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Can echinoculture be a feasible and effective activity? Analysis of fast reliable breeding conditions to promote gonadal growth and sexual maturation in *Paracentrotus lividus*

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ABSTRACT

Paracentrotus lividus gonads have been considered a delicacy since the time of ancient Greece; nowadays gonads are marketed fresh, frozen and pasteurized all over the world. Due to commercial fisheries and destructive harvesting methods employed to meet market demand, a dramatic depletion of *P. lividus* was registered in Europe, especially along the Mediterranean coasts, with a complete disappearance of urchins from areas of former abundance. In this study, we evaluated through different biological outcomes the efficacy of a specific breeding condition characterized by set light/dark regime, a controlled Recirculating Aquaculture System (RAS) and the supply of artificial diets (MD, Macroalgae; MSD, Maize and spinach and PD, a commercial pellet used in fish aquaculture) to ensure a rapid gonadal growth and promote an effective maturation of gametes of *P. lividus*. Date reported demonstrate that the breeding condition was extremely efficient after just three weeks in regard of the most effective diet MSD, with an increase of the Gonadal Index (GI) from a complete spent stage of 135%. The commercial and biological goodness of these gonads was tested with fecundation and embryo development tests, in addition to a scrupulous histological analysis. The maturation of gonads continued for the 9 weeks of treatment with a final GI value of 19.24 ± 2.95, an increase of about 340% if compared with the beginning of the treatments. The breeding conditions presented in this study proved that Echinoculture could be a feasible commercial activity and can cope in supplying healthy gametes for an eventual repopulation process.

1. Introduction

In the Atlantic and Mediterranean coasts of Europe, North Asia, New Zealand and Chile the fishery of different species of sea urchins as food resource has been performed since the beginning of XVII sec., with dramatic ecological consequences worldwide (Andrew et al., 2002). The most intensively exploited species were *Loxechinus albus* and *Strongylocentrotus spp.* whereas the Mediterranean fishery focused on the species *Paracentrotus lividus*, whose reddish-orange gonads owe their quality to the high nutritional content, especially for the considerable presence of carotenoids.

The request of gonads has significantly increased since the early 70s, reaching its peak in 1995 with a landing of 113,654 t, a value three times higher than the one reported for 1970, and it declined slightly to 100,000 t per year nowadays (Williams, 2002; FAO, 2011). Although the amount of sea urchin gonads commercialized in Europe is far lower than those attributable to Japan and US, the most important commercial activity in the old continent is localized in France. Here indeed, the overexploitation of this species between the 60s and the 70s caused a dramatic depletion in populations, with an estimated annual landing of 1000 t of live sea urchins. In the following years the annual catch declined to 250–300 t (Allain, 1972; Ledireac'h, 1987; Le Gall, 1987, 1990), reaching a complete disappearance of urchins from areas of former abundance (Southward and Southward, 1975; Régis et al., 1986). In Italy, despite the collection of this echinoid is regulated by law, *P. lividus* fishery is common in southern regions and it is performed throughout the year by illegal harvesters (Tortonese, 1965; Guidetti et al., 2004; Pais et al., 2012). As a consequence of this uncontrolled uptake, the population has been dramatically exploited in shallow subtidal rocky reef.

In this international background, an increasing number of studies aimed to improve the culturing of sea urchin has been published in the last two decades (Le Gall and Bucaille, 1989; Le Gall, 1990; Hagen, 1996; Grosjean et al., 1998; Kelly et al., 1998, Robinson and Colborne, 1998; Cook and Kelly, 2007a, 2007b), but up to the present no results have been reported to be remarkable enough to meet market demand and partially replace the steady decrease of natural stocks. Indeed, as reported by Grosjean et al. (1998), despite an annual release of 60 million of juvenile sea urchins in the sea, no significant improvement have been registered in Japan. According to the last data released by FAO (2013) the production of *P. lividus* with aquaculture systems is 10 t/year in Europe, and it has to sustain a landing activity of 108 t/year, as a consequence it is clear the urgent need of development of fast and reliable strategies aimed to respond to this over exploitation.

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Hence, in the last years our research group has focused on the assessment of a feasible strategy aimed to ensure a rapid and effective gonadal growth of healthy gametes of *P. lividus* in Recirculating Aquaculture System (RAS).

After significant preliminary results (Sartori et al., 2015), in the presented study the reliability of Echinoculture was evaluated with different biological parameters, such as fertilization and embryo development test in order to investigated the health of gametes, Gondadal Index calculation (GI) to measure the production of a commercially valuable product, and histological analysis of gonads to appraise the stage of gonadal maturation. Three possible diet treatments were tested: a maize (*Zea mays*) and spinach (*Spinacia oleracea*) diet (MSD), a macroalgae diet (MD) and a diet based on a commercial pellet normally used in aquaculture for warm-water species (Classic K®, PD).

The selection and efficacy of these diet treatments had been already tested by our research group in a previous study (Sartori et al., 2015).

The outcome of our trials would have important implications in the commercial aquaculture of this species, in addition to its usefulness in ecological studies aimed to assess a potential repopulation strategy. Thanks to our results indeed, we demonstrated the possibility to afford a continuous availability of healthy gametes and embryos for different applications outside the spawning period, which in the Mediterranean Sea generally runs from October to June (Giambartolomei, 1990).

2. Materials and methods

2.1 Sea urchin culture system

Three 200 l aquaria were set up and filled with Filtered Sea Water (FSW 0.45 μm) collected from an uncontaminated area. Each aquaria was equipped with an electronic temperature controlling device and a skimmer pump; in addition it was divided by a water tight movable septum into three smaller tanks (replica) of 65 l volume each with independent water supply. The rearing treatments were also provided of a double UV system (Tetratec UV 400) with a 10 watt low-pressure mercury vapor lamp. Seawater flow rate was kept constant at 1 l/min.

During the experimental period ammonia, nitrite, nitrate and phosphate concentrations were measured and verified every 48 h by spectrophotometer Hach Lange D3900 equipped with thermoreactor LT 230. Dissolved oxygen, pH and temperature were monitored by directly immersed sensor EUTECH PCD 650 in water. Salinity was measured by refractometer.

2.2 Experimental protocol

Adults of *P. lividus* (N = 150), with test diameter ranging from 40 to 45 mm were collected in April in a sub-littoral zone along the Tyrrhenian coast near Castiglioncello (Livorno — Italy) [43°25′31.79″ N, 10°23′37.51″ E].

Once in laboratory, organisms (N = 10) were immediately dissected to provide an initial baseline (T = 0) for measurements of both body weight and gonad index (GI). Thereafter, specimens were starved for six weeks (Grosjean et al., 1998) in order to promote the re-absorption of gonads and get them in phase regarding their reproductive cycle (Spirlet et al., 1998). Sea urchins were kept in aquaria with 12 ± 1 °C water temperature and exposed to a photoperiod 12 H L: 12 H D completely devoid of food (Grosjean et al., 1998). After six weeks, ten organisms were dissected and their maturation stage was evaluated by histological analysis. Each aquaria was divided in three replicates with approximately 50 individuals each by using glass plates, shifting the photoperiod to 10 H L: 14 H D and water temperature to 16 ± 1 °C. To promote the gonadal maturation, the effectiveness of the following three diets was evaluated: a pellet used in fish farming activities (Classic K® Hendrix S.p.A.) (PD), a natural diet based on macroalgae (MD) and a maize and spinach diet (MSD).

The biochemical composition of pellet Classic K® (Hendrix S.p.A) employed in our study, is shown in Table 1. The MD diet consisted of a mixture of the following species collected from the sampling site of sea urchins: *Dictyopteris* sp., *Padina pavonica*, *Dictyota* sp., *Ulva lactuca*, *Halopteris scoparia*, *Flabellia petiolata*, *Laurencia* sp., *Corallina elongata*, *Codium* sp. Finally, the MSD diet consisted of a mixture in equal proportion of maize kernel (*Z. mays*), previously crushed with a blender into grains of a few millimeters and chopped fresh organic spinach leaves (*S. oleracea*). Diets were administered ad libitum throughout the experiment, and prior to each food administration, fecal pellets and unconsumed nourishment were removed from each aquarium.

In order to evaluate the effectiveness of the different diets, multiple biological data were analyzed, in particular: Gonad Index (GI), histological examination of gonadic tissue; fertilization and embryo development test with a reference toxicant (Copper).

2.3 Gonad index (GI) and Histological examination.

Every three weeks, 10 organisms were let to drip for approximately 5 min, weighed and consequently dissected into body compartments (test, spines and gonad), then mean wet weights of gonads were used to calculate the gonad index (GI, Shpigel et al., 2004) as follows:

$$GI = [\text{gonads weight (g wet)}/\text{whole urchin (g wet)}] * 100$$

One in five gonads from each animal was fixed in 10% formalin to block the vital cellular activity by making insoluble structural components, stabilizing the proteins and inactivating the hydrolytic enzymes. After dehydration with alcohol solutions, roe was embedded in paraffin; 7 μm sections were dissected with a microtome and stained with Mayer's Haemalum and Eosin. Slides were then observed under a light microscope and classified as described by Byrne (1990) into six stages: stage I (recovery), stage II (growing), stage III (premature), stage IV (mature), stage V (partly spawned) and stage VI (spent); this classification is referred to the sea urchin gonads independently from the sex.

2.4 Fertilization and embryo development test

Experiments on fertilization and embryo development were performed on both reared sea urchins and animals from a natural unimpacted population (Livorno — Italy). Fertilization test was performed using as reference toxicant a standard copper solution for atomic absorption analysis [$Cu(NO_3)_2 * 3H_2O$ (1000 mg/l, Fluka, Switzerland] and FSW (0.22 μm) as negative control (Lera and Pellegrini, 2006). A volume of 100 μl of sperm solution was added to each test chamber, the final solution was then maintained at 18 ± 1 °C for 60 min. After exposure to toxicant, 1 ml of oocytes suspension was added to sperm solution; after 20 min the presence of the fertilization membrane was assessed in more than 95% of the zygotes, to ensure that fertilization had occurred homogeneously within the sample. The sperm:egg ratio was fixed at 15,000:1 with approximately 1000 eggs in a final volume of 10 ml in test chambers (Lera and Pellegrini, 2006). Adult sea urchins were induced to spawn by injecting 1 ml of 0.5 M KCl solution into the coelom through the peristome. Sperm from each male was collected dry using a Pasteur pipette, pooled and preserved at 4 °C, while oocytes from each female were shed into 50-ml beakers previously filled with 0.22 μm Filtered Sea Water (FSW) and then pooled into L beaker. Sperm density was determined by means of a hemocytometer (Thoma

Table 1
Biochemical Composition (%) of pellet Classic K® (Hendrix S.p.A.).

Composition	%
Protein	43.0
Crude fat	11.5
Crude fiber	3.2
Ash	8.0
Phosphorus	0.8
Digestible energy (MJ/kg)	14.8