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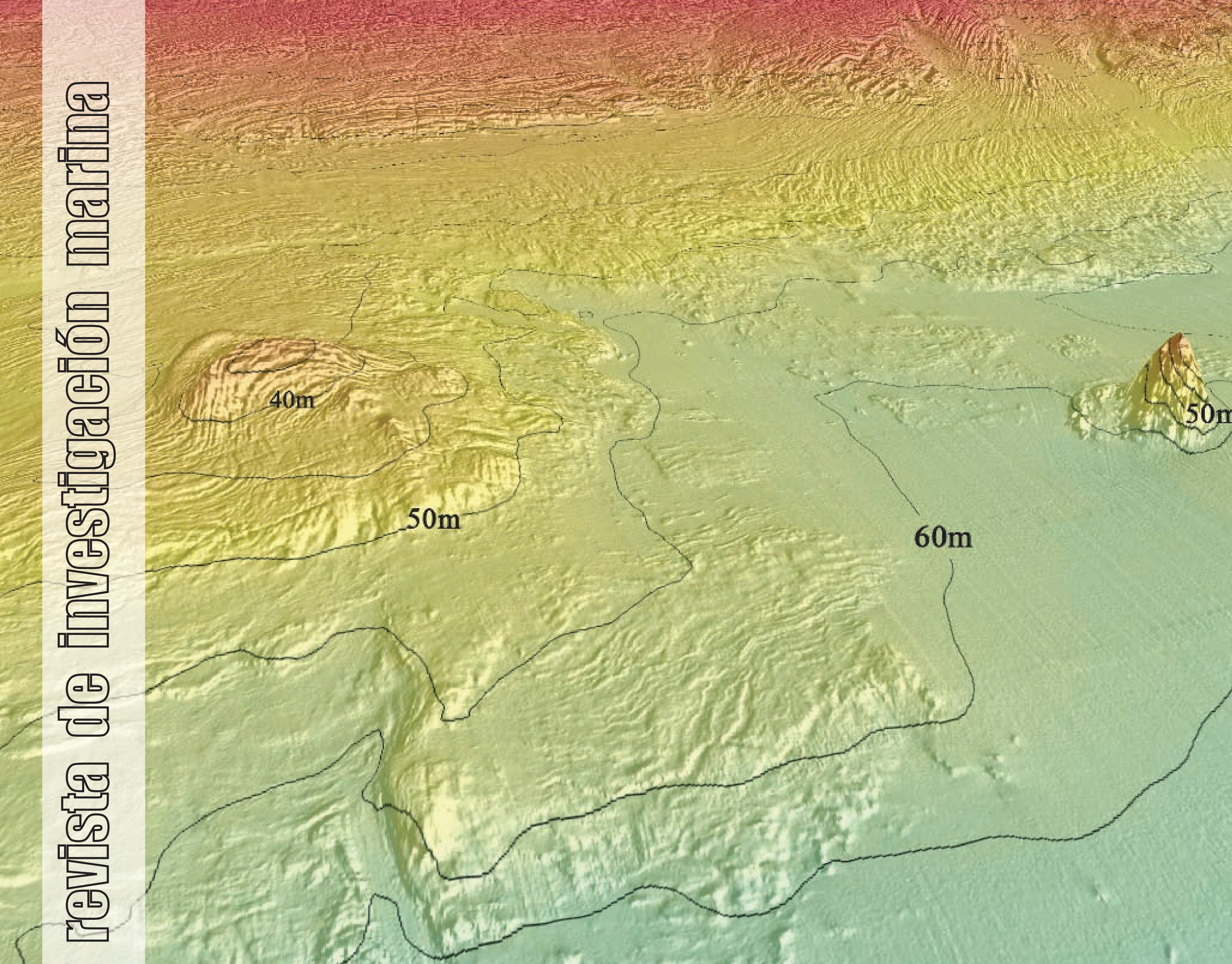

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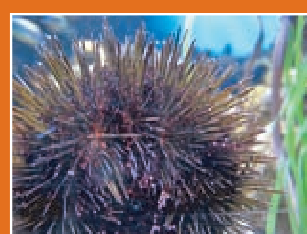

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Investigation on the annual availability of gametes in two populations (south-eastern Bay of Biscay, Spain) of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) for toxicity tests

Iratxe Menchaca*, María Jesús Belzunce, Javier Franco, Joxe Mikel Garmendia

Abstract

The reproductive cycle of the sea urchin, *Paracentrotus lividus* (Lamarck 1816), was studied on the Basque coast (innermost part of the Bay of Biscay), to establish the temporal availability and the quality of gametes to be used in routine toxicity tests. Samples were collected monthly from January to December 2007, at 2 relatively adjacent locations; these differ in their wave exposure and algal communities. According to one year results, spawning took place at the end of winter. The maturity period was longer and embryogenesis success was higher at the more sheltered site. Factors such as hydrodynamic conditions and food type could explain the observed differences in the reproductive cycles, between the locations. The sheltered site appears to be a suitable locality to sample the sea urchins, necessary for routine bioassays; this is related to the good quality and availability of gametes for almost the whole of the year.

Keywords

Basque coast; Gametes maturity; Gonads; *Paracentrotus lividus*; Reproductive cycle

Introduction

The sea urchin, *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: Echinoidea) is very abundant along the southern European coasts (Reboreda 1994; Barnes et al. 2001; Lustres 2006); its ecological and commercial importance is well-known. On the one hand, its grazing activity controls the macrophytic structure and biomass; such as it is considered a key species for the maintenance of equilibrium of sublittoral communities (Sala et al. 1998). In contrast, it is a prized food for its roe (Barnes et al. 2001), although the use of destructive harvesting methods has collapsed its fisheries in some countries (Andrew et al. 2002; Berkes et al. 2006; Pais et al. 2007).

Bioassays on the embryonic phase of this species are performed frequently to evaluate the toxicity of the contaminants present in marine waters and sediments; this is in relation to its large geographic distribution, easy manipulation, simplicity for achieving gametes and for performing in vitro fertilisation and the high toxicological sensitivity of its larvae (Casado-Martínez et al. 2006; Arizzi et al. 2006; Marín et al. 2007; Beiras and Saco 2006). In fact, such bioassays have been included in several guidelines for assessing toxicity in marine environments (e.g. Environment Canada 1992; USEPA 2002; Garmendia et al., 2009).

In order to undertake routine toxicity bioassays tests, an appropriate knowledge of the seasonality of gametes in the field is necessary. In Atlantic and Mediterranean waters, the reproduction cycle of *P.* lividus takes place during spring and early summer (Byrne 1990; Spirlet et al. 1998; Barnes et al. 2001; Gago et al., 2003; Sellem and Guillou 2007; Garmendia et al., 2010). However, in spite of the high abundance and the large distribution of this species on the rocky bottoms of the Basque coast, many aspects of its reproduction are still poorly known; in part, this is related to a lack of interest in the commercial exploitation of this species, over this area (Garmendia et al., 2010).

Analysis of temporal changes in gonad index is used often to study the reproductive cycle, the fluctuations in gonad size and the spawning periods (Lozano et al, 1995; Guettaf et al. 2000; Sánchez- España et al. 2004; Sellem and Guillou, 2007). In this sense, food availability and quality (Lozano et al. 1995), water depth (Himmelman 1986), the prevailing hydrodynamics (Guettaf et al. 2000), phytoplankton blooms (Himmelman 1986; Starr et al., 1990), water temperature and photoperiod (Kawamata 1997; Spirlet et al. 1998) are some of the most important environmental factors responsible for gonad index variations.

The aim of this investigation is to identify suitable locations, within the Basque coast, for the supply of sea urchin gametes, for toxicity bioassays, throughout the year. Hence, the annual cycle of gonad development of *P. lividus* is studied, through examination of the monthly variation of gonad index and gamete availability and quality from two different locations.

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Material and methods

Sampling locations

The study was carried out on sea urchins obtained from two relatively close locations (35 km): Donostia (43º 32’ N; 2º 02’ W) and Zumaia (43º 18,2’ N; 2º 15’ W), both in the Basque Country (northern of Spain), in the innermost part of the Bay of Biscay. The sampling site at Donostia was a shallow rocky zone (0.2 to 2 m water depth); this was emerged at low tide, being dominated by seaweeds, mainly *Lithophyllum incrustans, Corallina elongata* and *Gelidium corneum*. This site is sheltered from wave action and, therefore, exposed to low hydrodynamics [0-10 kw/m of wave energy (Borja et al. 2006)]. In contrast, the sampling area of Zumaia is a large intertidal area, with shallow pools occupied by *Lithophyllum incrustans*, *Corallina elongata, Cystoseira tamariscifolia, Plocamium cartilagineum* and *Stypocaulon scoparium*. The prevailing hydrodynamics of the area are high, being very exposed to wave energy [20-30 kw/m (Borja et al. 2006)]. At both of the sites, water temperature ranges from 12 °C to 23 °C and the salinity lies between 32 and 36 psu. None receives direct discharge from rivers or effluents; therefore, it is assumed that the sampled specimens come from unpolluted environments.

Sampling methods

Individuals (16), between 40- 65 mm diameter (excluding spines), were collected randomly on a monthly basis from January to December 2007, at both locations. All of the samplings were performed at low tide. The sea urchins were collected and then transported alive, in a cool box with seawater, being processed immediately in the laboratory. Temperature and salinity of the water were registered *in situ*. Day length was checked in the nautical calendar of the Defence Ministry of Spain (RIOA 2006).

Laboratory methods

Each individual was measured (height and diameter, excluding spines), its body wet weighed and dissected, to extract the 5 gonads. One of the gonads was wet weighed and dried with the remaining body, for 48 h at 60 °C, and then reweighted. Subsequently, the monthly gonad index was calculated, for both populations, as follows:

GONAD INDEX (GI): gonad dry weight x100 (g)/ body dry weight (g) (Spirlet et al., 1998)

Where, gonad dry weight was estimated by multiplying (by five) the value for a single gonad.

Sexes and maturity of the 16 sea urchins were determined according to the material obtained from the other four gonads (see above). A specimen was considered to be mature when sperm or eggs were present in the gonads (Lozano et al. 1995). Microscopic observations were necessary to check the quality of gametes: high mobility in male gametes and a minimum of 100 µm size and spherical shape in female ones. To confirm the viability of gametes and the reproductive capacity at both of the sites, monthly gonad material was used to carry out fecundation. Following Fernández and Beiras (2001) and Garmendia et al. (2009), gametes were obtained directly from gonads, after having dissected one mature male and one mature female. Eggs were transferred into a 100 mL measuring cylinder, containing 0.2 µm-filtered sea-water; 5 µL of sperm were added (ratio sperm/egg was approximately 20000:1). After 5 to 10 minutes, this mixture was checked and, only if a minimum of 90% of fertilized eggs had developed a fertilization membrane the fecundation was considered successful. Approximately 600 fertilized eggs were transferred into 10 vials (20 mL), containing 0.2 µm-filtered sea-water. These were incubated in darkness at 20 ºC. After 48 hours, the vials were fixed, by adding 3-5 drops of 40% formalin; then, they were observed directly under an inverted microscope, to check the percentage of well-developed larvae and to extrapolate the viability of the gametes.

Statistical analysis

Data were arc sin √p transformed, to achieve normality (p= probability). After checking normality by Shapiro-Wilks W and homogeneity of the variances, comparisons of different variables, over time and between sites and sexes were performed using ANOVA and the Tukey test. The Chi-Square test was used to compare the sea urchin maturity period, between populations. The Statgraphics® Plus 5.0 package was used for these analyses.

Results

Some individuals, mainly at Zumaia in autumn, were not sexually classified, due to the low level of gonad development. Comparing the GI between males and females and between months by two-way ANOVA, there was no significant difference at either site ($p>0.05$). Grouping both sexes, one-way ANOVA on the GI revealed a strong effect of the season (month) both at Donostia ($F= 12.36$, $p<0.001$) and at Zumaia ($F= 17.86$, $p<0.001$). The Tukey test between months at Donostia classified the gonad index into two different groups: values from October to February (5-6.3) were significantly higher than those from March to September (2-3.5) ($p<0.05$). In contrast, the IG lowest values at Zumaia were in February, June and August. Comparing the gonad index between locations, the GI at Donostia was significantly higher than at Zumaia only in February 2007 ($p<0.05$) (Figure 1).

At both sites the GI reached a maximum in December-January, when the day length was about 10 hours. At this period, temperature was lower than 14°C. Between January and March, when the photoperiod increased in three hours, the maturity percentage increased and the GI decreased drastically (Figures 1 and 2). At Zumaia, a second minimum GI value was observed in June. The GI increases from August to late winter in both populations, when the corresponding day length was shortening and the water temperature was decreasing.

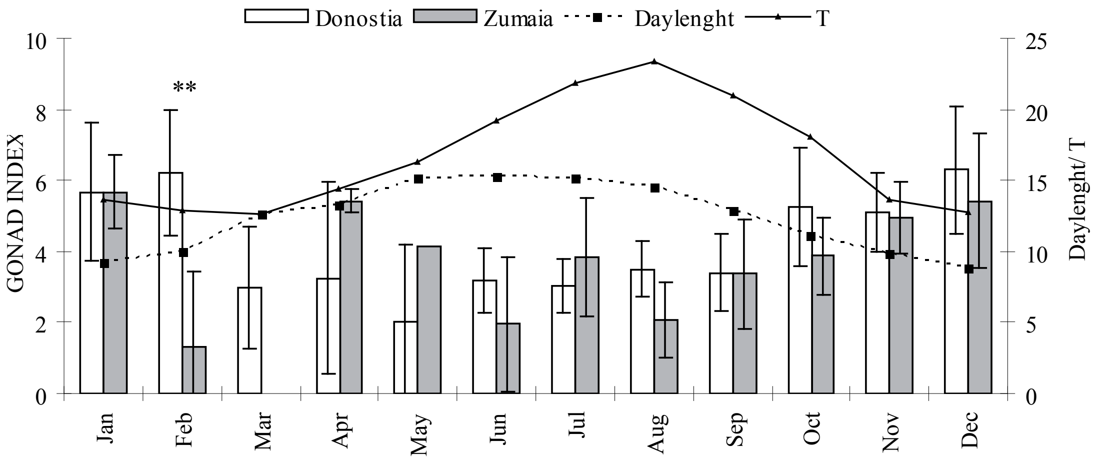

Figure 1. Mean values of gonad index (GI: gonad dry weight x100 (g)/ body dry weight (g)) in sea urchins from Donostia (DO) and Zumaia (ZU) from January to December 2007. Key: bars indicate standard deviation; and ** significant differences (p<0.05) between locations. Note: environmental variables (day length, in hours, and water temperature, in ºC) are also represented.

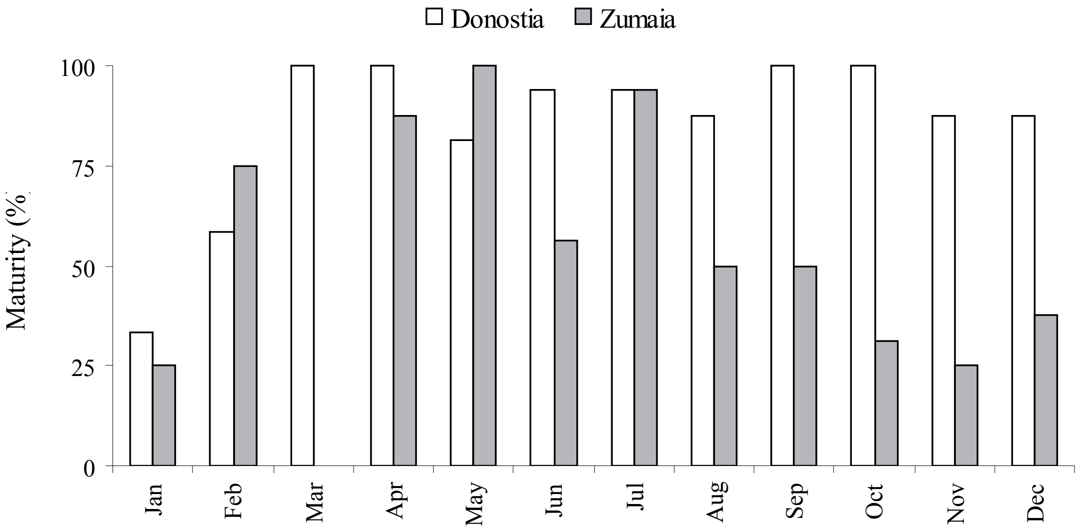

Figure 2. Percentage of mature sea urchins from Donostia (DO) and Zumaia (ZU), from January to December 2007.

At Donostia, the percentage of fertile sea urchins remained at high values (> 75%) from March to December (Figure 2). The Chi-Square test revealed that the period of maturity at Zumaia was significantly shorter than at Donostia ($p<0.05$). At Zumaia, high maturity percentages (> 75%) were found occasionally in spring and summer, whereas low maturity percentages (~ 50%) extended from late summer to winter. From August to December, high values at Donostia contrasted with low values at Zumaia (Figure 2).

According to the site, the maturity percentages differed by sex: they were high and not very different between male and female at Donostia (Figure 3A). At Zumaia, the percentage of mature females was low compared to the males during August, September and October (Figure 3B).

Embryo-larval success percentage at Donostia was more than 90% for almost all of the year (Table 1). In contrast, the reproductive capacity was lower at Zumaia, mainly because

Table 1. Embryogenesis success achieved with sea urchins from Donostia (DO) and Zumaia (ZU) from January to December 2007 (YES = more than 90% of the larvae developed the four arms; NO = less than 90% of larvae developed the four arms; * Lack or poor quality of gametes; NS = no sample).

	2007											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
DO	YES	YES	YES	YES	YES	NO	YES	NO	YES	NO	YES	YES
ZU	NO**	NO*	NS	YES	YES	NO	YES	NO*	NO*	YES	YES	NO*

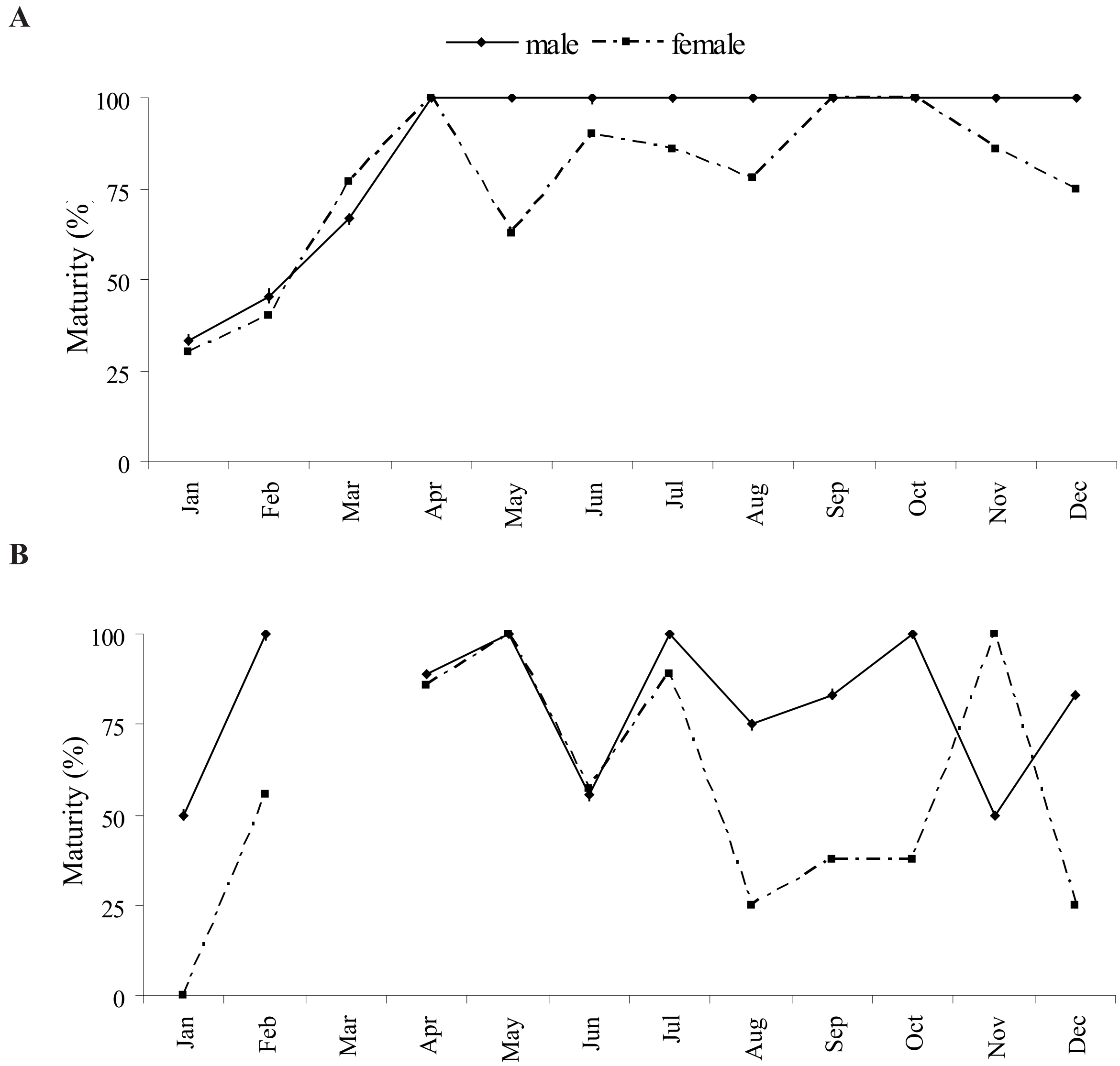

Figure 3. Percentage of mature individuals for each sex in (A) Donostia and (B) Zumaia, from January to December 2007.

of the lack of gametes. Here, 90% embryo-larval success was achieved only in less than half of the sampling occasions.

Discussion

According to one year results, the reproductive cycle, identified from changes in the gonad parameters, indicated a general annual pattern at both localities. This pattern follows the three main phases, identified by Spirlet et al. (1998): (i) the gonad-growing phase (late summer and autumn), when sea urchins accumulate reserve material in their gonads; (ii) the maturation phase, or gametogenesis (during winter), when nutritive cells turn into gametes; and (iii) the spawning phase, when gonad indices reduction takes place, in early spring. In the case of San Sebastian location, our results are in according to Garmendia et al. (2010), who affirmed that sea urchins were observed to behave like a typical Atlantic population, with generally high gonad indices between April and May.

Moreover, there was a drastic decrease of GI values one month earlier at the exposed site and the maturation period was longer at the sheltered site. In relation to this earlier spawning event at Zumaia, Lozano et al. (1995) observed that a drastic reduction in gonadal values could be explained as a result of adverse weather conditions that induce the reduction of gonadal content., Consistent with this observation, the bad weather during February and March coincided with that drastic decrease and could have been related to this premature spawning event.

Moreover, during winter, the sea urchins at the sheltered site had higher gonad indices than at the exposed site. The low gonad index found at Zumaia would confirm the hypothesis that higher hydrodynamism could reduce the reproductive potential due, in part, to a higher cost of maintenance and spine repair (Sellem and Guillou, 2007) and a limited food grazing in open sea exposure (King et al. 1994; Kawamata 1997).

A reduction in water temperature, concomittant with decrease in day length at the end of summer coincides with an increase in gonad index (Kelly 2001; Walker et al. 2001; Shpigel et al. 2004; James et al. 2007). However, for the beginning of spawning at the end of winter, the day length appears as a more decisive factor (Spirlet et al. 1998).

At Donostia, we found sea urchins with different gonad conditions during the same sampling period. This observation could be explained by the fact that gonads are not emptied completely, and that small spawnings are possible during the autumn and winter months. Therefore, this individual variability during the spawning period could be also related partially to differences in the acquisition and allocation of energy reserves to gametogenesis (Meidel and Scheibling 1998).

Conclusions

Finally, as conclusion, the present study suggests that the population of Donostia would supply gametes for almost all year. However, the number of sea urchins to be used will depend on the season, as a high percentage of individuals with spawning capacity were found from February to October, and a low percentage from November to January. In the case of Zumaia, the use of sea urchins is more limited, mainly because of the lack of gametes, especially due to the shorter period of maturity of the females.

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