6.76±0.28^{B}	6.00±0.26^{C}	6.17±0.40^{C}	0.00
C20:4 n3	0.33±0.05^{BC}	0.31±0.07^{BC}	0.36±0.04^{BC}	0.43±0.02^{B}	0.24±0.01^{C}	0.94±0.05^{A}	0.83±0.01^{A}	0.90±0.08^{A}	0.00
EPA	2.90±0.05^{G}	3.87±0.06^{F}	5.42±0.02^{E}	6.15±0.03^{D}	10.96±0.07^{C}	14.24±0.18^{A}	13.57±0.05^{B}	13.43±0.08^{B}	0.00
C22:4 n6	0.04±0.04^{D}	0.07±0.06^{CD}	0.11±0.01^{CD}	0.12±0.01^{C}	0.66±0.03^{A}	0.34±0.01^{B}	0.31±0.01^{B}	0.36±0.01^{B}	0.00
C22:5 n6	0.01±0.01^{E}	0.04±0.01^{E}	0.10±0.01^{D}	0.11±0.01^{D}	0.57±0.01^{A}	0.41±0.01^{C}	0.41±0.02^{C}	0.49±0.03^{B}	0.00
DPA	0.09±0.00^{F}	0.14±0.01^{E}	0.23±0.01^{D}	0.25±0.01^{D}	0.47±0.02^{C}	0.67±0.02^{B}	0.65±0.01^{B}	0.74±0.02^{A}	0.00
DHA	0.12±0.01^{G}	0.29±0.00^{F}	0.67±0.03^{E}	0.82±0.02^{E}	1.33±0.09^{D}	2.89±0.08^{C}	3.31±0.04^{B}	3.66±0.10^{A}	0.00

Data expressed as mean value ± standard deviation. Values in each roe with different superscript letters are to be considered as significantly different (A, B, C, D, E, F, G = $p<0.05$).
Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eiocosapentaenoic acid.

Table 3.15. Continued.

Fatty acid	C3R	C4R	C5R	C6R	C3W	C4W	C5W	C6W	Food
SFA	24.70±0.23^{D}	23.21±0.14^{E}	24.61±0.15^{D}	26.40±0.05^{C}	31.52±0.44^{A}	30.43±0.07^{B}	31.59±0.11^{A}	31.38±0.36^{A}	15.49
MUFA	19.98±0.14^{C}	21.50±0.24^{A}	21.50±0.06^{A}	21.92±0.16^{A}	20.75±0.08^{B}	21.55±0.28^{A}	21.48±0.24^{A}	21.39±0.39^{A}	25.25
PUFA	53.49±0.10^{A}	52.37±0.32^{B}	50.71±0.12^{C}	48.35±0.47^{D}	42.44±0.53^{E}	41.38±0.26^{F}	40.68±0.28^{FG}	40.28±0.41^{G}	59.10
Undefined	1.83±0.14^{E}	2.92±0.43^{D}	3.19±0.02^{D}	3.33±0.33^{D}	5.28±0.17^{C}	6.64±0.13^{AB}	6.26±0.11^{B}	6.95±0.33^{A}	0.16
∑ n3	20.90±0.10^{C}	20.15±0.22^{D}	21.48±0.04^{B}	21.14±0.20^{BC}	21.08±0.20^{BC}	25.97±0.28^{A}	25.83±0.08^{A}	25.75±0.09^{A}	27.23
∑ n6	27.59±0.08^{A}	26.76±0.19^{A}	23.97±0.09^{B}	22.29±0.43^{C}	17.92±0.21^{D}	11.95±0.52^{E}	11.27±0.39^{E}	11.25±0.41^{E}	31.87
n3/n6	0.76±0.00^{D}	0.75±0.01^{D}	0.90±0.00^{CD}	0.95±0.02^{C}	1.18±0.00^{B}	2.18±0.12^{A}	2.29±0.09^{A}	2.29±0.08^{A}	0.85
n6/n3	1.32±0.00^{A}	1.33±0.01^{A}	1.12±0.00^{B}	1.05±0.02^{C}	0.85±0.00^{D}	0.46±0.03^{E}	0.44±0.02^{E}	0.44±0.02^{E}	1.17
EPA/DHA	24.14±2.78^{A}	13.42±0.42^{B}	8.08±0.32^{C}	7.49±0.15^{CD}	8.29±0.54^{C}	4.93±0.08^{DE}	4.10±0.05^{E}	3.67±0.11^{E}	0.00
EPA+DHA	3.02±0.05^{F}	4.16±0.05^{E}	6.09±0.05^{D}	6.97±0.03^{C}	12.29±0.16^{B}	17.13±0.26^{A}	16.89±0.08^{A}	17.10±0.08^{A}	0.00

Data expressed as mean value ± standard deviation. Values in each roe with different superscript letters are to be considered as significantly different (A, B, C = $p<0.05$).
Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eiocosapentaenoic acid.

3.4 Discussion

In the past few decades, due to the overexploitation of the sea urchins populations all over the world, a series of experiments aimed to improve the echiniculture techniques have been carried out. One of the two most followed ways concerns the enhancement of the characteristic of the larval and post-larval forms artificially produced (Liu et al., 2007; Carboni et al., 2014, 2015). Other researchers focus their efforts on the optimization both of the somatic growth and of the quantitative and qualitative improvement of the roe via artificial foods (Watts et al., 1998; McLaughlin & Kelly, 2001; Spirlet et al., 2001; Robinson et al., 2002; McBride et al., 2004; Mortensen et al., 2004; Pearce et al., 2002, 2003, 2004; Vidal, 2004; Senaratna et al., 2005; Cook et al., 2007; Pantazis, 2009; Hammer et al., 2012). As sea urchins are predominantly grazers on macroalgae, many researchers have dealt with the effects of such a food item. With the exception of cultivated species, in this case the bottleneck is represented by the temporal variability in availability and by their nutritional value during time (Vadas et al., 2000; Pearce et al., 2002; Cook & Kelly, 2007b). Other studies have also included

photoperiod and water temperature in their experimental design, in order to evaluate their effect on both the somatic and the gonadal growth (Minor & Scheibling, 1997; Walker & Lesser, 1998; Pantazis et al., 2000; Shpigel et al., 2004; Siikavuopio et al., 2007). In any case, it seems that the major factor influencing somatic and gonadal growth of the sea urchins is represented by availability and quality of the food (Briscoe & Sebens, 1988; Caltagirone et al., 1992; Lawrence et al., 1992; Grosjean et al., 1996; Gago et al., 2001), but also other factors, such as water temperature and photoperiod, can affect sea urchins evolution both in the field and *in vitro* (Turon et al., 1995; Fernandez, 1996; Spirlet et al., 2000; Shpigel et al., 2004; McCarron et al., 2009, 2010; Sartori et al., 2015). Nevertheless, despite the numerous experiments carried out there are not companies operating in the sector of the intensive sea urchins aquaculture. At present in Europe the only exception is represented by Dunmanus Seafood Ltd. (Durrus, Ireland), where juvenile *Paracentrotus lividus* specimens are artificially cultured in order to increase them until they reach the commercial size and then to transfer them within rock pools or in subtidal areas for restocking (Kelly & Chamberlain, 2010).

In such a context, the aim of the present experiments was to evaluate the growth of both artificially produced and wild juvenile specimens of the edible sea urchin *P. lividus* using an artificial diet already used during an experiment performed with *Strongylocentrotus droebachiensis* specimens (Robinson et al., 2002).

The effects of the administered food in form of pellets to the experimental groups employed in this work showed once again the effectiveness and validity of artificial diets to be use in captivity. In fact, the pellet has allowed us to maintain the sea urchins in good health conditions for almost the entire period of the trials, ensuring the survival and partial growth under controlled conditions. The survival rate observed was quite high and in line with those of other research, reaching up to 100% for various size

classes (Robinson et al., 2002; McCarron et al, 2010; Sartori et al., 2015). In particular, as regards the UNISS experiment, smaller classes showed a lower survival rate in comparison to the biggest ones. Conversely, the SAMS trial put on evidence a more various trend, with high survival in all the classes examined. In this case, the biggest specimens exhibited a slightly higher mortality. This may be in part attributable to the different source of the sea urchins used for the 2 experiments. Actually, while the urchins used for the Scottish experiment were artificially produced at SAMS, the specimens of the UNISS trial were of natural origin.

Considering growth, the same trend was observed for both the test diameter (TD) and the total wet weight (TWW) in the 2 experiments: the littlest specimens were also in this case those who have showed the best results. Clearly, the sea urchins used for the SAMS experiment showed higher increase in comparison to those of the UNISS trials, but this could be a consequence of the longer rearing period: 16 weeks instead of 10. As reported by other researchers, during these experiments a decrease in test diameter has been registered. In fact, McCarron et al. (2010) noted a decrease in test diameter during the second and third months of their 6 months experiment in 21.2±0.32 mm TD *P. lividus* specimens held under a 16h light : 8h dark photoperiod. For the corresponding size class (C2; 20-25 mm TD) of the UNISS experiment neither decrease nor increase was registered until the third month. Nevertheless, in accordance with results of Fernandez & Boudouresque (2000), this class showed the best final result in test diameter growth. Instead, for the littlest specimens (C1; 15-20 mm TD) such a negative trend in test diameter was observed during the first month of the experimental period.

The dissections of the specimens highlighted the evolution of weight of the 3 considered anatomical structures: gonads, gut and test. The gonad weight showed a dramatic increase in both the experiments, and the littlest specimens reached considerably higher

values (in % growth) than the largest ones. In the case of gut weight, the trend was the same but with a lesser increase than that observed for the gonads. Conversely, the trend for the test weight exhibited a contrasting tendency: while in the SAMS trial, as for the other structures, the test weight showed a constant positive trend for the entire period of the experiment, in the case of UNISS specimens a decrease in 3 of the 6 considered classes was registered. Such a decrease, mostly observed in sea urchins measuring 25-30 and 35-40 mm in test diameter could be explained, at least in part, by a partially loss of spines in some specimens. Accordingly with Grosjean et al. (1998), the test represents an important fraction of the whole body weight. In our case it is undoubtedly the major fraction, with values ranging from 60% to 70% in SAMS experiment and slightly lower for the UNISS specimens.

The dissections' results are in contrast with those reported by Fernandez & Boudouresque (2000) and McCarron et al. (2009), which suggest that the allocation of energy is primarily in favour of somatic growth in small individuals and of gonadal growth in large ones. Our trials, in fact, have always underlined a major growth in smallest sea urchins, considering both the somatic and gonadal growth.

Finally, both the gonadal and the alimentary indexes showed the same trend thus far evidenced in the 2 experiments: little specimens showed once again the best results, with an increase in gonadal index of more than 10 times in various size classes, while the largest have just more than tripled their initial indexes. In terms of absolute values, the obtained GI from UNISS experiment were lower than those registered by McCarron et al. (2009), while the percentage growth was higher in the present trial. Among the sea urchins classes at SAMS, no one was comparable with those of the cited experiment. In any case, it should be highlighted that the specimens used by McCarron et al. (2009) were hatchery produced, and that the experiment lasted 6 months. The gonad yield per

week was of about 1%, a value slightly lower in comparison to those obtained by administering the same diet to *Strongylocentrotus droebachiensis* (Robinson et al., 2002) for a 65 days period. In the case of alimentary index, in general, the growth was less important but however remarkable.

During these trials the food was readily consumed by sea urchins, and the levels of production observed indicate that a sufficient amount of food was eaten. Due to the high variability of the combinations of water temperature and photoperiod used until now by various researchers, it is not easily understandable which of them can ensure the best results. In any case, in the light of these outcomes, it can be stated that a combination of 18°C and 12 hours light : 12 hours dark can improve somatic growth and gonad enhancement in juveniles *Paracentrotus lividus*. However, as underlined also by Spirlet et al. (2001), sea urchins eat slowly so that a degradation of pellets can occur, causing a loss of nutrients that, consequently, can increase water pollution and stress events for them. In RAS systems these problems can be faced via continued monitoring of the physical and chemical water variables, with frequent changes of water and, finally, taking care to equip the rearing system with easily removable modules, in order to facilitate the cleaning operations. As regards the food manufacturers, instead, they have to found a better solution to improve the pellet resistance in sea water.

From an analytical point of view, furthermore, the proximate composition of the gonads of reared specimens showed average values of moisture and lipids relatively similar to those of wild conspecifics of analogous size. However, in the artificially fed sea urchins, significantly lower average content of ash and protein than those of wild origin were detected. As regards the fatty acid profile of the 2 analysed groups, however, remarkable differences, especially in the content of some fatty acid groups such as SFA and PUFA, have been observed. In the case of SFA (Saturated Fatty Acid), a mean

value considerably higher in the gonads of wild specimens was evidenced, while for the PUFA (PoliUnsaturated Fatty Acid), the average value appeared significantly higher in individuals artificially fed. In particular, these latest results are almost certainly attributable to the composition of the food used during the trial. In fact, the latter is characterised by high percentage of C18:2 n6 and C18:3 n3, which were the most represented fatty acids, together with C16:0, in the gonads of reared specimens. Also Gago et al. (2009) found these fatty acids in the gonads of sea urchins fed with artificial diet. In partial disagreement with our data, in similar experiments Cook et al. (2007) and Carboni et al. (2013) found that C16:0, C18:2 n6, C14:0 and EPA were the most abundant fatty acids. Only a little amount of C20:5 n3 was found, probably due to its absence in the diet. As regards the wild specimens, Zlatanos et al. (2009) and Martinez-Pita et al. (2010) pointed out the preponderance of SFA in comparison to other fatty acids groups. In contrast, the group mainly represented was that of PUFA. Moreover, it must be emphasised the higher average percentage content of EPA (Eicosa Pentaenoic Acid) and DHA (Docosa Hexaenoic Acid) found in the wild sea urchins than that detected in the reared ones (also in this case due to the provided food). In accordance with several authors (Gago et al., 2009; Martinez-Pita et al., 2010; Carboni et al., 2013), this study confirmed that the acidic profile of the sea urchin gonads can be influenced by the diet.

The results obtained in the present experiments suggest that the above mentioned temperature-photoperiod combination, together with a suitable food, can provide optimal conditions for the rearing of juvenile *P. lividus* specimens in a RAS system, especially in the case of sea urchins ranging from 15 to 30 mm in test diameter. However, it has to be considered that longer breeding periods could produce different results with respect to those discussed here.

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4. Profitability and sustainability of *Paracentrotus lividus* fishery in Sardinia

4.1 Introduction

Profitability and sustainability are frequently considered as conflicting alternatives of fisheries development strategies in the European Union (EU), thus creating serious dilemmas for policy makers (Surìs-Segueiro et al., 2002; European Commission, 2001, 2009). For this reason, the main challenge is to preserve profits and employment for fishery firms without compromising the marine population's turnover capability and, at the same time, by reducing the risk of overexploitation of marine resources (Frost & Andersen, 2006; European Commission, 2009; Khalilian et al., 2010; Markus, 2010). This is an issue particularly critical for the Mediterranean Sea, where incomes of many local coastal communities are strictly dependent on the economic performances of fisheries (European Commission, 2000, 2002; Madau et al., 2009). Nevertheless, an adequate level of profitability is not achieved by several fishery firms, as a result of operating under very small-scale size and due to strict regulations that limit small-scale and multispecies fisheries.

The edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) fishery is a representative example of this contradictory policy issue. This species is the most intensively harvested Echinoid in several Mediterranean regions, particularly in the southern part of the Basin (Régis, 1986; Guidetti et al., 2004; Pais et al., 2007; Ceccherelli et al., 2011; Matsiori et al., 2012). The fishery of this species, which usually concerns specimens larger than 40 mm in test diameter, is traditionally carried out along the European (both Mediterranean and Atlantic) and Maghreb coasts due to the high economic value and delicacy of its gonads (roe; Régis, 1986; Byrne, 1990; Barnes & Crook, 2001; Guidetti et al., 2004; Gianguzza et al., 2006; Sellem & Guillou, 2007; Antoniadou & Vafidis, 2009; Sellem et al., 2011; Fernandez-Boan et al., 2012; Grisolia et al., 2012; Pais et al., 2012; Furesi et al., 2014).

At present, *P. lividus* is subjected to intensive harvesting activities by both professional and recreational divers, with the risk of compromising its natural stocks in different areas of the Mediterranean (Guidetti et al., 2004; Gianguzza et al., 2006; Tessier et al., 2010; Ceccherelli et al., 2011; Pais et al., 2007, 2012). On the other hand, rigorous normative constraints on edible sea urchin fishery (addressed to minimize the risk of overexploitation) might put at risk the profitability in harvesting this resource for professional fishermen (Furesi et al., 2014).

In Sardinia Island (central western Mediterranean, Italy), the fishery of this species has been historically practiced by both professional and non-professional fishermen and sea urchin roe is the principal ingredient for a number of typical dishes (e.g., spaghetti, pizza and croutons; Furesi et al., 2014). Consequently, Sardinian policy makers have established several measures in order to regulate this activity with the aim of minimizing the risk of overexploitation of the resource and, above all, of fighting illegal fishing. In particular, according to the measures allowed by the Common Fisheries Policy (CFP) for the conservation and sustainable exploitation of fisheries resources in the Mediterranean, for the fishing season 2015-2016 the Sardinian Regional Government has imposed strict rules on edible sea urchin fishery aimed at reducing the fishing effort on this resource as follows: 1) by granting a limited number of firm authorizations for this activity (about 200); 2) by limiting the fishing season from 1st November 2015 to 10th April 2016 (every day from dawn to 15.00 p.m.); 3) by fixing a maximum daily amount of captures of 1,500 sea urchins (for each professional fisherman); and 4) by providing a minimum harvestable size (50 mm of the test diameter without spines). In addition, recreational fishers can harvest (without breathing apparatus) a maximum of 50 sea urchins per day only 3 days a week (Wednesday, Saturday and Sunday) and holidays during the fishing season.

Nevertheless, these type of constraints greatly limit the annual profits from sea urchin fishery for professional fishermen and force this fishery to be a seasonable activity. Among the effects arising from this condition, a strong pressure to overcome the normative constraints is in place due to market demand that has been rapidly growing over the last few decades. Due to this last fact, illegal sea urchin fishing and poaching have increased relevance, becoming a severe problem for guaranteeing the sustainability of this resource.

On the other hand, it is worth noting a lack of knowledge on economic issues of edible sea urchin fishery in the Mediterranean. In fact, while there is a large literature on the biological management of sea urchins along the European coasts (Guidetti et al., 2004, 2005; Gianguzza et al., 2006; Tessier et al., 2010; Ceccherelli et al., 2011; Pais et al., 2007, 2012), only a few studies have investigated the economic outcomes and implications at fishery firm level arisen from harvesting restrictions provided by the public authorities. Some authors have attempted to estimate the economic value of edible sea urchins by evaluating the willingness to pay for eating a based sea urchin dish (use value), or for guaranteeing conservation (no-use value; Grisolia et al., 2012; Matsiori et al., 2012; Furesi et al., 2014). Other studies throughout the world (even if not specifically on *P. lividus*) have investigated on other economic issues associated to edible sea urchin fishery and with the aim of estimating the value of sea urchin market on the whole and not at fishery firm level (Muraoka, 1990; Reynolds & Wilen, 2000). Nevertheless, at present, in the scientific and economic literature there is no empirical confirmation of the economic profitability of sea urchin fishery in the Mediterranean Sea and, in particular, in Sardinia.

This means that these policies have been often promoted without the support of scientific evidence, particularly with reference to the relationship between sea urchin

fishery profitability and sustainability. As a consequence, empirical data on the economic convenience of sea urchin harvesting need to be collected for improving the effectiveness of policy makers' decisions to reduce the risk of overexploitation without compromising the profitability of edible sea urchin fishery.

In the light of these concerns, a study on this topic coordinated by the Department of Science for Nature and Environmental Resources (DIPNET) of the University of Sassari and under the control of the Regional Agency of Agriculture (LAORE Sardegna) was conducted. This study was aimed at: 1) estimating the economic convenience for edible sea urchin fishery in Sardinia, and 2) evaluating if both environmental (in terms of preservation of natural wild stocks) and economic pillars of sustainability can be achieved by firms given the actual policy restrictions. In addition, the role played by the technological differences among firms in affecting profitability was investigated. These differences primarily concern the harvesting system (from boat or from shore) and the level of vertical integration adopted (processing part of the product or any processing). To the best of my knowledge, this is the first attempt to investigate edible sea urchin fishery data directly collected from professional fishermen in the Mediterranean Sea. Therefore, these empirical evidences might allow to define several policy implications for improving the efficacy of policies in positively affecting both economic and environmental sustainability of sea urchin fishery in Sardinia.

As already discussed in the previous chapters, the edible sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) is widely distributed throughout the Mediterranean Sea and along the North eastern Atlantic coast from Scotland and Ireland to southern Morocco (Boudouresque & Verlaque, 2001). This herbivorous species lives on rocky substrates and in seagrass meadows, from shallow waters down to about 20 m depth. The annual cycle of the gonad maturation of *P. lividus* shows one or two seasonal peaks

and spawning has been reported to occur either once or twice in a year (Boudouresque & Verlaque, 2001).

Although sea urchin fishery has been traditionally practiced in several coastal areas of Sardinia Island, at present there are no studies that describe the relative economic and market issues (Lei Spano, 1977). Nevertheless, detailed information on technical and structural characteristics of this type of fishery and of the market of this fish product can be very useful for understanding how sea urchin harvesting is really carried out in Sardinia.

Until a few years ago, edible sea urchins were harvested on foot or from flat-keel boats, using a rod (usually a reed), a landing net and a seascope. This activity was mainly carried out by non-professional fishermen and the sea urchins harvested were generally used to satisfy household consumption or to the local market (e.g., kiosks or stands along the streets). In addition, sea urchins were almost always eaten fresh with bread and their harvest was limited to a small period of the year (mainly in winter, from January to February).

During the last years, the demand for edible sea urchins has dramatically grown, mainly because of the high consumption by residents and tourists (Pais et al., 2007). Recent reports pointed out that, on average, about 30 million *P. lividus* specimens (approximately 1,800 t) were consumed in the last years in Sardinia, providing a value of sold product by traders equal to more than 10 million € (Carboni et al., 2012). Consequently, considering a population of about 1.7 million residents, Sardinia's annual *per capita* consumption was about of 1.1 kg that corresponds to approximately four times the consumption of Japan. On the other hand, an exact assessment of the edible sea urchins annually harvested along the Sardinia coast (1,849 km) is considerably difficult, due to the large quantity collected by local illegal fishermen and poachers

(Ceccherelli et al., 2011).

To meet the increasing demand, the fishing technique for this species has been significantly improved in the last few decades: the traditional system based on the reed as collection tool has practically disappeared and most of the fishing activities are now carried out by divers (mainly SCUBA divers and much less free divers without breathing apparatus). In particular, SCUBA diving is performed directly from the shore or using small boats (4-6 m in length and with a crew of 2-3 members). However, this technique, with or without boat, involves a greater investment in terms of equipment and higher costs of production (both fixed and variable costs) than traditional system or free-diving. On the other hand, this fishing method has allowed firms to significantly increase the total catches amount: especially hookah diving with the aid of a boat allows to considerably enhance the productivity of labour and the quantity of edible sea urchins harvested per day.

Some noteworthy changes have also occurred in the consumption of edible sea urchin roe. Beside the consumption of fresh product, the demand of the processed one (obtained by both refrigeration and vacuum preservation of the roe) has considerably increased. The use of processed roe has allowed to expand consumption to restaurants, to broaden sea urchins food uses (not only as fresh roe but also as seasoning of pasta and pizza), and to extend the consumption during periods in which the harvesting of sea urchins is not permitted (late spring and summer). In addition, it is worth noting that processed roe is sold at much higher prices than the fresh one, with very high profits for sellers.

4.2 Materials and methods

Economic and structural data were collected from a sample of professional Sardinian

sea urchin fishery firms in 2012. The sample size was defined by stratifying the sample on the basis of the criteria explained below, and taking into account the availability of fishermen in providing data and information. The data collected are not limited for the fishing year considered, but represent economic and structural features that, on average, can describe firms' activities in the last few years. A questionnaire – preliminarily pre-tested on a small sample of firms – was submitted to fishermen in order to collect information on their activities.

The selection was based on stratified sampling design according to 3 specific criteria. Firstly, data were collected in 4 different coastal zones of Sardinia coasts (South, West, North western and North eastern) particularly devoted to this fishing activity. Secondly, the sample was selected by taking into account technical differences between the harvesting methods used (although each fishing system is typically related to an artisanal/small-scale activity). In particular, 32 firms in which fishermen harvest sea urchins exclusively by diving from the boat (13), or exclusively from the shore (14), or from both (5) were sampled. The rationale at the basis of this distinction lies on the idea that differences in the fishing methods used might affect volume of captures, costs and revenues of the firms. Lastly, both types of firms that entirely sell the unprocessed product (23) and that only partially process the gonads (9) were selected. This choice was made to evidence putative differences in profitability between vertical integrated firms and not. Nevertheless, it must be emphasized that all Sardinian firms usually process all the sea urchins harvested and that the most of this product is sold as "fresh sea urchin roe".

On the basis of the data and information collected by interviewing the Sardinian sea urchin fishermen, the costs and revenues of all firms were analyzed in order to estimate the economic convenience in harvesting and selling edible sea urchins. Special attention

was put on highlighting the structural characteristics of sea urchin fishery at firm level and on estimating cost production and profitability of this activity. A summary of the analyzed balance items with the corresponding categories of inputs and outputs is illustrated in Table 4.1.

Table 4.1. Summary of cost and revenue items analyzed.

Costs	Revenues
Labour (wages)	Revenue from sea urchins selling
Variable expenditures (e.g., cost for fuel, cost for insurances)	Other revenues (e.g., financial aid)
Capital (depreciation charge for vessel, fishing tackle, car, etc.)	
Taxes	
Other expenditures	
Processing*	

*Only for firms that process part of the harvested sea urchins

As far as labour is concerned, both explicit and implicit wages were taken into account. This was done because labour is partially or totally carried out by the entrepreneurs in each selected firm. Consequently, the cost of labour was calculated both on the basis of wage paid to employers and of salary ideally associated to the labour of entrepreneur (hire wages). The aim is to emphasize the real cost of labour by avoiding possible discrepancies among firms with different intensity of labour provided by employers.

Variable expenses are for fuel (vessels and cars), insurances, packaging and other expenditures for no capital inputs, except for taxes that were computed as separate cost item. Cost for capital endowment accounts the depreciation charges associated to the value of capital inputs involved in sea urchin fishery, such as vessels, cars, fishing tackle, etc. Additional expenditures are due to costs incurred for immaterial inputs concerning firm activity on the whole (e.g., costs for professional advices, extension service, etc.), whereas processing costs affects only firms that process part of the sea urchins harvested.

Finally, break-even point analysis was performed to relate profitability to sustainability thresholds allowed by regional policy makers of Sardinia. Basically, the daily quantity of sea urchins harvested were estimated in order to achieve the break-even point of firms and, consequently, the eventual profitability increase associated to a possible growth of the maximum daily cap of captures. This analysis allows to assess if a re-modulation of quotas provided by the Sardinian regional legislation could be suggested according to empirical evidences achieved from the estimate of economic outcomes of the sea urchin fishery.

4.3 Results

A high variability of structural characteristics and economic results was detected among the firms examined (Table 4.2). During the fishing season 2012, on average, the annual captures amounted to about 114,000 sea urchins per firm, a little less than 1,700 specimens per day. Nevertheless, this value is significantly higher (about threefold) for firms which perform harvesting operations from the boat in comparison to those that practice fishing from the shore.

Table 4.2. Annual and daily captures, on average, by fishing system (n. of trips, n. of sea urchin specimens).

Fishing system	Daily trips per year	Captures	Daily captures*
Diving from boat	68.8	188,250	2,650
s.d.	*(24.0)*	*(88,055)*	*(790)*
Diving from shore	67.9	62,571	1,029
s.d.	*(25.7)*	*(35,004)*	*(706)*
Both systems	66.0	82,000	1,250
s.d.	*(26.1)*	*(29,496)*	*(252)*
Total	**67.7**	**113,608**	**1,669**
s.d.	***(24.3)***	***(83,453)***	***(972)***

*The value is calculated as average on daily captures reported by each vessel

This marked difference is almost entirely attributable to the daily amount of *Paracentrotus lividus* specimens collected (2,650 and 1,029, respectively), as the number of fishing days available is almost equal to 68 for both the types of firms. The higher daily productivity of diving from the boat (especially using SCUBA equipment) is due to the greater ability and speed of spatial movements in looking for areas with high concentration of sea urchins. In addition, by considering the time spent at sea by each diver, it was observed that on the whole the former firms employ a higher number of hours per fishing day than the latter ones (18.3 hours vs. 7.9 hours per day). This means that firms operating from the shore usually employ only 1 diver per fishing day, while firms that harvest from the boat simultaneously employ, on average, 2 divers (8-9 hours per diver).

From an economic point of view, significant differences between the 2 fishing systems were also observed (Table 4.3).

Table 4.3. Costs, revenues and profits, on average, by fishing system (€).

Fishing system	Costs	Revenues	Profits*
Diving from boat	17,924	34,672	**14,643**
s.d.	*(8,277)*	*(21,670)*	*(13,110)*
Diving from shore	6,688	8,732	**2,600**
s.d.	*(3,129)*	*(5,956)*	*(6,275)*
Both systems	10,179	10,798	**699**
s.d.	*(3,347)*	*(4,338)*	*(2,561)*
Total	**11,798**	**18,505**	**6,988**
s.d.	***(7,748)***	***(16,349)***	***(11,193)***

*The value is calculated as average on profits reported by each vessel

In particular, costs are significantly higher in firms that utilize boat (more than 2.5 times in comparison to firms which harvest sea urchins from the shore). This fact mainly depends on the higher number of daily fishing hours totally employed and also by the

larger amount of equipment used (i.e., boat, boat-truck, breathing apparatus, etc.) that influence value of salaries, allowance for capital consumption and cost for materials. In addition, there are important differences between the 2 fishing systems in terms of structures and production scale. These differences are fundamentally related to different volumes and incidence of capital endowments that characterize these 2 sea urchin fishing methods.

With reference to this issue, it was found that, on the whole, about 20% of the cost in firms that harvest sea urchins from boat corresponds to cost for capital, while the incidence of this item is close to 14% in firms operating from the shore (Table 4.4). Nevertheless, salary is the item that mainly affect total expenditures of the sample examined, with an incidence of more than 50%. This aspect means that sea urchin fishing is a highly labour-intensive activity and it must be emphasized that harvesting intensity tends to be noticeably higher in firms that fish *P. lividus* from the shore (60% vs. 52%) owing to inherent under-capitalized structure.

Table 4.4. Distribution of costs, on average, by items (€).

Fishing system	Cost items					
	Labour	**Capital**	**Variable expenditures**	**Taxes**	**Other expenditures**	**Total**
	Euros (€)					
Diving from boat	9,352	3,543	3,898	656	475	**17,924**
Diving from shore	4,019	932	842	591	303	**6,688**
Both systems	5,275	2,592	1,453	551	307	**10,179**
Total	**6,382**	**2,252**	**2,179**	**611**	**374**	**11,798**
	%					
Diving from boat	52.2	19.8	21.7	3.7	2.7	**100**
Diving from shore	60.1	13.9	12.6	8.8	4.5	**100**
Both systems	51.8	25.5	14.3	5.4	3.0	**100**
Total	**54.1**	**19.1**	**18.5**	**5.2**	**3.2**	**100**

By considering only the firms that partially process and/or package the sea urchins collected, the incidence of processing costs amount to 17%, a little higher for firms operating from the shore.

As far as revenues is concerned, their amount primarily depends on selling price and on the number of sea urchins harvested. The rationale at the basis of the differences in prices is the level of processing of the roe sold (fresh or processed). Actually, it was found that processed sea urchin roe reaches a market price significantly higher (from 50% to 300%) in comparison to the equivalent amount of the fresh one.

The 9 sampled firms that process part of the *P. lividus* specimens harvested are almost equally divided in offshore and inshore firms, but empirical evidence demonstrates that the level of processing – in terms of quota of processed product on the total product – is significantly higher in firms operating from the boat than the other ones (on average, 35% vs. 13%), thus meaning that the former tend to attain more profitable prices than the latter.

As regards the second reason, the number of sea urchins harvested varies considerably between the 2 fishing systems used. Consequently, the firms operating from the boat achieve more important revenues (almost fourfold) than the other ones. Therefore, even if harvesting sea urchins from the boat is more costly than fishing them from the shore, the noticeable revenues allow firms operating from the boat to have profits significantly higher than the other ones (about 14,600 vs. 2,600 €; Table 4.3).

Profits are also significantly higher when part of the sea urchins harvested is processed and packaged. More exactly, profits of the integrated firms are, on average, equal to 16,300 € while raw sea urchins sellers gain only 3,400 € each one. This is a clear evidence of the premium price granted by customers to post-harvest activities. Therefore, the opportunity to employ sea urchin roe to prepare and consume several

dishes (e.g., pasta and pizza) during a season in which the fresh product cannot be harvested and sold is well remunerated.

The break-even point was found, on average, to be close to 43% of the total captures (about 50,000 sea urchins per year). This fact suggests that a relatively small amount of sea urchin is needed in order to get even revenues with costs. In terms of annual trips, equality between costs and revenues might be achieved, on average, at about 70% of the observed trips (about 46).

The comparison between the break-even point analysis and the economic balance analysis allows to highlight relevant implications on the economic convenience in harvesting sea urchins. On the one hand, indeed, it was found that the amount of captures observed does not ensure, on average, satisfactory profits for firms. On the other hand, the break-even point was found at a low level of captures. This fact means that sea urchin fishery firms rapidly tend to attain a correspondence between revenues and costs but, at the same time, a high level of captures is needed to guarantee an adequate profitability.

This is because the estimated elasticity of profit with regard to the level of captures is sensitively high (1.48), given the invariability of output and input prices. Actually, labour is the only cost item that varies when daily captures increase, contrary to the use of other variable inputs that depend on factors not directly related to daily captures (e.g., number of trips) and of capital inputs.

Essentially, it implies that profit would increase more proportionally than captures do. This result indicates that the cap on daily captures provided by the Sardinian regional regulation (as it stands today) does not allow firms to employ inputs in their own disposability in an economically effective and profitable way. Consequently, the labour productivity is low with marked implications on economic results achieved by the sea

urchin fishery firms in Sardinia, due to the scarce production efficiency.

Based on the estimated profit/captures elasticity, a simulation of the effects on profits arisen from a possible increase of the maximum amount of captures allowed was performed. How a slight increase of the daily captures allowed might generate a significant increase in profits is illustrated in Fig. 4.1 (e.g., in case of cap increase by 20%, it switches from 1,500 to 1,800 sea urchins/day/fisherman, profit would increase by about 30%).

This would be an important result in order to provide scientific information to support policy makers in implementing daily quotas of captures. In fact, the analysis takes into account the need of preserving the marine resource (reducing the risk of overexploitation) and, at the same time, the need of guarantee a suitable profitability for the Sardinian sea urchin fishery firms.

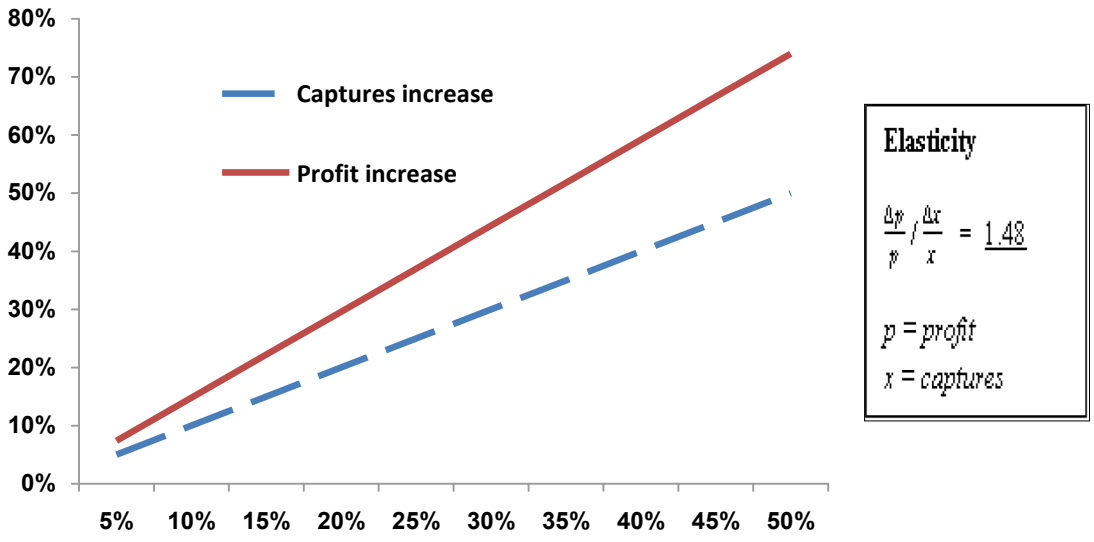

Figure 4.1. Relationship, on average, between eventual increase of captures and profit.

4.4 Discussion

During the last few years, the harvesting of edible sea urchin *Paracentrotus lividus* has dramatically increased along the Sardinian coasts. Therefore, this study can enhance our knowledge on the economic nature of this phenomenon and regional policy makers could get useful suggestions from the empirical findings described.

Firstly, the policy for sustainability of the sea urchin fishery should better fit both the needs of conservation of natural population and economic profitability. Conversely, the results reported clearly indicate that the current constraints on this fishery do not allow commercial sea urchin harvesting to be profitable enough. The whole value created by resources legally exploitable by fishermen each year could be higher with a different distribution of quotas. More specifically, the maximum daily amount of captures could be adjusted on the basis of economic size of each activity. This could be accomplished, for example, allowing the greater scale firms to fish higher quantity of sea urchins than the smaller scale ones. This remodulation could be realized spontaneously under the hypothesis of a rearrangement of rights among firms as to minimize the risk of overcapacity of very small scale firms (under utilization of the captures allowed) and to authorize great scale firms to increase captures without effects as regards the total amount permitted. The costs and benefits of such a management are widely and deeply known in catch share fisheries (Gordon, 1954; Essington, 2010). Among the former ones, the concentration of the rights and the marginalization of small firms play an important role in this specific case (Eythorsson, 1996). Consequently, special attention and caution are required before introducing these management tools for Sardinian sea urchin fishery.

In addition, the rotation of fishing areas and the control of the accessibility of fishing locations could minimize the risk of overexploitation of the natural resource. Such a

measure implies a comprehensive knowledge of the state of the different stocks and, of course, a continuous monitoring activity by marine biologists and ecologists. In this way, both measures – i.e., rights modulation and redistribution and fishing areas rotation – could make Sardinian sea urchin fishery management more rational, with positive effects on incomes and without compromising the health of natural stocks.

In Sardinia, the annual fishing season for this species is not well scheduled also in time. Indeed, the harvesting of *P. lividus* is a seasonal activity due to a regulation that allows fishing only from November to April. This fact certainly affects profitability, because the local fresh product cannot be supplied during the summer to satisfy the considerable potential demand by tourists and to achieve more profitable prices.

According to Pais et al. (2006) and taking into account the biological cycle of *P. lividus* in Sardinia, a technical solution could be found in changing the fishing season in order to allow captures also during the summer months (without increasing the total number of fishing days), although for a limited period of time. On the other hand, it must be emphasized that policy measures aimed at improving economic convenience and preserving sea urchin populations have to focus on enforcing controls and providing stricter disciplinary sanctions to reduce illegal fishing. The results acquired suggest that Sardinian fishermen have solid economic reasons for breaking the rules imposed by the Regional Authorities: in such a condition of affairs, command and control regulation lacks of effectiveness if not supported by additional measures which take into account the economic nature of an activity.

In this perspective, promotion of community based fisheries and sea urchin co-management by policy makers and stakeholders – according to CFP addresses (European Commission, 2009) – can reduce the "free rider problem" and the risk of illegal behaviours. Among the diverse types of co-management, the cooperative one,

where fishers and authorities are equal partners in developing suggestions for management improvement (Nordic Council of Ministers, 2009), seems the most effective. This decisional line should be a radical reform of the Sardinian sea urchin fishery policy which, until now, has only been carried out in the top-down direction. Such a management plan is not new for edible sea urchin fisheries: a long experience of co-management in Galicia (North western Spain) is an useful source of information on the benefits and the problems arising from this approach (Fernandez-Boan et al., 2012). Also this management practice needs the help of an updated and comprehensive report of the state of sea urchins local populations.

Moreover, the fishery co-management approach could force fishermen to collaborate in promoting common management and marketing strategies. Therefore, a possible edible sea urchin overexploitation in Sardinia could be tackled only by an effective driving of the market forces (Miyata, 2010). The results reported above indicate that firms which process and package the edible sea urchin roe are significantly more profitable than the other ones. This information means that marketing policies should be aimed at promoting the quality and traceability of the product in order to achieve a higher selling price. The co-management approach to fishing and/or the cooperation between local firms could enhance the improvement of a truly market-oriented sector. Thus, promotion of high quality local brands could support this strategy. This fact implies the reinforcement of an integrated production system where fishermen (preferably grouped in Producer Organizations) and traders share the same rules, strategies and objectives.

In conclusion, it is clear that the fishing pressure on edible sea urchin populations can be reduced by supporting sea urchin farming, although some critical technical points have so far limited the development of this aquaculture practice (Kelly, 2004). Further research is therefore needed on this subject as well as on other important topics, like the

technological innovations in processing raw materials and the agronomic utilization of industrial byproducts (Garau et al., 2012; Akino et al., 2015).

4.5 Conclusions

The aim of this chapter was to examine the economic convenience of fishing the edible sea urchin *Paracentrotus lividus* in Sardinia and to assess if profitability and sustainability can be guaranteed, given the regulatory measures adopted by Regional Authorities to control the harvesting of this species. Heterogeneous findings on the economic outcomes of sea urchin fishery firms were observed. Nevertheless, empirical evidences indicate that, at present, sea urchin fishery is far to achieve both the economic (remunerative profits) and environmental (conservation of the stocks) needs.

On the basis of the issues raised by the results of this study, the regional policy makers have some margins to establish normative modifications with the purpose of increasing policies effectiveness and to find a more effective equilibrium between economic and environmental spheres of sustainability.

To the best of my knowledge, the present work is the first attempt aimed at estimating profitability, distribution of costs, and role of the fishing systems in conditioning the economic results of sea urchin harvesting activity in the Mediterranean Basin. It provides some useful economic insights on this type of fisheries and put emphasis on several policy implications, particularly with reference to the relationship between possible modification of the normative constraints on fishing and expected profits.

Nonetheless, further research in this field is needed to increase the knowledge on this topic and to support policy makers' decisions in Sardinia as well as in other Mediterranean areas.

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