Gonadal growth and reproduction in the sea urchin

*Sphaerechinus granularis* (Lamarck 1816)

(Echinodermata: Echinoidea) in southern Spain

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SUMMARY: The gonadal index and reproductive state of the sea urchin *Sphaerechinus granularis* were studied for a year in three populations from southeast Spain. The development of the gonad during the period of study was assessed using histological methods and four maturity stages were considered for female specimens and two for male specimens. The study of gonad development showed a clearly defined annual cycle of gametogenesis with a single spawning period in summer-autumn. It begins in June at Torremuelle and Palmeral and a month later at La Herradura. The three populations exhibited similar reproductive patterns, having mature gonads in almost all the months. Though the environmental conditions were similar, the population from La Herradura had the highest Gonadosomatic Index value (GSI) and that from Torremuelle the lowest one.

Keywords: Echinodermata, Echinoidea, gonadosomatic index, *Sphaerechinus granularis*, reproductive cycle, sea urchin.

RESUMEN: Crecimiento gonadal y reproducción del erizo de mar *Sphaerechinus granularis* (Lamarck, 1816) (Echinodermata, Echinoidea) en el sureste de España. – Se ha estudiado el índice gonadosomático y los estados reproductivos del erizo de mar *Sphaerechinus granularis* durante un año en tres poblaciones del sureste de España. El desarrollo de la gónada se estudió usando métodos histológicos; además, el estado de los individuos se determinó utilizando cuatro categorías de desarrollo gonadal para las hembras y dos para los machos. Existe un único periodo de puesta a lo largo del año, entre verano y otoño. En las estaciones de Torremuelle y Palmeral la puesta comienza en junio mientras que en La Herradura tiene un retraso de un mes. Las tres poblaciones muestran patrones reproductivos semejantes y se caracterizan por tener gónadas maduras casi todos los meses, aunque en proporciones distintas. Los valores del índice gonadosomático son mayores en La Herradura y menores en Torremuelle a pesar de que las condiciones ambientales son similares.

Palabras clave: ciclo reproductivo, Echinodermata, Echinoidea, índice gonadosomático, erizo de mar, *Sphaerechinus granularis*.

INTRODUCTION

The echinoid *Sphaerechinus granularis* (Lamarck, 1816) (Toxopneustidae) is a species which occurs from the French coast of the western English Channel in the north to the Gulf of Guinea in the south (Köhler, 1921) as well as in the Mediterranean Sea (Tortonese, 1965). It is found from the intertidal zone to 130 m depth (Cherbonnier, 1958) in varied habitats. Its density is variable, but rarely high. In the English Channel density is often low (Cabioc, 1968). However, in southern Brittany density is higher than 50 per m²; in this area several studies dealing with the reproductive biology, growth and feeding behaviour of *Sphaerechinus granularis* have been made (Guillou and Michel, 1993, 1994;

The sea urchin’s feeding behaviour has been studied on the European coasts of the Mediterranean Sea (Fenaux, 1972; Ballesteros, 1981; Verlaque, 1981; Campos-Villaca, 1984) and its age and growth were briefly investigated in the Algerian coast (Soualili *et al.*, 1999). *Sphaerechinus granularis* is a grazer consuming mostly encrusting coralline algae on rocks (Verlaque, 1981; Sartoretto and Francour, 1997). In meadows of *P. oceanica, S. granularis* also consumes dead seagrass leaves and their algal epiphytes, probably from the litter. It also grazes on the rhizomes and roots of *P. oceanica* when these are accessible (Verlaque, 1981; Campos-Villaca, 1984). In the Mediterranean Sea, it sometimes shares its rocky habitats with other sea urchins such as *Arbacia lixula* and *Paracentrotus lividus*.

With respect to the reproductive cycle, a single spawning period in spring has been observed (Guillou and Michel, 1993; Guillou and Lumingas, 1998). However, other sea urchins such as *P. lividus* have one or two spawning periods (Lozano *et al.*, 1995; Martínez *et al.*, 2003; Sánchez-España *et al.*, 2004). This variability may be related to environmental factors such as temperature or quantity and quality of food (Guettaf *et al.*, 2000; Bayed *et al.*, 2005; Sellen and Guillou, 2007.)

*Sphaerechinus granularis* is a valuable product for the fisheries industry of the Mediterranean coast, so the knowledge of the reproductive cycle will be useful for the management of the sea-urchin fisheries and the establishment of regulatory measures that the Andalusian government would like to introduce. The present study aims to provide basic data on gonad development over an annual cycle at three localities of the southern Spanish Mediterranean coast on the shores of Andalusia.

**MATERIAL AND METHODS**

**Sampling sites**

Specimens were collected from December 2000 to November 2001 at three different localities: Torremuelle (36°34′39″N 4°34′02″W), La Herradura (36°44′14″N, 3°45′29″W) and Palmeral (36°49′17″N, 2°26′28″W) (Fig. 1). All sampling stations were located at 3-10 m depth.

Torremuelle site: The population of *S. granularis* was located at 4-5 m depth. There are large rocky blocks over sandy bottoms. The rocks have smooth surfaces covered mainly by the antozoan *Anemonia sulcata*. Algae are not abundant (*Halopteris scoparia*, crustose coralline algae). The oceanic currents and swell are not strong. Extractive human activity is very low. *Arbacia lixula* and *Paracentrotus lividus* are also abundant at this locality.

La Herradura site: The sampling station was located at depths between 5 and 10 m. It is a steep zone and the bottom is mainly rocky with sandy patches. It is a sheltered area, exposed to low hydrodynamic stress. The assemblages are dominated by seaweeds, mainly *Corallina elongata* and some brown algae, although the latter are scarce. *A. lixula* and *P. lividus* are also abundant.

Palmeral site: This sampling station was situated at 5-10 m depth. Close to this zone there is a small and discontinuous stream. This community consists of a sandy bottom with stone barriers in which *P. lividus* and *A. lixula* are very abundant. The specimens are easily accessible but on the southeastern Spanish coast, the demand for sea urchin gonads is low, which prevents their depletion. On the sandy bottom, beds of the seagrasses *Posidonia oceanica*, *Cymodocea nodosa* and *Zostera* sp. are found. The rocky surface is covered by brown algae.

Adult specimens were collected monthly between December 2000 and November 2001 at Torremuelle, La Herradura and Palmeral. In October and November it was not possible to collect animals at Palmeral because of unfavourable weather. On each sampling...
occasion, 20 specimens were collected randomly by two Scuba divers and brought back to the laboratory alive. Measurements were taken of height (oral-aboral axis), diameter (perpendicular to the oral-aboral axis) and wet weight of the animals. Specimens were then dissected and the five gonads were weighed and fixed in 10% formaldehyde for histological studies. The gonads were dehydrated in alcohol, embedded in paraffin wax and divided into sections of 7 µm, which were stained with hematoxylin and eosin. The sex and development stage were then determined. Throughout the study we always found a sex ratio of 3:2 or 3:3.

Maturity levels of the gonads were estimated according to stages established by Lozano et al. (1995) and Sánchez-Españo et al. (2004), which included four phases of development in the female and two in the males (Fig. 2).

Fig. 2. – Histology of the gonads of Sphaerechinus granularis. Males: A M0, spent condition; B M1, mature gonad with sperm. Females: C, H1, spent condition; D, H2 phase of oocyte maturation; E, H3, mature state with ova; F, H4, phase of gonadal reconstitution with maximum thickness of the nutritive layer. AW, ascinal wall; NP, nutritive phagocytes; O, mature ova; OO, oogonia; OP, primary oocytes; RO, relict ova; S, spermatozoa; SG, spermatogonia.
Female stages:

- Stage I (H1): spent stage. The ascini are devoid of relict gametes and the ascinal walls are thin, but the lumen is either empty or filled with nutritive phagocytes. The primary oocytes are either very small in number or absent along the ovary wall.

- Stage II (H2): phase of oocyte maturation. With the onset of vitellogenesis the primary oocytes increase in size. They still remain attached to the ascinal walls and are surrounded by nutritive phagocytes. Larger primary oocytes start migrating towards the centre of the ascinus and displace the nutritive phagocytes.

- Stage III (H3): mature stage with ova. This is a prespawning stage. Ovaries are filled with closely-packed ova. The nutritive phagocytes form a thin layer along the ascinal wall.

- Stage IV (H4): gonad reconstitution with maximum thickness of the nutritive layer. The ova are loosely packed with spaces vacated by spawned ova. The ascinal wall is almost devoid of cells and very thin.

Male stages:

- Stage I (M1): having sperm. Mature testes are packed with spermatozoa and the nutritive phagocytes are either absent or limited to the periphery.

- Stage 0 (M0): lacking sperm. Spent testes devoid of relict gametes and the nutritive phagocytes are around the periphery.

The Gonadosomatic Index (GSI) was calculated as: GSI= 100 x wet weight of gonads / wet weight of whole animal. As no difference was detected between males and females using a t-test (p<0.05), all the GSI data were grouped.

Statistical analyses were performed mainly by one- and two-way ANOVA for unbalanced designs and post-hoc Tukey tests. Prior to parametric tests, the assumption of normality was tested by the Kolmogorov-Smirnov test. No transformation of the data was necessary. STATISTICA for Windows (v 4.2) and Jandel SYGMASTAT (v 2.0) software were used for the analyses.

RESULTS

Gonadosomatic Index (GSI)

The relationship between GSI and size (diameter) (all months pooled) is presented in Figure 3. The lowest value of GSI was observed in the 41-50 mm size class at Torremuelle, while the highest value was observed in the 71-80 mm size class at La Herradura. La Herradura showed the highest values for the size classes comprised between 40 and 80 mm, while Torremuelle showed the lowest values in all size classes.

GSI increased with body size at Palmeral, whereas at La Herradura the values were very similar in the three first classes (41-50, 51-60, 61-70). At these stations the largest specimens belonged to 81-90 and 71-80 mm size classes, respectively. The GSI value at Torremuelle was very low in the smallest size class (41-50) but it showed a clear increase in the 51-60 mm size class. Then it reached a plateau until the 81-90 mm size class, before finally decreasing in the 91-100 mm size class.

The temporal trend of GSI for each station is presented in Figure 4. Torremuelle showed the lowest GSI values in the study period while La Herradura showed the highest ones. The GSI tended to increase from December to June at Palmeral and Torreme-
lle, although at the latter station there was a decrease in March and April. The index decreased in July at both localities, indicating that spawning had occurred. Nevertheless it showed a second increase in September at Palmeral, maintaining its low values at Torremuelle. At La Herradura the GSI values increased until July and then decreased until October.

Comparison of GSI (both sexes combined) across localities by 1-way ANOVA showed a statistically significant difference (p<0.001). Furthermore, all pairwise multiple comparison procedures (Tukey test) indicated that there were also significant differences (p<0.001). To determine whether there were significant effects among the months for each locality, one-way ANOVA was applied to the three stations. All localities showed a significant effect (p<0.005 at Torremuelle, p<0.001 at La Herradura, p<0.05 at Palmeral). Tukey tests among each month revealed that at La Herradura October differed from January, April, July, August and November, and September differed from November. July and November were the months with the highest GSI, while October was the month with the lowest value. At Palmeral, September, which had the highest value, differed from December and July, both with the lowest values. Tukey tests showed that at Torremuelle June differed from November. The GSI reached the maximum values between May and June at this station.

Gonadal cycle

Histological observations of the gonads over an annual cycle were made for all sites, and four stages for the females and two stages for the males were distinguished. Male mature gonads with spermatozoa (M1) were found at each locality at all times. At La Herradura the stage M0, lacking gametes, was not observed. At Palmeral this stage was most abundant during December and March and at Torremuelle from October to December and from April to May. Immature specimens (lacking gonads) were found at Palmeral from January to March and in August (Fig. 5-7). These individuals showed a small size, below 30 mm.

The cycle began for females when the ascini were devoid of relict gametes (spent stage H1). At La Herradura and Torremuelle this process occurred in autumn, while at Palmeral it was later, during the winter. At Palmeral this spent stage was also present in July and at Torremuelle in April. During winter–early spring the primary oocytes grow due to the onset of vitellogenesis (stage H2). At La Herradura and Palmeral this stage was frequent in July and September respectively and at Torremuelle between June–October. The mature stage (H3) appeared during the whole year but at Torremuelle it was most
frequent from spring to late summer (May-September). Finally, the phase of gonad reconstitution with maximum thickness of the nutritive layer (H4) occurred frequently in October and January at La Herradura and Palmeral, respectively. At Torremuelle it took place from October to January and from May to July although it was less important during this latter period.

**DISCUSSION**

The reproductive cycle of the sea urchin *S. granularis* has been well described on the French Atlantic coast (Guillou and Michel, 1993; Guillou and Lumingas, 1998). It has been studied in the Mediterranean Sea by Fenaux (1972), who obtained data about spawn period but none about growth or size. In the present study the changes in the GSI and the gonad stage of *S. granularis* at three localities of the Mediterranean coast of southeastern Spain were analysed for a period of one year.

*Sphaerechinus granularis* has a reproductive cycle with a single spawning in summer-autumn. It began in June at Torremuelle and Palmeral, and one month later at La Herradura. At Palmeral it took place between June and August and at Torremuelle between July and November, indicating that at the latter station the spawning event lasted longer. For the populations at La Herradura, the spawning lasted from July to October. This pattern resembles the cycles of other sea urchins such as *Echinus esculentus* (Nichols et al., 1985), *Diadema antillarum* (Hernández et al., 2006) and *P. lividus* (Byrne, 1990; Lozano et al., 1995; Spirlet et al., 1998, in British waters and Sánchez-Españo et al., 2004, in Spanish waters). *Sphaerechinus granularis* also has a single spawning season in Brittany (Guillou and Michel, 1993; Guillou and Lumingas, 1998). However, these authors state that spawning occurred in early spring. They observed that the gonadal index had a peak in spring (from March to May, depending on the year) before dropping to a minimum, which occurred in July-August. The spring decrease in the GSI values was attributed to changes in the composition of the gonad, in which the nutritive material is used and replaced by mature gametes, which are released at spawning. On the other hand, the cycle of the same species in the Mediterranean Sea (Fenaux, 1972) shows two spawning events (a small one in late spring and a second one in late summer). Some studies state that the reproductive cycle of *P. lividus* in the Mediterranean Sea also has two spawning events (Fenaux, 1968; Régis, 1979; Semroud and Kada, 1987; Fenaux and Pedrotti, 1988). However, Lozano et al. (1995) and Sellem and Guillou (2007) criticize this assertion. These authors found several GSI increases throughout the year but they described a single breeding season of *P. lividus* on the Tunisian coast. They, like other authors (Byrne, 1990; Guillou and Michel, 1993; King et al., 1994), relate these variations to changes in gonad reserves rather than changes in gamete release. At Torremuelle the GSI tendency suggests the possibility of two spawning events but, as has been pointed out previously, some GSI peaks appear to have no relation with the gametogenic cycle. Guillou and Michel (1993) found a second decrease in the French Atlantic coast population of *S. granularis* in December and February but they also thought that it did not reflect a spawning event.

Thus, the annual cycle of *S. granularis* is similar to the cycle described by Guillou and Michel (1993, 1994) and Guillou and Lumingas (1998) with a breeding season with a gonad growth which extends from autumn to early spring followed by the spawning period (from March to May), and finally a post-spawning recovery. In the Mediterranean Sea the mature
one of them showed

La herradura. this peak coincides with those of the populations of *P. lividus* at La Herradura studied by Martínez et al. (2003). The highest GSI values of *S. granularis* were reached in April and September at Palmeral and from May to June at Torremuelle. This is probably due to the availability of food. Pearse and Cameron (1991) stated that although food quantity and quality affect gonad size, these nutritional factors do not initiate gametogenesis. On the other hand, Pearse (1969) suggested that gametogenesis needed to attain a “critical level” of nutrients within the storage tissues to ensure gamete growth.

With respect to the relation between GSI and age, previous studies on *Strongylocentrotus droebachiensis* (Munk, 1992; Meidel and Scheibling, 1998) and *Strongylocentrotus purpuratus* (Gonor, 1972) have reported that the gonadal index increased exponentially in small sea urchins and reached an asymptote in larger individuals. Gonor (1972) and Lumingas (1994), however, stated that the relationship between the relative weight of the gonads and individual size shows a positive slope. Furthermore, in *S. granularis*, several studies (Guillou and Michel, 1993; Jordana et al., 1997; Guillou and Lumingas, 1998) have indicated that the growth of this species is faster during the first years of life than that of other temperate sea urchins. The decrease in reproductive effort is common in all echinoderms and could be due to the decrease in feeding activity and metabolism with age. This is consistent with our results at Torremuelle.

Besides the age, there are other factors which influence gonad growth, such as temperature, hydrodynamics and the quantity and quality of food (Fuji, 1967; Ebert, 1968; Vadas, 1977; Harrold and Pearse, 1980; Larson et al., 1980; Lawrence and Lane, 1982; Thompson, 1982; Byrne, 1990; Edwards and Ebert, 1991; Kenner, 1992; Hernández et al., 2006). In fact, several studies have demonstrated that food plays an important role in echinoid growth even for populations located only a short distance from each other. Hernández et al. (2006), observed that in two populations of *D. antillarum* one of them showed higher tests because of the availability of food. Under scarce food conditions, both somatic and gonad growth decrease and can lead to a reduction in test diameter and gonad volume due to re-absorption of material from both tissues (Ebert, 1967, 1968; Dix, 1972; Régis, 1979; Thompson, 1982). Guillou et al. (1999) observed that in sea urchin food-limited populations of *S. granularis*, where gonad production was low and the reproductive competence showed a delay, there was an increasing gonadal growth with age. This could be the case at La Herradura and Palmeral.

On the other hand, in food-rich habitats, the growth of the gonad decreases with age. Buchanan (1966) and Sivertsen and Hopkins (1995) showed in *S. droebachiensis* that specimens in habitats with abundant food attain maturity at a smaller size than in habitats with low ration. Thompson (1982), in a study of *S. droebachiensis*, suggested that allocation of energy to reproduction at the expense of somatic growth was an appropriate response when food was scarce, i.e. growth to a large size is only possible with energy surplus to reproduction (Thompson, 1982). Lozano et al. (1995) showed that the gonadal index of a population of *P. lividus* living under unstable conditions (with strong hydrodynamics, the proximity of a river mouth and highly fluctuating salinity and temperature) was higher than that of a population living in a more stable environment. In more sheltered habitats the individuals can delay the attainment of maturity to support somatic growth before the onset of reproductive activities while at exposed localities the sizes remain low. This contributes to larger sizes in the population living under stable conditions. However, Martínez et al. (2003) and Sánchez-España et al. (2004) reported that *P. lividus* from an unstable environment had higher GSI and sizes than from a stable habitat. This difference is possibly due to different quality of the seaweeds. In our study, although the environmental conditions were similar at La Herradura and Torremuelle (low hydrodynamics, low extractive human activity and low abundance of algal food due to dominance of crustose coralline algae), La Herradura showed the highest GSI values while Torremuelle showed the lowest ones.

Kelly et al. (1998) studied the populations of *Psammechinus miliaris* from Loch Fyne (Scotland). They reported that the food availability and quantity affect GSI and an increase in nutrients, even in winter, results in a rapid increase in gonad mass. This probably occurs at La Herradura and Palmeral during autumn, where weather conditions (rainfall)
could supply an input of food (i.e. drifting algae, dead leaves of *Posidonia oceanica*, rhizomes, etc., are always more abundant after storms). The increase of GSI in July at Palmeral was probably due to a summer storm.

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