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Year-round captive spawning performance of the sea urchin *Paracentrotus lividus*: Relevance for the use of its larvae as live feed

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Abstract – Field studies describe echinoplutei not only as grazers but also as prey of naturally occurring fish and shellfish larvae. This finding suggests their potential as live feed in aquaculture. This paper reports on consistent spawnings of the captive sea urchin *Paracentrotus lividus* (Lamarck 1816) (Echinodermata: Echinoidea) throughout the year using diets of plant origin (yellow maize and/or dried seaweed) with fixed environmental conditions equivalent to field conditions during late spring (14 h of daily illumination and 18 °C of temperature). Broodstock maturation without unwanted spontaneous spawnings was achieved in two ways: extending the natural season of reproduction and inducing out-of-season wild specimens to mature. Controlled spawnings of captive sea urchins were induced every month of the year by KCl 0.5 M injections. The diet maize/seaweed combination gave the best results (79% of the tested urchins) in terms of consistent large spawnings throughout the year, followed by the pure maize diet (50%) and the pure seaweed diet (36%). When out-of-season wild sea urchins were induced to maturation, the majority (72%) of tested individuals required at least 60 days to spawn under KCl injection when fed the combination diet. The results demonstrate the feasibility of producing larval *P. lividus* in that high numbers of fertilized eggs (up to 5 million per female) can be obtained year round. The main limitation of exploiting *P. lividus* as planktonic feed seems to be the mortality of broodstock after injection with 1 ml KCl 0.5 M, which prevents reutilization. The 1-month post-injection survival rate was 30 ± 8% (mean ± SE). All surviving sea urchins spawned again after re-injection 1 month later, with a 1-month survival rate of 29%.

Key words: Spawning / Gonad maturation / Planktonic feed / Sea urchin / *Paracentrotus lividus*

Résumé – Performance de pontes de l'oursin, *Paracentrotus lividus*, tout au long de l'année : intérêt de ses larves en tant qu'aliment vivant. Des études in situ décrivent les larves d'échinodermes non seulement comme brouteuses mais aussi comme proies de larves de poissons, crustacés, mollusques. Ce qui laisse présager de leur potentialité en aquaculture, en tant qu'aliment vivant. Nous présentons ici la reproduction tout au long d'une année de l'oursin violet en captivité *Paracentrotus lividus* (Lamarck 1816) (Echinodermata : Echinoidea) nourri d'aliments d'origine végétale (maïs et/ou graines séchées) en conditions environnementales contrôlées équivalentes à celles observées in situ durant le printemps (14 h d'éclairage et une température de 18 °C). La maturation des géniteurs sans ponte spontanée a été obtenue selon 2 procédés : en étendant la saison de reproduction et en induisant des spécimens sauvages à atteindre leur maturité sexuelle hors saison. Des pontes contrôlées d'oursins ont été induites chaque mois de l'année par injections de KCl 0.5 M. Le régime alimentaire combinant maïs/algues a donné les meilleurs résultats (79 % des oursins), en terme de pontes importantes tout au long de l'année, suivi par un aliment constitué de maïs (50 % des oursins) et d'algues (36 % des oursins). Lorsque l'induction de la maturation d'oursins sauvages a été effectuée, la majorité des oursins (72 %) testés ont demandé 60 jours au moins pour pondre sous injection de KCl et nourris du régime alimentaire mixte. Les résultats démontrent la faisabilité de produire des larves de *P. lividus* d'un grand nombre d'œufs fertilisés (jusqu'à 5 millions par femelle) qui peuvent être obtenus tout au long de l'année. La principale limite à l'exploitation de *P. lividus* en tant que nourriture planctonique semble être la mortalité des oursins reproducteurs après l'injection de 1 ml KCl 0.5 M. Le taux de survie des oursins, un mois après injection, était de 30 ± 8 % (mean ± SE). Tous les oursins survivants ont pondu à nouveau après ré-injection un mois plus tard, avec un taux de survie de 29 %.

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1 Introduction

Since the early 20th century, many university laboratories have reared sea urchin larvae for embryological studies or practical classes. Embryos or pluteus of *Paracentrotus lividus* are currently being explored as bioassays for toxicological studies (Bougis et al. 1979; His et al. 1999; Radenac et al. 2001). However, *P. lividus* larvae have seldom been the main objects of rearing studies. Fenaux et al. (1985) established equations for larval growth, including gross biochemical composition, of *P. lividus* reared to metamorphosis. Studies of the exploitation of sea urchin larvae for feeding other fish and shellfish larvae are lacking, although a recent study pointed out their potential use (Hubbard et al. 2003).

Ecological studies have shown the importance of echinoplutei, at least seasonally, in the composition and biomass of zooplankton communities. Although copepods generally dominate, 1980s plankton surveys in the central North Sea (Fransz et al. 1984) showed that echinoplutei were just as numerous; subsequent plankton surveys through the early 1990s showed the reverse situation, in which echinopluteus and ophiopluteus became more abundant than any single holoplanktonic species (Lindley et al. 1995). Rassoulzadegan and Fenaux (1979) used particulate carbon consumption data to estimate that during periods of larval abundance, echinoplutei account for 3% of the phytoplankton biomass. A 1998 report of plankton samples from the Mediterranean (López et al. 1998) recorded peak *P. lividus* larval densities of up to 33 m^{-3}. Such high densities suggest that echinoplutei are important not only as grazers but as prey of naturally occurring fish and shellfish larvae. McEdward and Miner (2001) suggest that predation might be one of the major causes of echinoid larval mortality in the field and can range from 6% to 27% per day.

It follows that if echinoplutei are natural prey, they may also be useful in the growth of fish and shellfish larvae as a mass-produced source of zooplankton. To be suitable for such mass production, any species of zooplankton must meet most of the following criteria:

- Year-round availability;
- Large number of eggs and larvae;
- Short period of embryonic development;
- Herbivory quality;
- Nutritional quality.

Thus far, only two species have met these criteria: the rotifer *Brachionus plicatilis* (length, 123–292 μm) (Snell and Carrillo 1984) and the nauplii of *Artemia* brine shrimp (length, 420–475 μm). However, some fish and crustacean larvae do not accept these species as zooplankton food, which has prompted the search for alternative and reliable sources of living zooplankton. The class Copepoda (length, ~2000 μm) has been studied for several decades, but the reliability of copepods has yet to be proved, especially in terms of achieving the numbers required for nourishment of the early developmental stages of fish larvae (Stottrup 2000). During endotrophic development, *P. lividus* larvae are 125–350 μm in length, which is comparable with the dimensions of prey commonly used in aquaculture.

Any assessment of *P. lividus* larvae as a reliable source of planktonic feed must first prove that captive broodstock can produce large numbers of fertilized eggs year-round and for extended periods. There are reports of gametogenesis occurring throughout the year among captive broodstocks of *P. lividus* (Grosjean et al. 1998; Spirlet et al. 2000; Shpigel et al. 2004). However, we lack details concerning the organism's spawning performance over the course of a year, and most studies have centered on the promotion of vitelogenesis for human consumption or have been short-term. Our report of year-round controlled spawnings of *P. lividus* provides evidence that *P. lividus* larvae could be a reliable source of living zooplankton, a source that meets at least four of the abovementioned criteria.

2 Material and methods

All the sea urchins used in the present work were collected during full-moon low tides from pools on the central west coast of Portugal near Cascais (Lisbon). A previous study of this population (Gago et al. 2003) established the annual variability of gonad index, spawning periods, and influence of food availability on gonad size.

Evaluation of the spawning performance of captive *P. lividus* involved three experiments. The first tested the hypothesis that large numbers of fertilized eggs could be produced year round by broodstock held captive for extended periods (long-term experiment). The second experiment was devised as a backup in case the first yielded negative results: wild immature sea urchins also were evaluated to see how fast they could mature in captivity (short-term experiment: induction of maturation during out-of-season gametogenesis). The final experiment evaluated broodstock mortality following injections with a spawning trigger (short-term experiment: survival tests to KCl injections).

Since gonad production of *P. lividus* is seasonal and follows an annual cycle (Gago et al. 2003), control over the maturity of broodstock and spawnings involves, as with many other seasonally reproducing marine species, rigorous attention to three parameters: photoperiod, temperature, and diet.

All experiments used a fixed photoperiod of 14L:10D cycle to mirror the prevailing conditions of mid-spring, when most sea urchins are mature in the field. Daylight fluorescent tubes of 58 W provided artificial illumination over each tank or aquariumm, generating 700 lux as measured (Gossen Lunasix 3) at the water surface of the tanks. Sea water was kept at 18 ± 0.5 °C in all three experiments, which also reflects the conditions of late spring and early summer in the field. We expected that such a narrow range would favor gonad maturation but not spontaneous spawning in captivity, which could be easily checked by the presence of gametes over the aboral hemisphere of sea urchins.

P. lividus is basically an herbivorous echinoid (Verlaque and Nédelec 1983; Boudouresque and Verlaque 2001; Gago et al. 2003) and thus was fed appropriate diets of plant origin. Basuyaux and Blin (1998) established the suitability of maize-based feeds coupled with algae for the survival and somatic growth of *P. lividus*. Likewise, three convenient plant-derived diets were tested during the long-term spawning experiment. The first diet consisted of commercial yellow grains of the maize *Zea mays* (13.5% moisture). The second diet consisted of the commercial dried seaweed *Laminaria ochroleuca*

(Kombu, Algas de Galicia, Algamar, Spain; 13.4% moisture). The third diet was a combination of the two basic diets wherein the components were alternated. Maize was presented as dry grains that were randomly distributed by tank water current as they sank. Dried seaweed fronds were pre-cut into small pieces, wetted for 15 min, and then also randomly distributed. Both of these basic diets are easily purchased and rich in carbohydrates (69.8% and 52.1%, respectively). The literature describes the biochemical compositions (gross, minerals, vitamins, and amino acids) of these diets. Crampton and Harris (1969) tabulated maize composition, and Sáa (2002) analyzed the dried seaweed. Protein (8.4% for maize and 6.9% for seaweed) and lipid contents (4.3% and 1.1%, respectively) were not very dissimilar. Maize is devoid of vitamins B_{12} and C and of the amino acids tryptophan and cystine, but maize has carotene at a concentration of 1.8 mg kg^{-1}. Most fatty acids from total lipids are polyunsaturated in maize (linoleic acid, 45%) and monounsaturated in the seaweed (oleic acid, 30%).

Gonad indices (measured in grams of wet gonad weight/grams of whole animal weight × 100) were determined immediately after spawning and in the same individuals to ensure a close correspondence between gonad size and range of spawnings. This technique thus might have underestimated gonad indices. However, pre-tests with wild sea urchins revealed this method yielded gonad indices similar to the ones reported by Gago et al. (2003).

Spawning was induced by injecting 1 ml KCl 0.5 M through the peristomial membrane using a 0.9 mm (external diameter) × 50 mm (length) needle coupled to a 5 ml syringe; the main environmental trigger of spawning is not clearly known (López et al. 1998). Each sea urchin was placed for 30 min in individual plastic beakers filled with 2 L of aerated sea water at 18 °C, where oocytes or sperm were eventually emitted. Light microscopy (×40) was used to evaluate spawning. Results were measured qualitatively according to the area occupied by gametes and concurrently compared with electronic counts. The resultant semi-quantitative classification of spawning consisted of *no spawning* (−); *spawning up to 500 thousand oocytes or 200 million spermatozoa* (+); and *spawning greater then previous values* (++). Precise gamete counts and oocyte size measurement were obtained with a Coulter Counter (model ZM with 140 μm cell aperture) coupled to a multichannel particle analyzer (Channelyser 256) during the gametogenesis season (May to September), allowing the spawning performances of wild and captive sea urchins to be compared simultaneously.

Each batch of emitted oocytes was checked for maturity (lack of central nucleus) and viability (formation of a fertilization membrane after fecundation). Fertilization rates were not precisely measured, but the large majority of emitted oocytes (in both wild and captive urchins) were mature and displayed fertilization membranes.

2.1 Long-term experiment: Year-round production of fertilized eggs

The evaluation of controlled spawning performance of broodstock held captive for extended periods took place over 12 months (October 2001 to September 2002) in a recirculating rearing system with a total volume of 3480 L of unfiltered natural sea water whose salinity was kept at 34–35 ppt. Sea urchins were tested in eight cylindrical black fiberglass tanks that were 0.80 m (base diameter) × 0.80 m (depth), equivalent to 402 L. No UV water sterilization or chemical/mechanical filtration was used; sea water treatment was achieved through biological filtration and high-efficiency protein skimming (Tunze Aquarientechnik, Venturi type model 3160). Plastic media (spiny balls) were used as substrate for nitrifying bacteria. Water changes took place during feces removal (15% of monthly total volume). Each tank was stocked with an initial density of 47 sea urchins/tank, equivalent to 94 sea urchins m^{-2} (area of base) or 117 sea urchins m^{-3} with a test diameter of 47 ± 0.7 mm (mean and SE) and a weight of 29 ± 1 g (mean and SE). Each diet was given to two tanks while the urchins in the remaining two tanks served as unfed controls. According to the method described by Spirlet et al. (1998), sea urchins were fed discontinuously twice a week for a period of 12 months. Every month, seven urchins were sampled from each treatment and subjected to induced spawnings immediately followed by measurement of gonad indices.

A simple rearing method provided a check of larval development to early four-armed pluteus. Fertilized eggs from captive and wild sea urchins, taken from May to July 2002, were washed on a 30 μm mesh and then placed into 2 L plastic beakers filled with aerated sea water for 3 days (endotrophic phase) at a temperature of 18–20 °C and salinity of 35 ppt.

2.2 Short-term experiments

The short-term experiments, induction of maturation during out-of-season gametogenesis and survival tests to KCl injections, took place in stand-alone aquaria. Wild sea urchins for both short-term experiments were tested in five glass tanks (0.425 × 0.345 × 0.250 m) filled with 37 L of natural sea water kept at 34–35 ppt. Four tanks were allocated as replicates, and one tank served as a control. Sea water treatment was provided through biological, mechanical, and chemical filtration enclosed in a hang-on external wet/dry filter (Millenium 1000, Aquarium Systems). Protein skimming was also performed (Berlin Air-Lift 60, Red Sea Fish Pharm). Additional water currents were produced by a power-head (Eheim 1005, 300 L h^{-1}). Nutrient export was ensured by water changes during weekly tank siphoning to remove feces.

2.2.1 Induction of maturation during the out-of-season gametogenesis

The induction of maturation and spawning of sea urchins during out-of-season gametogenesis (October to April) took place over two trials of 38 days (from December 2001 to January 2002) and 61 days (from February to April 2002). Tested sea urchins were stocked at 15 wild sea urchins per tank (100 sea urchins m^{-2}). Except for the unfed control group, all sea urchins were fed the combination diet twice a week. At the end of each trial, sea urchins were sampled from each replicate and subjected to induced spawnings immediately followed by determination of gonad indices.

2.2.2 Survival tests to KCl injections

Survival tests to 1 ml KCl 0.5 M injections took place during four consecutive trials from April to August 2002, the first three with recently collected wild sea urchins (0, 5, and 10 days of previous captivity) and the last trial with long-term captive sea urchins. Apart from the control group, sea urchins were pre-subjected to injections of 1 ml KCl 0.5 M and their survival was checked over the following 30 days. The surviving sea urchins were again injected and followed for another 30 days. All sea urchins were fed the combination diet twice a week and stocked at densities of 33–67 sea urchins m^{-2}, depending on the number of surviving sea urchins. At the end of each trial, surviving sea urchins from each replicate were counted and again subjected to induced spawning. To elucidate the diverse survival patterns following KCl injections, internal water volumes of selected wild sea urchins were measured ($N = 19$, test diameter ≥38 mm, ≤60 mm). Internal water was extracted through the peristomial membrane with a hypodermic syringe equal to the one used to inject KCl. Linear regression analysis of volume on diameter showed that V (ml) $= -32.013 + 0.916 \times D$ (mm) ($R = 0.942$, $t = 11.617$, $p < 0.001$).

Data were tested for normality, and one-way ANOVA (with diet as the source of variation) was used to analyze both semi-quantitative (arcsine transformed) and quantitative spawning results. Variations among tanks (duplicates) were tested by ANOVA, Model II. Differences between two data means (wild vs. captive, males vs. females) were analyzed using Student's t-distribution for independent values. A t-test was used to determine the significance of the regression coefficients of weights on diameters (Bailey 1976).

3 Results

3.1 Long-term experiment: Year-round production of fertilized eggs

P. lividus is a species quite suited to long-term captivity. Over 12 months, none of the 376 initially stocked sea urchins died, including the unfed control group. Pooled data for captive fed urchins showed an increase in mean diameter from an estimated 47 ± 0.7 mm (mean and SE) at the beginning of the experiment to 51 ± 0.4 mm at the end of the experiment ($p < 0.001$) and in mean weight from 29.1 ± 0.9 g to 33.6 ± 0.7 g ($p > 0.05$). Differences in regression coefficients for the same test diameter showed that captive fed sea urchins weighed more than wild sea urchins ($p < 0.05$), a possible indication of suitability. No correlation was found between weight and gonad index in wild or captive sea urchins ($p > 0.05$).

Gonad indices did not differ significantly between the sexes ($p > 0.05$), as Guettaf and San Martin (1995) and Gago et al. (2003) had reported earlier. Pooled data always showed that gonad indices were higher in the captive fed groups (mean, 10.5%) than in the concurrent wild population (mean, 6.1%) during every month of out-of-season gametogenesis (October to April) ($p < 0.001$). Although significantly different, the gonad indices of the wild sampled sea urchins (6.1%) and the captive unfed group (5.7%) were very close. Gonad indices over 10% were easier to achieve using maize or the combination diet than dried seaweed alone ($p < 0.001$). There was no significant difference in gonad indices between maize and combination treatment. Variations among tanks (duplicates) were not significant ($p > 0.05$). Gonad indices reached as high as 24% in sea urchins fed the combination diet compared with a maximum of 15% with dried seaweed alone.

Large (++) and/or small (+) spawnings were registered all the year round for both male and female pooled captive fed sea urchins (Table 1). Overall analysis showed that when large and small spawnings are considered simultaneously, pooled male and female captive sea urchins performed better than wild sea urchins ($p < 0.05$). Captive sea urchins also seemed to perform better than wild ones when only large spawnings are considered, but the differences were not significant ($p > 0.05$). However, no large spawnings were obtained with the wild sea urchins between October and January.

When diet is used to compare the results of induced spawnings of captive sea urchins (Table 2), the combination diet (maize/seaweed) performs best ($p < 0.05$) in terms of consistent large spawnings (++) of pooled males and females over a year (79% of tested urchins), followed by the maize diet (50%). The same applies for large spawnings when only females are considered ($p < 0.01$). The seaweed diet was clearly less efficient ($p < 0.05$): only 36% of the tested sea urchins emitted large quantities of oocytes or spermatozoa.

Checks for completeness of larval development from May to July 2002 showed that, for both wild and captive urchins, fertilized eggs at 18–20 °C developed into endotrophic early larvae (free-living blastula, prism, and prepluteus with 200 μm) within 36 h and then to planktotrophic four-armed plutei (350 μm long) within 72 h.

Precise counts of oocytes and spermatozoa emitted by captive sea urchins during the gametogenesis season (May to September) and compared with concurrent wild samples are represented in Figure 1. Captive sea urchins performed better than the wild ones over the entire experimental period. Table 3 shows that the maize diet (mean sperm count, 311 million spermatozoa per male) was more favorable ($p < 0.05$) for sperm emission than the two other diets (276 and 270 million spermatozoa per male). Sperm counts from sea urchins fed any of the three diets were higher ($p < 0.05$) than counts from emissions of wild sea urchins. Table 3 also shows that the maize diet (mean oocyte count, 1788×10^3 per female) was also more favorable ($p < 0.01$) for oocyte emission than any other diet (1582×10^3 and 863×10^3 oocytes per female). Oocyte counts obtained from females fed the seaweed diet were not significantly different from those obtained from wild sea urchins.

Oocyte sizes, measured electronically as modal frequencies, were similar for all three diet groups and similar to those obtained from wild urchins. During the maturation season, wild and captive sea urchins emitted small (~65 μm in diameter) and large oocytes (~90 μm), except during June 2002, when only large oocytes were produced. Oocyte size distributions were sometimes bimodal or even trimodal regardless of the origin of sea urchins. Except with wild males, the number of emitted gametes did not correlate with test diameters for either captive or wild sea urchins ($p > 0.05$).

Table 1. Induced spawnings in wild and captive fed sea urchins. Each group included 21 sea urchins sampled each month. Results express % of total sea urchins in each sub-group.

	Wild								Captive fed							
	Males				Females				Males				Females			
	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)
October	12	58	42	0	9	56	44	0	13	31	23	46	8	12	38	50
November	10	90	10	0	11	100	0	0	9	0	78	22	12	58	42	0
December	7	100	0	0	14	100	0	0	10	60	20	20	11	18	55	27
January	8	62	38	0	13	100	0	0	11	0	82	18	10	30	50	20
February	8	38	62	0	13	54	38	8	11	0	73	27	10	0	50	50
March	11	0	82	18	10	0	90	10	10	0	80	20	11	9	73	18
April	7	0	29	71	14	0	50	50	13	0	15	85	8	0	50	50
May	9	56	11	33	12	25	58	17	9	0	33	67	12	0	8	92
June	10	0	0	100	11	0	0	100	7	0	43	57	14	0	7	93
July	7	0	0	100	14	0	7	93	11	0	18	82	10	0	30	70
August	13	0	0	100	8	0	50	50	10	0	10	90	11	0	64	36
September	10	0	20	80	11	0	100	0	8	0	13	87	13	0	54	46
	112				140				122				130			
Mean (%) ± se			17 ± 1	37 ± 4			30 ± 2	20 ± 3			40 ± 1	52 ± 1			42 ± 1	45 ± 1

Pooled males and females (+ and ++): wild = 25.5 ± 0.6%, captive = 44.6 ± 0.2%. t = 2.29 with df = 76, $p < 0.05$.

Pooled males and females (++): wild = 27.9 ± 1.5%, captive = 48.4 ± 0.5%. t = 1.51 with df = 37, $p > 0.05$.

(#) Number of sampled sea urchins.

(-) No spawning; (+) spawning up to 500 thousand oocytes or 200 million spermatozoa; (++) spawning greater than previous values.

Table 2. Comparative results of induced spawnings in captive sea urchins fed two basic diets and a combination. Each group included 7 sampled sea urchins each month during one year. Results express % of total sea urchins in each sub-group.

	Seaweed								Maize (yellow, grain)								Combination (maize/seaweed)								Control (unfed)							
	Males				Females				Males				Females				Males				Females				Males				Females			
	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)	(#)	(-)	(+)	(++)
Oct.	6	0	17	83	1	0	100	0	5	40	40	20	2	0	100	0	2	100	0	0	5	20	0	80	2	0	50	50	1	0	0	100
Nov.	3	0	100	0	4	75	25	0	2	0	50	50	5	60	40	0	4	0	75	25	3	33	67	0	2	100	0	0	5	60	40	0
Dec.	1	100	0	0	6	33	67	0	4	50	50	0	3	0	67	33	5	60	0	40	2	0	0	100	4	100	0	0	3	100	0	0
Jan.	2	0	100	0	5	20	60	20	4	0	100	0	3	67	33	0	5	0	60	40	2	0	50	50	4	100	0	0	3	100	0	0
Feb.	3	0	67	33	4	0	50	50	3	0	33	67	4	0	50	50	5	0	100	0	2	0	50	50	3	100	0	0	4	100	0	0
Mar.	3	0	100	0	4	25	75	0	3	0	100	0	4	0	100	0	4	0	50	50	3	0	33	67	2	100	0	0	5	100	0	0
Apr.	5	0	40	60	2	0	100	0	3	0	0	100	4	0	50	50	5	0	0	100	2	0	0	100	4	100	0	0	3	100	0	0
May	2	0	0	100	5	0	20	80	4	0	25	75	3	0	0	100	3	0	0	100	4	0	0	100								
Jun.	2	0	0	100	5	0	20	80	2	0	0	100	5	0	0	100	3	0	0	100	4	0	0	100								
Jul.	4	0	0	100	3	0	33	67	4	0	25	75	3	0	33	67	3	0	0	100	4	0	0	100								
Aug.	3	0	0	100	4	0	75	25	3	0	0	100	4	0	50	50	4	0	0	100	3	0	33	67								
Sep.	3	0	0	100	4	0	100	0	1	0	0	100	6	0	17	83	4	0	0	100	3	0	0	100								
	37				47				38				46				47				37				21				24			
Mean (%)			29	59			70	16			31	60			45	40			12	72			10	85								
± sem			4	4			2	2			3	3			2	3			3	3			1	2								
(after angular transformation)																																

F-tests: Females (++) = 6.51 with df = 2, 33 $p < 0.01$; Males (++) = 0.15 with df = 2, 33 $p > 0.05$;

Pooled females and males (++) = 3.41 with df = 2, 69 $p < 0.05$. Combination diet (78.5% ± 1.7) > maize diet (49.8% ± 1.5) > seaweed diet (36.1% ± 1.3).

Pooled females and males (+ and ++) =0.01 with df = 2, 141 $p > 0.05$. Combination diet (42.7% ± 0.9); maize diet (43.7% ± 0.7); seaweed diet (41.8% ± 0.8).

(#) Number of sampled sea urchins in each batch of 7 sea urchins.

(-) No spawning; (+) spawning up to 500 thousand oocytes or 200 million spermatozoa; (++) spawning greater than previous values.

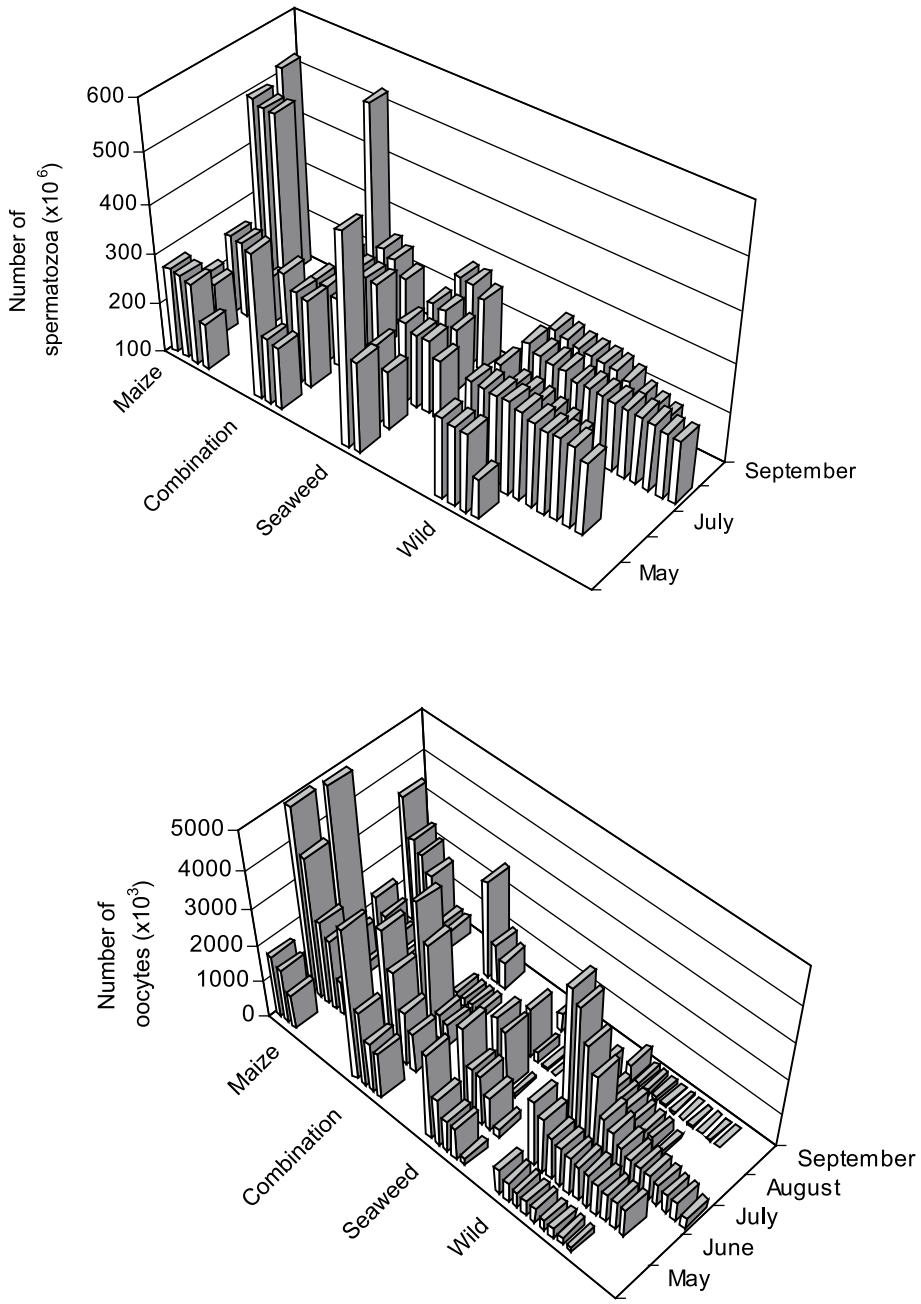

Fig. 1. Male (above) and female (below) sea urchin induced spawnings. Quantitative analysis during the gametogenesis season.

3.2 Short-term experiments

3.2.1 Induction of maturation during out-of-season gametogenesis

Gonad maturation can be induced in wild sea urchins during out-of-season gametogenesis (October to March), as shown in Table 4. Both trials showed that 20% of tested urchins spawned as quickly as 30 days, but most animals (72%) required at least 60 days to spawn when fed the combination diet. The gonad index decreased by a factor of 3 in unfed controls, which did not spawn. Once again, sea urchins were shown to adapt quite well to the experimental conditions of the stand-alone small aquaria (40 L of volume), given that no animals died and they readily consumed the combination diet.

3.2.2 Survival tests to KCl injections

Survival rates 1 month after injection varied between 17% and 44% over the three trials (30.1% ± 7.8%, mean and SE), whereas no mortality was registered for any sea urchins assigned to the control groups. Survival results from the first three trials seemed to suggest that the previous captive period (0, 5, and 10 days respectively) improved survival. However,

Table 3. Comparison of emitted number of spermatozoa and oocytes during the gametogenesis season by captive fed and wild sea urchins. # – number of sampled sea urchins.

	Male		Female	
Diet	Emitted spermatozoa ($x10^6$) (mean ± SE)	#	Emitted oocytes ($x10^3$) (mean ± SE)	#
Maize	311 ± 33	14	1788 ± 310	21
Combination	276 ± 19	17	1582 ± 279	19
Seaweed	270 ± 20	14	863 ± 176	21
Wild	244 ± 4	44	788 ± 119	53
	$F = 2.88$ $df = 3.67$; $p < 0.05$ All means are significantly different except the pair combination/seaweed		$F = 5.31$ $df = 3.92$; $p < 0.01$ All means are significantly different except the pair seaweed/wild	

Table 4. Induction to maturation of wild sea urchins during out-of-season gametogenesis. Trials took place in five stand-alone 40 L glass tanks. All sea urchins, except the unfed control group, were fed the combination diet and were stocked at 15 sea urchins per tank (100 sea urchins m^{-2}). First trial took place during December 12, 2001 to January 18, 2002 (38 days). The second trial took place from February 5 to April 6, 2002 (61 days) with two sampling dates.

	Sampled sea urchins			Mean test	Mean GI (%)		Induced spawning		
	Male	Female	Total	diameter (mm)	Initial	Final	Male	Female	Total
1st trial	24	15	39	43	6.1	6.7	1 (4%)	4 (27%)	5 (13%)
2nd trial									
Mar-08	14	11	25	47	4.7	5.4	4 (29%)	3 (27%)	7 (28%)
Control	3	2	5	48	4.7	4.4	0	0	0
Apr-06	15	10	25	49		4.4	10 (67%)	8 (80%)	18 (72%)
Control	3	2	5	49		1.6	0	0	0

mean survival rates were not significantly different ($p > 0.05$). In the fourth trial, exclusively carried out on long-term captive sea urchins, no animals survived. Surviving reinjected sea urchins spawned again, with a survival rate of 29% after 30 days.

Analysis of the mean test diameters and weights of all the surviving sea urchins from the first three trials ($n = 24$) revealed that they were larger (mean diameter, 60 mm vs. 50 mm; $p < 0.001$) and heavier (mean weight, 42.6 g vs. 30.7 g; $p < 0.001$) than the average captive sea urchin ($n = 244$). Therefore, although 1 ml KCl 0.5 M effectively induced spawning, the higher post-injection survival rate in these larger sea urchins could be due to the dilution of KCl. We estimated that 1 ml KCl 0.5 M injected into a sea urchin of 50 mm diameter (13.8 ml of measured internal volume) was equivalent to an internal KCl concentration of 0.27%. The same volume of KCl injected into urchins of 40 mm diameter (4.6 ml of internal volume) and 60 mm diameter (22.9 ml of internal volume) was equivalent to an internal KCl concentration of 0.81% and 0.16% KCl, respectively.

4 Discussion

Current research on the aquaculture of sea urchins seeks to learn how to *suppress* gametogenesis in order to produce good-quality gonads for consumption or how to *promote* gametogenesis for increased production of seed stock.

Our studies concern gametogenesis promotion, but only up to the stage of production of endotrophic plutei, disregarding metamorphosis-competent larvae. In the laboratory, common predators more readily consume embryos and early-stage larvae than late-stage larvae (Rumrill et al. 1985; Pennington et al. 1986). Our aim was to evaluate the feasibility of exploiting sea urchins in the near future as reliable sources of living zooplankton for rearing fish and shellfish larvae.

The results show that mass production of the early pluteus stage of the purple sea urchin *P. lividus* is feasible given that (1) high numbers of fertilized eggs (up to 5 million per female) can be obtained daily all the year round, (2) oocyte sizes are similar to those obtained from wild urchins, (3) fertilized eggs emitted by captive broodstock fully develop into endotrophic early larvae and then to planktotrophic four-armed plutei. Therefore, free-living endotrophic prey of different sizes (125−350 μm) can be produced from the same spawning within 3 days. Large-scale larvae production has also been reported for *Psammechinus miliaris* (Kelly et al. 2000) and *Echinus esculentus* (Jimmy et al. 2003), but such production has been for echinocultural purposes.

P. lividus broodstock are well suited to long-term captivity in that they showed no mortality and were easy to feed, readily consuming any of the tested diets. Tank cleaning is also relatively simple and not time-consuming in that water siphoning performed during water changes easily removes the abundant excreta produced by the urchins. Stocking densities could certainly be increased from the test numbers of about 100 sea urchins m^{-2} of floor space (3 kg m^{-2}) to the maximum density in the field of 234 urchins m^{-2} (Gago et al. 2003) or even up to 650 urchins m^{-2} (20 kg m^{-2}), probably without impairing survival or gonad index (Fournier 2001).

The gonad indices of wild sea urchins, determined immediately after induced spawnings and in the same individuals, were not dissimilar from the ones reported by Gago et al. (2003). It seems that full spawnings are rare given that even after large induced gamete releasing, gonad indices were always significant in captive and wild sea urchins. Spring and summer spawnings with more than one event have been reported for the present field population of *P. lividus* (Gago et al. 2003) and for another Atlantic population (Crapp and Willis 1975). The number of oocytes released per spawning can likely be improved further because it appears that egg production is far from realizing its potential with current procedures.

Throughout the year, all feeding treatments stimulated significantly higher gonad indices and larger induced spawnings in captive animals than with urchins concurrently sampled from the field. Urchins fed maize or maize/seaweed had significantly higher gonad indices (11.7 ± 0.5% and 11.5 ± 0.6%, respectively) than urchins given seaweed alone (8.2 ± 0.5%). The combination diet of maize/seaweed achieves dominance (78.5 ± 1.7% of tested sea urchins) in terms of the ultimate goal of this study: large spawnings throughout the year. In terms of cost and availability, maize alone (49.8 ± 1.5% tested urchins) is also a good diet if very large spawnings are not required, and during the maturation season (May to September) the maize diet was superior even to the combination diet in terms of large spawnings of oocytes and sperm.

In their natural environment, the studied *P. lividus* population feeds primarily on erect algae or encrusting algae, with the former leading to better somatic growth and gonad size (Gago et al. 2003). The Mediterranean purple sea urchin is also mostly herbivorous, with 86%–96% of gut contents being of algal origin (Phaeophyceae, 41%; Rhodophyceae, 19%) (Verlaque and Nédelec 1983). However, the role of consumed algal components in the promotion of gonad growth is not yet understood. Gonad production is similar when *Strongylocentrotus franciscanus* is fed algal or prepared diets (McBride et al. 1997, 2004) but lower when *Loxechinus albus* (Lawrence et al. 1997) or *Psammechinus miliaris* (Otero-Villanueva et al. 2004) is fed an algal diet compared with a prepared diet. Our results also show consistently lower gonad indices (8.2 ± 0.5%) and lower spawnings (36.1 ± 1.3%) with sea urchins fed the seaweed diet alone. Prepared feeds, including extruded feeds, have been used successfully for gonad production with *P. lividus* (Lawrence et al. 1989, 1992, 2001; Fernandez et al. 1996). On the other hand, Frantzsis and Grémare (1992) suggested similar qualitative nutrient requirements for somatic and gonadal growth of *P. lividus*. Algal diets seem to be superior to prepared diets when fed to juvenile *Strongylocentrotus droebachiensis*, suggesting that sea urchins may not require animal protein for growth (Kennedy et al. 1999).

Basuyaux and Blin (1998) proposed carbohydrate-rich compounds like maize as practical diets for rearing *P. lividus*. Maize, with 4804 cal g^{-1} (Crampton and Harris 1969), is as rich in energy as common prepared diets, but much lower in protein content (8.4% of dry matter compared with at least 20% of dry matter in prepared diets). Optimal gonad production with *P. lividus* may depend more on high energy than high protein, which may not be crucial. Frantzsis and Grémare (1992) also reported that somatic and gonadal growth depended more on the amount of ingested organic matter than on the amount of ingested protein. Therefore, with similar efficacy, maize is more economical than prepared feeds as a source of high energy and protein for *P. lividus*.

The adopted conditions of photoperiod (fixed cycle of 14L:10D) and temperature (18 ± 0.5 °C) seemed to work well for *P. lividus*. Broodstock was kept mature throughout the year without unwanted spontaneous spawnings, as no gametes were ever observed on the aboral hemisphere of captive sea urchins. Grosjean et al. (1998) were able to maintain the maturity of *P. lividus* broodstock throughout the year at 18–20 °C but in total darkness. More recently, Shpigel et al. (2004) presented evidence that temperatures of 18–22 °C enhanced gonad growth in *P. lividus* but that gametogenesis was controlled by photoperiod: long days reduced rates of gametogenesis and short days increased reproductive development. On the other hand, Spirlet et al. (2000) found temperature to be the main determinant of the reproductive cycle of *P. lividus*. Obviously, current data on the best combination of temperature and photoperiod to achieve continuous gonad growth and gametogenesis with *P. lividus* are ambiguous. It seems that any photoperiod works with captive *P. lividus* broodstock at the temperature range of 18–22 °C as long as diet is appropriate.

P. lividus was clearly able to produce mature gonads and spawnings in the relatively short period of at least 60 days.

With the same species, Fernandez et al. (1996) reported full gonad development in captivity using similar periods.

4.1 Limitations of method

The main drawback of the present method is the mortality of broodstock after injection of 1 ml KCl 0.5 M to induce spawning. Survival tests showed that only 30% of tested urchins could survive and spawn again after KCl injection, thus preventing extensive reuse of the same broodstock. Internal dilution of KCl in larger sea urchins can be an explanation to their survival to injections. A study reported full survival of juvenile green sea urchins at external water KCl concentrations less than or equal to 5%, but survival decreased to 0% at a 10% concentration (Hagen 2003a). Although the literature generally recommends injections of 1−2 ml of KCl 0.5 M, results suggest that lowering the dose of KCl improves smaller broodstock survival without impairing spawning. Electrostimulation is also common but is ineffective at inducing spawning, although survival rates (68.6%–91.4%) are better than with KCl (Hagen 2003b). Other inducers could be sought given that López et al. (1998) have proposed photoperiod, phytoplankton blooms, and turbulence as natural triggers of spawning. These authors also suggested that temperature is the main trigger of spawning episodes in field populations of *P. lividus*.

Another critical limitation may be the nutritional value of sea urchin larvae. While a full biochemical analysis of their food value is beyond the scope of the present study, the issue is already being addressed. However, at least in terms of lipid composition, prospects look good. Pantazis et al. (2000) and Bell et al. (2001) found that the gonads of *P. miliaris* can elongate-desaturate considerably from diets low in polyunsaturated fatty acids into highly unsaturated fatty acids, including 20:4n-6, 20:5n-3, but only vestigial amounts of 22:6n-3.

A final limitation could rest on the hypothesis that echinoderm larvae contain chemical defenses, as the larvae apparently have no structural defenses. Laboratory experiments with filter-feeding benthic predators showed that echinoderm larvae experienced the highest survival, which constitutes preliminary evidence of chemical defense mechanisms (Cowden et al. 1984). But experiments with zooplanktonic predators, including fishes and crustaceans, showed that predation rates differed with the stage of embryonic and larval development, with embryos and early larvae being more susceptible than later-stage echinoplutei (Rumrill et al. 1985; Pennington et al. 1986). Echinoplutei have several defenses that reduce predation (Rumrill 1990), such as the development of swimming behavior (Rumrill et al. 1985).

5 Conclusion

The combination of three factors—an appropriate diet composed of maize grains coupled with a dried seaweed, 18 °C sea water temperature, and a photoperiod of 14 h of artificial illumination—produced large numbers of viable *P. lividus* oocytes and spermatozoa over 1 year, including the period of out-of-season gametogenesis. However, broodstock survival following spawn induction must be improved before *P. lividus* can be fully considered as a suitable source of larval foods.

Our methods for obtaining the fertilized eggs and larvae of *P. lividus*, although targeted to aquaculturists and public aquarium professionals, might also aid toxicological studies since we have shown that embryos or larvae can be produced year round regardless of the availability of mature wild adults. In the same way, these results could help experimental classes in high schools and undergraduate courses in universities, which use sea urchin oocytes and sperm to introduce in vitro fertilization and embryology.

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