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Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbé (Galicia, north-western Spain)

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Seasonal variations in the gonad index and the biochemical composition of gonad and gut tissue of the sea urchin, *Paracentrotus lividus* (Echinodermata: Echinoidea) were determined. Samples were collected from Lorbé (Galicia, north-western Spain) between November 1994 and October 1995. The gametogenic cycle of the sea urchin was annual, with a long, single spawning period from May to August 1995. Most of the biochemical components in gonad showed clear seasonal variation, which was related to the gonad cycle. The main component was protein, which ranged between 36 and 60% of the dry weight, with maximum levels coinciding with the minimum levels of glycogen. Gonad glycogen therefore seemed to be the energy source employed for protein synthesis during gametogenesis. Seasonal variation in total lipids in the gonad was less evident; the main lipidic component was triacylglycerol (around 10% of the dry weight), whereas total cholesterol accounted for less than 1%. The gut does not seem to act as a storage tissue in *P. lividus* and seasonal variation in biochemical components may be a consequence of fluctuations in the availability of food.

INTRODUCTION

The sea urchin *Paracentrotus lividus* (Lamarck) is the most abundant echinoid in the Boreo-Mediterranean zone. Commercial exploitation of this echinoid has increased in recent years, and the gonads are particularly appreciated in Japan and France. However, the wild population has been drastically depleted in some areas, due to over-exploitation (Serrao, 1988). In Spain, Galicia is the main producer, with an average rate of production of 500 tn per year between 1996 and 1998 (Catoira, 1999).

The gonad cycle of *P. lividus* has been widely studied in different regions. An annual cycle with two spawning periods has been observed in Mediterranean populations in France (Fenaux, 1968), although a single spawning period has also been described in populations from Brittany (Dominique, 1973), Ireland (Byrne, 1990) and the north-western Mediterranean (Lozano et al., 1995).

Studies related to seasonal variation in the biochemical composition and the distribution of the components in body tissues have been carried out with various species of echinoids in different environments, such as tropical (Lawrence & Guille, 1982), temperate (Fernandez, 1998) and polar (McClintock & Pearse, 1987) waters. These studies have demonstrated that the biochemical components (mainly proteins) of the gonad and gut of these echinoids vary seasonally, whereas the test mainly comprises inorganic compounds, which do not vary seasonally. The biochemical changes which take place in the sea urchin *Arbacia lixula* during starvation have also been studied (Fenaux et al., 1977), as has the effect of diet on the biochemical composition of *P. lividus* from a Mediterranean lagoon (Fernandez, 1997).

Apart from these studies, and despite the economic importance of these species, little information is available on *P. lividus* in Galicia, where only gonad index and biometric parameters of different populations have been analysed (Catoira, 1999). The aim of the present study was to investigate seasonal variation in the contents of: water, ash, proteins, carbohydrates (free glucose and glycogen) and lipids (total lipids, triacylglycerols and cholesterol) in the gonad of *P. lividus*. The possible relationships between these variables and gonad index were also investigated. The variation in these biochemical components in the gut was also estimated. Results were compared with those of previous studies carried out with sea urchins from different habitats.

MATERIALS AND METHODS

Sea urchin sampling

Sea urchins, *Paracentrotus lividus* (Lamarck), of commercial size (total wet weight 83.34 ±0.6 g) were collected from depths of 4 to 8 m by SCUBA-diving, in Lorbé (Galicia, north-western Spain). Samples of 32 animals were collected at approximately monthly intervals between November 1994 and October 1995. Animals were taken immediately to the laboratory where biometric parameters (test diameter, test height, total wet weight and gonad wet weight) were recorded; dissection was performed within 24 h to avoid spawning. The test was discarded and body components, gonads and guts separated. Gonads from each animal were damp-dried and the wet weight (WW) was determined in order to calculate the gonad index. The gonads and guts

were pooled separately, frozen in liquid nitrogen and stored at −70°C. The samples were lyophilized and then analysed to stablish the main biochemical components.

Gonad index, dry weight and ash

Aliquots (3–5 g) of lyophilized tissue were weighed and the dry weight (DW) was determined after drying at 100°C for 24 h. Ash content was obtained after combustion at 500°C for 12 h in a muffle furnace. The gonad index was calculated for each sea urchin as the gonad wet weight (gonad WW) divided by the wet weight of the intact animal (total WW) and multiplied by 100, following Fenaux et al. (1977):

$$\mathrm{GI} = \left(\frac{\text{gonad WW}}{\text{total WW}}\right) \times 100 \qquad (1)$$

Biochemical composition

Glycogen (G) was extracted according to Keppler & Decker (1984) and hydrolysed enzymatically to glucose (with amiloglucosidase from *Aspergillus niger*, Boehringer Mannheim). Glucose was estimated by a colorimetric method using a commercial enzymatic (glucose oxidase–peroxidase) kit (Glu-cinet, Diagnostics Sclavo). Total lipids (L) were extracted using chloroform: methanol (2:1), following the method of Folch et al. (1957) modified by Beninger & Lucas (1984). Total lipid content was determined gravimetrically (Bligh & Dyer, 1959). Triacylglycerols (TG) were measured using the Triglycerides GPO-PAP commercial kit (Boehringer-Mannheim), based on the method of Nägele et al. (1985). Free and total cholesterol (CH) were estimated using the FCholesterol CHOD-PAP and Cholesterol CHOD-PAP kits, respectively (Boehringer-Mannheim) following the procedure of Siedel et al. (1985). An Elemental Analyser (Carlo Erba, model 1108) was used to determine nitrogen content and a conversion factor of 6.25 was applied to give the protein (P) content (Beukema & de Bruin, 1979).

The biochemical analyses were carried out in triplicate and the results were expressed as percentages of dry weight of tissue (%DW), except for water, which was calculated as percentage of wet weight of tissue (%WW). Triacylglycerols and cholesterol levels were also expressed as percentage of total lipids (%TL).

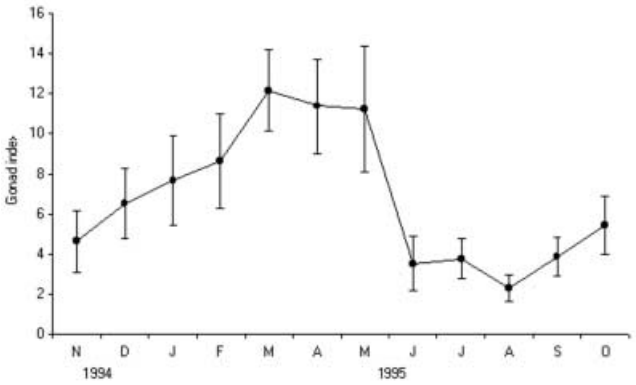

Figure 1. Seasonal changes in the gonad index in *Paracentrotus lividus*. Mean values ±SD, N=32.

Statistical analyses

Results were expressed as the mean ±standard error, except for biometric measurements and gonad index, which were calculated as the mean ±standard deviation. Data were normalized using the arcsine transformation and then compared by one-way analysis of variance (one-way ANOVA) followed by test of Tukey-B, with a significance level of 0.05 (Grant & Tyler, 1983). Prior to ANOVA, normality and homoscedasticity were verified by Kolmogorov–Smirnov and Levene tests. The degree of association between the different parameters was estimated by the Spearman rank-order correlation coefficients (r_s). Statistical analyses were performed using the SPSS statistical package (v. 6.1) for Windows.

RESULTS

Biometric measurements and gonad index

The biometric measurements made on 32 animals were recorded monthly throughout the experimental period and the corresponding mean values calculated. Mean diameter of the test (without spines) ranged between 54.5 and 60.3 mm and mean height of the test between 25.4 and 34.7 mm. Mean total WW ranged between 74.4 and 92.0 g in January and October 1995, whereas mean gonad WW ranged between 1.7 and 10.7 g in August 1995 and March 1995, respectively.

Gonad index (GI) for *Paracentrotus lividus* showed a clear seasonal variation (Figure 1). Values increased steadily from November 1994 (4.6), peaked at 12.2 in March 1995 and remained at this level for three months; GI then decreased rapidly until reaching minimum levels in June 1995 and remained at this level until August 1995 (one-way ANOVA, $P<0.05$). A new increase in the gonad index began in September 1995 (Figure 1).

Water and ash levels

Water (W) levels in the gonads of *P. lividus* exhibited a clear variation during the year ranging between 70.3 and 80.5% of WW (one-way ANOVA, $P<0.05$) (Table 1). The minimum ash content (A) of the gonad was found in December 1994 (7.3%) and the maximum in June 1995 (14.0%), the pattern of changes being similar to that observed for the water percentage (one-way ANOVA, $P<0.05$) (Table 1).

The percentage of water in the gut was higher than in the gonad, ranging between 83.1% in December 1994 and 88.4% in October 1995 (one-way ANOVA, $P<0.05$) (Table 1). Higher percentages of ash were also found in the gut, ranging from 28.6% in March 1995 and 38.1% in October 1995; these levels coincided with low values detected in the gonad (one-way ANOVA, $P<0.05$) (Table 1). Seasonal variation in ash and water content was therefore confirmed for the gonad of *P. lividus*, but was less evident in the gut.

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Table 1. *Seasonal changes in water (expressed as percentage of wet weight of tissue, %WW), ash (expressed as percentage of dry weight of tissue, %DW) levels in the gonad and in the gut of* Paracentrotus lividus. *Mean values ±SE (N=3 experimental replicates of the pooled gonads or guts of 32 individuals collected each month).*

	% Water		% Ash	
Date	Gonad	Gut	Gonad	Gut
22-11-94	70.3 ±0.1	85.0 ±0.5	7.3 ±0.2	31.9 ±1.9
26-12-94	70.4 ±0.1	83.1 ±1.1	7.0 ±0.6	30.4 ±1.2
27-01-95	74.2 ±0.1	86.3 ±0.5	8.3 ±0.3	31.3 ±0.7
28-02-95	74.7 ±0.1	85.9 ±0.4	8.3 ±0.9	28.9 ±3.0
30-03-95	76.1 ±0.1	86.1 ±0.7	9.1 ±0.8	28.6 ±2.2
28-04-95	76.9 ±0.2	86.6 ±0.2	11.1 ±0.6	33.4 ±1.7
26-05-95	78.6 ±0.2	86.9 ±0.2	12.1 ±0.5	31.2 ±2.3
28-06-95	80.1 ±0.1	85.3 ±0.1	14.0 ±1.0	30.9 ±1.3
21-07-95	80.5 ±0.1	86.4 ±0.2	12.3 ±0.5	32.2 ±1.9
28-08-95	78.2 ±0.1	88.2 ±0.2	12.4 ±0.1	33.7 ±2.3
26-09-95	73.8 ±0.1	87.3 ±0.3	10.6 ±1.0	34.5 ±1.9
30-10-95	72.7 ±0.1	88.4 ±0.4	7.8 ±0.5	38.1 ±1.7

Carbohydrate and protein levels

Glycogen and free glucose levels were determined in *P. lividus* gonad and gut. However, free glucose was impossible to detect in the samples analysed and, therefore, glycogen was considered to be the main carbohydrate component in both tissues.

Seasonal variation in glycogen (G) levels was evident in the gonad, ranging between 25.4% in December 1994 and 7% in June 1995 (Figure 2) (one-way ANOVA, $P<0.05$). Levels remained high between November 1994 and January 1995 and then fell gradually, reaching low values in June–July 1995. There was a gradual recovery with values in September–October 1995 being similar to those observed in November 1994 (Figure 2).

The glycogen levels in the gut were always lower than in the gonad (Figure 2). In the gut the glycogen content increased from November 1994 peaking at about 6% in January 1995, it then fell until reaching 3% in February then remained at this level for the rest of the experimental period (one-way ANOVA, $P<0.05$). The ratio between maximum and minimum levels in the gut was 2.7 and in the gonad, 3.6.

Protein was the major component of both, gonad and gut (Figure 2). The percentage levels ranged between 36 and 60% in the gonad, and between 24 and 42% in the gut. The level of total proteins (P) in gonads increased significantly between November 1994 (36.1%) and May 1995 (60.1%), followed by a significant decrease between May and September 1995 (one-way ANOVA, $P<0.05$) (Figure 2). Changes in gonad protein levels were similar to those of gonad index, but there was no significant correlation between these two parameters throughout the experimental period. As with glycogen, protein levels in *P. lividus* gonad, were higher than protein levels in the gut, with maximum and minimum levels, in the gut, of 42% and 24%, respectively, compared to 60.1% and 36.1% in the gonad (Figure 2). However, the ratio between maximum and minimum values was very similar in both tissues (1.6 for guts and 1.7 for gonads). Protein content of the gut increased steadily from November 1994, reaching about 42% by March–April 1995; it then fluctuated between 24 and 36% for the rest of the experimental period. The percentage levels of protein in the gut in November 1994 and October 1995 were similar (Figure 2).

Total lipid, triacylglycerol and cholesterol levels

Changes in lipid levels throughout the study period were less obvious than those of proteins and glycogen. The total lipid content (L) in the gonads increased from 12.6% in November 1994 to a maximum of 15.9% in February 1995 (one-way ANOVA, $P<0.05$) (Figure 2), then dropped until reaching 12% in April 1995. The lipid content remained constant for six months until a new increase was detected in September 1995, followed by another decrease in October 1995. Furthermore, lipid contents measured in February and in September 1995 were similar (15.9 and 16.1%, respectively) (Figure 2).

Total lipid content in the gut was lower than in the gonad as also seen for the other biochemical components. Total lipids in the gut increased steadily from December 1994 to April 1995, peaking at 9.9% and then fell to a minimum of 6.7% in June 1995 (one-way ANOVA,

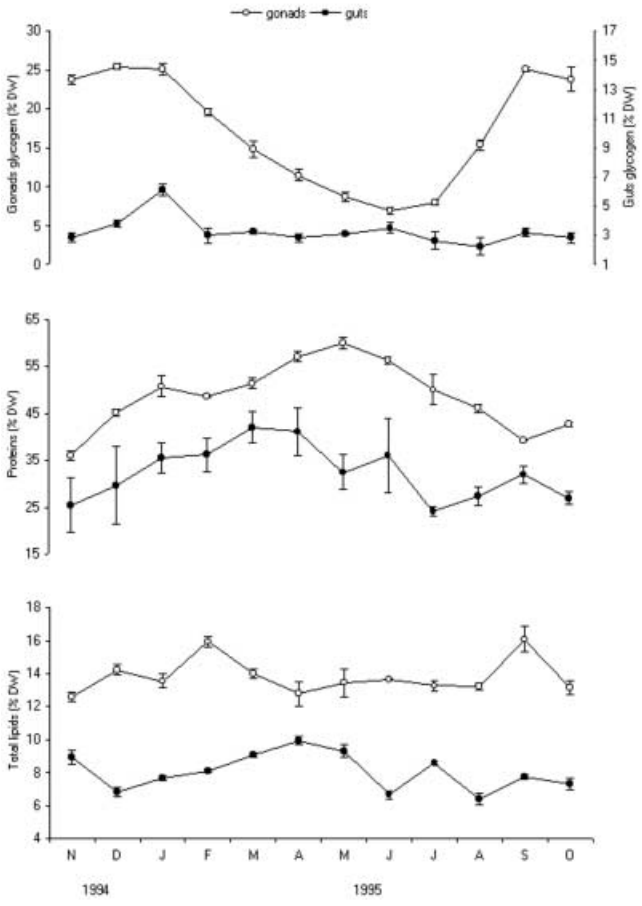

Figure 2. Seasonal changes in glycogen (G), total protein (P) and total lipid (L) levels (expressed as percentage of dry weight of tissue, %DW) in the gonad and the gut of *Paracentrotus lividus*. Mean values ±SE, (N=3 experimental replicates of the pooled gonads or guts of 32 individuals collected each month).

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$P<0.05$). The level then fluctuated between 6.7 and 7.2% throughout the remainder of the experimental period, although an unexpectedly high level of 8.6% was observed in July 1995 (Figure 2).

Triacylglycerols were the main lipid component in the gonad of the sea urchin. The maximum TG content (expressed as %DW) of the gonad was found in December 1994, the level being about 40% higher than the minimum, obtained in April–May 1995 (one-way ANOVA, $P<0.05$) (Figure 3). However, when the TG contents of gonad were calculated as %TL, the maximum levels obtained in December 1994 were 32% higher than the minimum levels, found in May 1995.

The TG content (%DW) of the gut increased rapidly from a minimum in December 1994 to a maximum of about 3.5% in May 1995 (one way ANOVA, $P<0.05$). It then dropped to a low level in June 1995, then increased over the next four months, before decreasing again in October 1995 (Figure 3). When the TG content was expressed as %TL, levels were ten times higher than when expressed as %DW, with values ranging between 27.4 in February 1995 and 42.7 in August 1995.

The TG levels in the gonads were three times (11.1% compared to 3.5%) and two times higher (78% compared to 42.7%) than in the guts of the sea urchin *P. lividus* when expressed as % DW and as %TL, respectively.

Free cholesterol was not detectable in either gonads or guts of *P. lividus*. Total cholesterol (CH) levels were ten times lower than triacylglycerol levels in the gonad (Figure 3). Total CH levels (in %DW) in gonad increased from 0.8% in November 1994 to a maximum of 1.2% in July–August 1995, and then fell, reaching about 0.6% in October 1995 (Figure 3). A similar pattern was observed for total CH in gonads when expressed as %TL. The ratio between maximum and minimum levels of total CH in the gonad was 2 (whether expressed as %DW or %TL).

An unexpected result was observed for total CH levels in the gut; CH content in this tissue was twice that in the gonad (Figure 3), in contrast to the results obtained for the other biochemical components. The maximum level of total CH (in %DW) was found in April 1995, coinciding with the maximum value for total lipids. High values of CH were also obtained in July 1995, after a short period of low levels from April to June 1995 (Figure 3). However, when these levels of CH were expressed as %TL, the minimum value corresponded to May 1995 and the maximum to August 1995. The ratio between maximum and minimum level in the gut was 1.4 (whether expressed as %TL or as %DW).

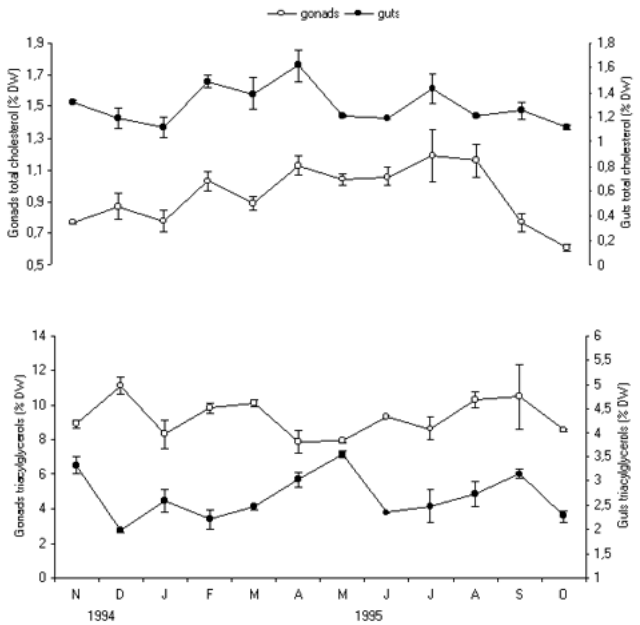

Figure 3. Seasonal changes in triacylglycerol and total cholesterol levels in the gonad and in the gut of *Paracentrotus lividus*, expressed as percentage of dry weight (%DW). Mean values ±SE, (N=3 experimental replicates of the pooled gonads or guts of 32 individuals collected each month).

Degree of association between different parameters

The Spearman correlation coefficients were obtained for comparisons between the GI and the different biochemical components of the gonad and gut of *P. lividus*.

Water content in the gonad correlated negatively with glycogen content ($r_s=-0.891$, $P<0.001$), positively with cholesterol in %DW ($r_s=0.765$, $0.001<P<0.01$) and with protein content ($r_s=0.777$, $0.001<P<0.01$). Ash levels in the gonad correlated positively with water content ($r_s=0.940$, $P<0.001$) and with total CH in %DW ($r_s=0.748$, $0.001<P<0.01$) and negatively with the glycogen level ($r_s=-0.818$, $P<0.001$). There was also a positive correlation between water and ash content of the gut ($r_s=0.662$, $0.01<P<0.05$).

There was a negative correlation between glycogen and protein levels in the gonad during the same period ($r_s=-0.713$, $0.001<P<0.01$). Although there was apparently no relationship between glycogen level and GI, it was seen that the decrease in glycogen levels in February 1995 corresponded approximately with the highest values obtained for the GI (just prior to the reproductive and spawning periods in May–June).

No significant correlation between protein content in the gonad and GI was observed, although data revealed that the maximum level of proteins occurred in May 1995, when the GI was high, and before the decrease in these levels observed during the spawning period. There was a positive correlation between the level of proteins in the gut and gonad ($r_s=0.592$, $0.01<P<0.05$) and also between GI and gut protein levels ($r_s=0.669$, $0.01<P<0.05$).

Positive correlations were found between total lipids in the gonad and glycogen in the gut ($r_s=0.583$, $0.01<P<0.05$). There was also a clear correlation between total lipids and total CH in the gut (%DW) ($r_s=0.733$, $0.001<P<0.01$). Moreover, as found for gut proteins, there was a relationship between total lipids in the gut and GI ($r_s=0.695$, $0.01<P<0.05$). Total cholesterol levels in the gonad were negatively correlated with glycogen levels ($r_s=-0.711$, $0.001<P<0.01$).

DISCUSSION

Different aspects of the gonad cycle in the sea urchin, *Paracentrotus lividus* have previously been investigated. A single annual spawning period has been described in

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populations from the Atlantic coast of France (Spirlet et al., 1998), Ireland (Byrne, 1990) and Mediterranean Spain (Lozano et al., 1995). However, two annual spawning periods have been reported in another Irish population (Crapp & Willis, 1975) as well as in a Mediterranean population (Fernandez, 1998). In Galicia, preliminary studies have shown the existence of an annual gonad cycle in *P. lividus*, with a single spawning period (Catoira, 1995).

Our results confirmed the presence of a single annual spawning in the population of *P. lividus* analysed. Spirlet et al. (1998) highlighted the similarity of the reproductive pattern in echinoids located in temperate areas, with a nutrient storage period in autumn and winter and a long spawning period during summer. Populations from northern areas show greater differences between minimum and maximum GI, probably due to higher temperature differences between summer and winter leading to greater nutrient storage during winter. The greater differences in northern compared to southern populations have also been reported for Galician populations (Catoira, 1995), with ratios between maximum and minimum GI values of 1.9 and 1.4 corresponding to northern and southern areas, respectively. The area studied here is in northern Galicia and the corresponding ratio was even higher (5.3).

In addition, it has been demonstrated that a decrease in temperature during autumn is an important factor for initiating gonad growth in Mediterranean and Irish populations of *P. lividus* (Régis, 1979; Byrne, 1990). However, an increased temperature in spring acted as a catalyst for gametogenic processes, and spawning took place just prior to when the temperatures were highest (between 13°C and 16°C) (Spirlet et al., 1998). Nevertheless, too high (24°C) or too low (8°C) temperatures inhibited spawning in Mediterranean populations of *P. lividus* (Fenaux, 1968). Spawning induction was observed with an increase in water temperature of 5°C (data not shown). However, further studies are necessary to confirm the influence of temperature.

Food availability is another important factor in gonad development. It has been demonstrated that sea urchins located in intertidal areas, where food is limited, had smaller gonads than sea urchins from subtidal areas, where food is abundant (Byrne, 1990). The population studied here came from an intertidal area with abundant food available, and therefore high GI values were expected.

Levels of biochemical components

The biochemical composition of the gonad and gut of the sea urchin, *P. lividus*, from Galicia is similar to that observed in other Echinoidea species. McClintock & Pearse (1987) also observed that the pattern of biochemical composition in the Echinoidea, was independent of species and geographical location. Protein appears to be the main component in animals belonging to this class, whereas lipid and glycogen levels are always lower.

The water content of *P. lividus* gonads was similar to that observed by Giese (1966) in gonads of *Strongylocentrotus purpuratus*. However, the gut water content was slightly higher in *P. lividus* than in *S. purpuratus* (Giese, 1966). Ash percentage in gonad tissue of *P. lividus* (between 7.0 and 14.0%) is in the same range found by Fernandez (1998) for the same species (between 5 and 10%). However, the ash content of the gut was four times higher than that of the gonad of *P. lividus*, and much higher than those measured in similar tissues of different echinoid species (Fenaux et al., 1975; Fernandez, 1998).

Glycogen is mainly stored in the gonads of sea urchins and in the gonads and pyloric caecae of sea stars. It seems likely that stored glycogen is used as a primary energy source in ovogenesis (Khotimchenko et al., 1988). Minimum levels of glycogen in the gonad of *P. lividus* were similar to those found in other species (about 5%) (Giese, 1966; Fenaux et al., 1975; McClintock & Pearse, 1987). However, maximum levels were 60% higher than those measured in the same species from a lagoon environment (Fernandez, 1998) and five times higher than that measured in the same tissue of *Arbacia lixula* (Fenaux et al., 1975). On the other hand, glycogen levels in the gut were 3–4 times lower than in the gonad, as previously observed in other echinoid species. These results contrast with those of a previous study, by Fernandez (1998) in which similar levels of glycogen were observed in the gonad and the gut, as a consequence of the use of the gut as a reservoir organ in conditions of limited food (lagoonal environment). In the present study, the *P. lividus* specimens were collected from an area of open sea where food was available and the gut should not have been used as a nutrient storage tissue.

Protein content in *P. lividus* was similar to other echinoids. Protein levels ranged between 36.0 and 60.1% in the gonad and between 24.1% and 42.0% in the gut of *P. lividus*. Proteins were found to be the main component of *P. lividus*, and their structural role in the sea urchin was confirmed. Such a role has been suggested in the sea star *Asterias rubens* (Jangoux & van Impe, 1977) because of a decrease in the protein level observed in the pyloric caecae when gonad demand for structural material, e.g. amino acids, was high.

Seasonal variation in total lipids was less evident than for the other biochemical components. Total lipid levels in the gonad of *P. lividus* were similar to those of other echinoids, whereas the levels in the gut were between two and three times lower (Fenaux et al., 1975; Serrazanetti et al., 1995).

It has been suggested that triacylglycerols are the main storage lipids in *A. rubens* (Oudejans & van der Sluis, 1979), as they are used for gametogenic and gonad growth purposes. We found higher levels of triacylglycerols in the gonad than in the gut, thus confirming the role of the gonad as a nutrient storage organ in *P. lividus*.

Seasonal variation in biochemical components, their inter-relationships and association with the reproductive cycle

There was clear seasonal variation in glycogen levels in the gonad of *P. lividus*, with the maximum in December 1994 and a minimum in June 1995, whereas gut levels remained fairly constant. Glycogen storage in gonads during gametogenesis has previously been observed in sea urchins (Fenaux et al., 1977; Fernandez, 1998), there being a clear relationship between GI and glycogen content. In the present study, this relationship was not so clear, but it was found that the decrease in gonad glycogen levels from the maximum in December 1994 coincided with the increase of the GI to a maximum level, between March

and May 1995. This inverse relationship may indicate the use of glycogen as a primary source of energy for gonad growth and gametogenesis. However, there was no relationship between glycogen in the gut and the reproductive cycle, probably because of the small proportion of gut compared to gonad tissue (Lawrence & Lane, 1982). Moreover, glycogen in the gut accounted for only a small percentage of total organic matter, and possibly only glycogen from intermediary metabolism, or with a structural role, were involved (Bishop & Watts, 1992).

Protein levels in gonad tissue exhibited significant variations throughout the period of study, ranging between 36.0% in November 1994 and 60.10% in May 1995. Maximum levels of proteins in the gonad coincided with the highest GI values, there being evidence of gonad protein requirements during gametogenesis, as previously observed in *Arbacia lixula* (Fenaux et al., 1977) and in *P. lividus* (Fernandez, 1998). It is also important to point out the inverse relationship between glycogen and total proteins in the gonad of *P. lividus*, which confirms the use of glycogen storage to obtain the energy for gamete production. Similar profiles were obtained for protein in the gut and the gonad of *P. lividus*, with maximum levels in spring, when algae availability was high and gametogenesis occurred.

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REFERENCES

Beninger, P.G. & Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* (Jeffreys) and *Tapes philippinarum* (Adams and Reeve). *Journal of Experimental Marine Biology and Ecology*, **79**, 19–37.

Beukema, J.J. & Bruin, W. de, 1979. Calorific values of the soft parts of the tellinid bivalve *Macoma balthica* (L.) as determined by two methods. *Journal of Experimental Marine Biology and Ecology*, **37**, 19–30.

Bishop, C.D. & Watts, S.A., 1992. Biochemical and morphometric study of growth in the stomach and intestine of the echinoid *Lytechinus variegatus* (Echinodermata). *Marine Biology*, **114**, 459–467.

Bligh, E.G. & Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

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

Catoira, J.L., 1995. Spatial and temporal evolution of the gonad index of the sea urchin *Paracentrotus lividus* (Lamarck) in Galicia, Spain. In *Echinoderm Research 1995. Proceedings of the Fourth European Echinoderms Colloquium, London, United Kingdom* (ed. R. Emson et al.), pp. 295–298. Rotterdam: A.A. Balkema.

Catoira, J.L., 1999. Outros recursos e outras economías, ourizos. In *Cultivando o mar, a acuicultura do milenio* (ed. J. Fernández et al.), pp. 47–60. Spain: A Coruña.

Crapp, G.B. & Willis, M.E., 1975. Age determination in the sea urchin *Paracentrotus lividus* (Lmk), with notes on the reproduction cycle. *Journal of Experimental Marine Biology and Ecology*, **20**, 157–178.

Dominique, F., 1973. *Contribution a l'étude du cycle annuel de reproduction de deux espèces d'echinoides (echinodermata) des côtes de Bretagne.* BSc thesis, Université Libre de Bruxelles, Belgium.

Fenaux, L., 1968. Maturation des gonades et cycle saisonnier des larves chez *A. lixula*, *P. lividus* et *P. microtuberculatus* (echinides) a Villefranche-sur-Mer. *Vie et Milieu*, **19**, 1–52.

Fenaux, L., Malara, G. & Charra, R., 1975. Effets d'un jene de courte durée sur les principaux constituants biochimiques de l'oursin *Arbacia lixula*. I. Stade de repos sexuel. *Marine Biology*, **30**, 239–244.

Fenaux, L., Malara, G., Cellario, C., Charra, R. & Palazzoli, I., 1977. Evolution des constituants biochimiques des principaux compartiments de l'oursin *Arbacia lixula* (L.) au cours d'un cycle sexuel et effets d'un jeune de courte durée au cours de la maturation sexuelle. *Journal of Experimental Marine Biology and Ecology*, **28**, 17–30.

Fernandez, C., 1997. Effect of diet on the biochemical composition of *Paracentrotus lividus* (Echinodermata: Echinoidea) under natural and rearing conditions. *Comparative Biochemistry and Physiology*, **118A**, 1377–1384.

Fernandez, C., 1998. Seasonal changes in the biochemical composition of the edible sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. *Marine Ecology*, **19**, 1–11.

Folch, J., Lees, M. & Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497–509.

Giese, A.C., 1966. Some methods for study of the biochemical constitution of marine invertebrates. *Oceanography and Marine Biology. Annual Review*, **5**, 159–186.

Grant, A. & Tyler, P.A., 1983. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices. *International Journal of Invertebrate Reproduction*, **6**, 259–269.

Jangoux, M. & Impe, E. van, 1977. The annual pyloric cycle of *Asterias rubens* L. (Echinodermata: Asteroidea). *Journal of Experimental Marine Biology and Ecology*, **30**, 165–184.

Keppler, D. & Decker, K., 1984. Glycogen. In *Methods of enzymatic analysis*, vol. VI (ed. H.U. Bergmeyer), pp. 11–18. Weinheim: Verlag Chemie (VCH).

Khotimchenko, Y.S., Zalutskaya, E.A., Deridovich, I.I. & Kreimer, D.I., 1988. Glycogen synthase activity of the gonad of the sea urchin *Strongylocentrotus intermedius* and the starfish *Asterias amurensis* (Echinodermata). *Comparative Biochemistry and Physiology*, **89B**, 9–13.

Lawrence, J.M. & Guille, A., 1982. Organic composition of tropical, polar and temperate-water echinoderms. *Comparative Biochemistry and Physiology*, **72B**, 283–287.

Lawrence, J.M. & Lane, J.M., 1982. The utilisation of nutrients by post-metamorphic echinoderms. In *Echinoderm nutrition* (ed. M. Jangoux and J.M. Lawrence), pp. 331–371. Rotterdam: A.A. Balkema.

Lozano, J., Galera, J., López, S., Turon, X., Palacín, C. & Morera, G., 1995. Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series*, **122**, 179–191.

McClintock, J.B. & Pearse, J.S., 1987. Biochemical composition of antarctic echinoderms. *Comparative Biochemistry and Physiology*, **86B**, 683–687.

Moss, J.E. & Lawrence, J.M., 1972. Changes in carbohydrates, lipids and proteins levels with age and season in the sand dollar *Mellita quinquiesperforata*. *Journal of Experimental Marine Biology and Ecology*, **8**, 225–239.

Nägele, U., Wahlefeld, A.W. & Ziegehorn, J., 1985. Fatty acids and derivates. Tryglicerids. In *Methods of enzymatic analysis*, vol. VIII (ed. H.U. Bergmeyer), pp. 3–18. Weinheim: Verlag Chemie (VCH).

Oudejans, R.C.H.M. & Sluis, I. van der, 1979. Storage and depletion of lipid components in the pyloric caeca and ovaries of the seastar *Asterias rubens* during its annual reproductive cycle. *Marine Biology*, **53**, 239–247.

Régis, M.B., 1979. Analyses des fluctuations des indices physiologiques chez deux échinoïdes (*Paracentrotus lividus* (Lmk) et *Arbacia lixula* (L.) du Golfe de Marseille. *Thétys*, **9**, 167–181.

Serrao, E., 1988. Contribuçào para conhecimento da distribuçao e cartografía dos ouriços (Echinoïdea) a Norte do Cabo Espiches. *Recursos Faunísticos Marinhos Portugeses*, **II**, 1–80.

Serrazanetti, G.P., Pagnucco, C. & Cattani, O., 1995. Hydrocarbons, sterols and fatty acids in sea urchin (*Paracentrotus lividus*) of the Adriatic Sea. *Chemosphere*, **30**, 1453–1461.

Siedel, J., Wolfgang, R., Röschlau, P. & Zierenhorn, J., 1985. Cholesterol. In *Methods of enzymatic analysis*, vol. VIII (ed. H.U. Bergmeyer), pp. 139–149. Weinheim: Verlag Chemie (VCH).

Spirlet, C., Grosjean, P. & Jangoux, M., 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*. Analysis by means of the maturity index. *Invertebrate Reproduction and Development*, **34**, 69–81.

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