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Technical note

Long-term maintenance of the sea urchin *Paracentrotus lividus* in culture

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ABSTRACT

The common sea urchin *Paracentrotus lividus* (Lamarck, 1816) is an important commercial species in the Mediterranean Sea for the consumption of its gonads (roe). This species has also long been used as an animal model in developmental biology and as an indicator in the assessment of environmental quality. In recent decades, the exploitation of this marine resource has become increasingly intensive, causing the depletion of wild stocks. The ripple effect observed in the laboratory use of this species has been the growing difficulty in finding valiant mature animals in the wild. We focused on the long-term maintenance of wild *P. lividus* and on the essential question of diet to maintain the animals and improve gonad development. The use of practical ration blocks which are nutrient-rich and show stability, easy storage and handling, resulted reduction in labor requirement and time for feeding streamlining the feeding practice. A significantly higher gonad production and a prolonged period of reproduction were obtained compared to wild caught individuals over the same period of time.

1. Introduction

The common sea urchin *Paracentrotus lividus* (Lamarck, 1816) is a regular edible echinoid, which is very widespread throughout the Mediterranean coasts and in the north eastern Atlantic, from Scotland to southern Morocco (Tortonese, 1965; Boudouresque and Verlaque, 2013). Over the years, several laboratories have chosen this species as an animal model. Molecular biology and eco-toxicology studies, which require the use of gametes and embryos at various stages of development (Giudice, 1973; Pagano et al., 1986; Pagano et al., 1993; Privitera et al., 2012), have been added to the classic studies on fertilization and development (Monroy, 1986). One of the basic requirements demanded by an experimental model is its availability throughout the year.

P. lividus living along the Italian coasts has a single reproductive period, which generally lasts from October to June with a peak from December to March. Gonads vary in size and gametogenetic state according to this annual cycle. These seasonal fluctuations lead to a limited availability of gametes at certain times of the year, which is a major limitation to using this model system in biological experimentation.

Sea urchins are also a valuable resource for the high commercial value of gonads (roe), and there is an international demand for the production of marketable quality gonads. *P. lividus* gonads are esteemed as a luxury sea food by Mediterranean countries. Due to its importance in research as an animal model and in aquaculture as seafood, much research has been carried out on this species to determine all the phases of the reproductive cycle and relate them to environmental characteristics (Byrne, 1990; Lozano et al., 1995; Spirlet et al., 1998; Sanchez-Espana et al., 2004; Sellem and Guillou, 2007; Garmendia et al., 2010). Three factors are universally cited as important to the reproductive cycle: diet, photoperiod and temperature. Copious work has been produced on the modification of the gametogenic cycle through experimental manipulation while rearing the sea urchins in confinement, to obtain gonads with features that increase their commercial value (Lawrence et al., 1997; Walker and Lesser, 1998; Spirlet et al., 2000; Shpigel et al., 2004; Shpigel et al., 2005; Kirchhoff et al., 2010; McCarron et al., 2010; Marsh et al., 2013; Sartori et al., 2015). This field is still underdeveloped because of each species of sea urchin has its own environmental or chemical cue (Kirchhoff et al., 2010). Food appears to play a pivotal role in the regulation of the reproductive cycle and it has been attested that the gonadic growth is strongly correlated with the availability, quantity and quality of food (Fernandez et al., 1995; Boudouresque and Verlaque, 2013 and ref. therein). Several studies have shown that sea urchins fed with high rations of good quality food improve their reproductive capacity. Therefore, one of the critical aspects in maintaining productive individuals in the laboratory is the determination of an optimal or at least efficient feeding regime.

This study was at first addressed towards the enhancement of the research status of *P. lividus*, improving their use as laboratory animal. A coveted result in this latter direction is control the reproductive cycle, maintaining individuals in a “ready to spawn” condition. This allows us to quickly obtain gametes (on demand) for their application in different

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fields, such as ecotoxicology and developmental biology. Thus, the objective of this paper is to describe a simple system and management focused mainly on the feeding practice to induce and control gonadal growth in a rapid timespan.

2. Methods

2.1. Sea urchin collection

Sea urchins were hand collected by scuba-diving from a rocky site of the gulf of Naples, along the southern Tyrrhenian coasts of Italy. A group of 100 *P. lividus* was collected in September 2013 (week 0) from a natural population and reared in a culture system for 18 weeks (rearing test period). Other groups of 10 individuals were collected from the field population at intervals of 6 weeks (week 6; week 12; week 18) to establish the population condition in the wild and for comparison with cultured population. Sea urchins were chosen to be relatively uniform in size (diameter 40.6 ± 1.5 mm, mean ± SD), and presumably in age, to minimize variation in growth potential, feed consumption potential and initial gonad weight. Specimens with mean diameters above 40 mm normally correspond to adult stages.

Captured animals were placed in a cooler and were carried to the laboratory under moist conditions within 2 h. In the laboratory, the sea urchins were measured and acclimatized for 1 week to confined rearing conditions before starting the feeding regime in the culture system.

2.2. Culture system

The culture system (Fig. 1) was addressed to the long-term maintenance of sea urchins and was tested during this study (rearing test period). It is still running without major changes at the Marine Resources for Research Facility of the Stazione Zoologica of Naples.

Sea urchins were held in suspended baskets (50 cm × 35 cm × 25 cm) in a recirculating system that received low flows of make-up seawater (2–3%) for compensating water losses associated with routine tank cleaning. Our system consisted of 2 square tanks (500 L), each containing up to 4 suspended baskets. Stocks up to 50 sea urchins can be maintained in a single basket. A centralized Life Support System (LSS) maintain optimal sea water conditions. This consisted of a reservoir equipped with cartridge filter, protein skimmer, ultraviolet sterilizer and refrigerator; a centrifugal pump recirculated natural seawater at a rate of 7.5 L min^{-1} to each tank. Aeration in the tanks provided additional water movement and air supply for the urchins.

Dissolved oxygen (> 90% saturation), pH (8.0 ± 0.1), and salinity (38.0 ± 0.2) were measured 3 times a week by a multi-parameter probe (YSI-85, USA). Seawater temperature, which was recorded daily, was 16 ± 1 °C and the photoperiod was set for 12 light: 12 dark. Ammonia, nitrite, nitrate and phosphate concentration were checked every week by a spectrophotometer (HACH USA, DR/2500) and values matches parameters required for a healthy recirculation system (Huguenin and Colt, 2002). Twice a week the tanks were cleaned removing uneaten food and fecal pellets by siphoning.

Table 1
Ingredients and the ratio at which they were mixed to prepare the Ration Blocks of Food (RBF) and proximate nutrient analysis (per g dry matter). Energetic level of food was calculated as gross energy by burning sample of wet food in a bomb calorimeter.

Ingredients	Ration (%)
Algae	38
Mussels	25
Corn	17
Supplements	12
Agar-agar	8
Nutrients	% dry matter
Carbohydrate	21
Crude protein	20
Crude fat	1.5
Crude fibre	9
Minerals	14
Ash	20
Gross Energy (MJ Kg^{-1})	12

2.3. Feeding practice

We drew up a mixed diet based on animal meal (seafood) and vegetable meal (natural algae) and used this to formulate a prepared "ready to use" food. We produced a compound combining dry powdered ingredients with agar–agar as binder (modified by Nagai and Kaneko, 1975), to form a moist pellet with sea water. The resulting mixture was molded before solidifying in Plates 1 cm thick, from which Ration Blocks of Food (RBF) were cut by hand. Diet ingredients included mussel meal, corn, natural macro-algae (*Ulva lactuca*) and microalgae (*Spirulina platensis*), fish oil and mineral supplement (calcium carbonate) (Table 1). Nutritional analysis was conducted at the Laboratory "ANALISIS" of Angri (Salerno; Italy). The formulated food was analyzed in duplicate to determine its crude protein, fat, moisture, ash, fibre, carbohydrate and gross energy contents (Table 1) using protocols according to the regulation of the DM 18/03/09 (Directive 2008/100/CE).

We shaped RBF weighing ~1 g to feed sea urchins in culture twice a

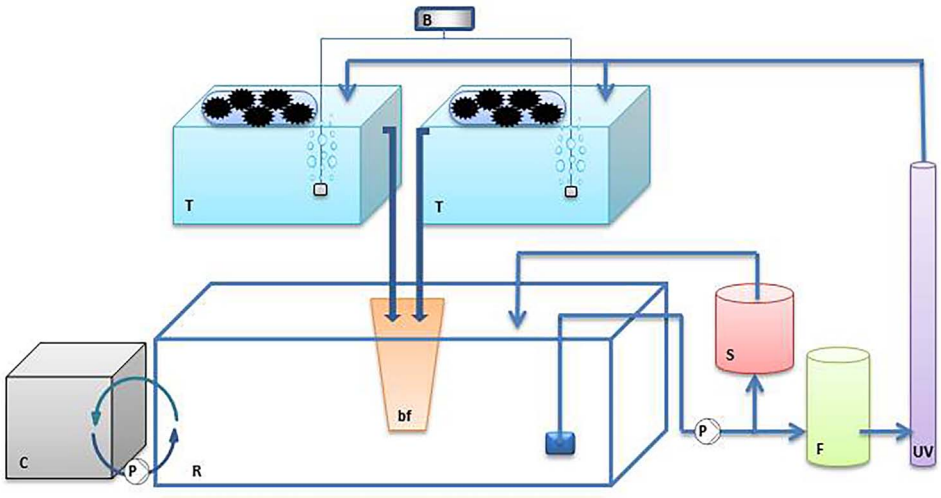

Fig. 1. Schematic diagram of sea urchins culture system. **T** = Tanks; **C** = Chiller Unit; **R** = Reservoir; **bf** = bag filter; **S** = Protein Skimmer; **F** = Cartridge Filter; **UV** = Ultraviolet sterilizer; **B** = Blower; **P** = Centrifugal Pump.