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Effects of food type and feeding frequency on the performance of early juveniles of the sea urchin *Loxechinus albus* (Echinodermata: Echinoidea): Implications for aquaculture and restocking

P. Francisco Cárcamo [1]

Departamento de Medio Ambiente, Instituto de Fomento Pesquero, Doctor Marín 340, Coquimbo, Chile

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ABSTRACT

Sustainable aquaculture and restocking of the sea urchin *Loxechinus albus* will demand a reliable source of high-quality juveniles. Culture techniques for *L. albus* must be refined on several levels to scale up to commercial production. In this study, the effects of different natural foods (six algal foods and one animal food) and the feeding frequency (three regimes: one continuous and two intermittent) on the growth and feeding of *L. albus* juveniles with a test diameter of ca. 5 mm belonging to a single 5-month-old cohort were investigated. Juveniles fed with Rhodophyta: *Porphyra* sp. and *Gracilaria chilensis* grew significantly faster (wet body weight and test diameter) than those fed other foods, although these food items are not frequently observed in the gut contents of wild populations. Juveniles fed with *Macrocystis pyrifera* showed the lowest growth rates. Differences in ingestion rates and the food conversion ratio (FCR) were principally associated with organic matter and protein content in foods. Although *L. albus* have been described as generalist herbivores, in this study, they ingested an animal food (marine sponge) and were able to grow significantly better than juveniles fed with *M. pyrifera*. Juveniles did not show evidence of a compensatory mechanism with a decrease in the frequency of food availability. The growth, ingestion rates and FCR in early juveniles were positively related with feeding frequency, suggesting that continuous feeding is the best and most efficient dietary treatment in terms of growth. *Porphyra* sp. and *G. chilensis*, as single species diets, are promising alternatives for the development of cultures of *L. albus* juveniles; however, further studies are required to compare the efficient use of algae as food (e.g., versus artificial feeds).

1. Introduction

The edible sea urchin, *Loxechinus albus* (Molina, 1782), is the only commercially valuable sea urchin species in Chile, and its fishery is the largest in the world (Moreno et al., 2007). This species is distributed from the coast of Peru (6° S) to the southern tip of Chile (55° S) and from the intertidal zone down to 106 m in depth (Larraín, 1975; Moreno and Molinet, 2013). Along the Chilean coast, it is harvested exclusively by artisanal fishermen and is a species of great economic and social importance (Moreno et al., 2007). Landings have decreased from 60,000 tons in 2002 to 29,000 tons in 2012.[2] The depletion of traditional fishing grounds has encouraged the migration of fishermen to distant parts of southern Chile in search of new natural beds to exploit (Moreno et al., 2007, 2011; Stotz, 2004). The overexploitation of this sea urchin under the present management strategies makes its sustainability uncertain over the long term (Moreno et al., 2007; Stotz, 2004). However, Chilean fishermen have recently shown a great interest in the implementation of sea urchin restocking programs (Cárcamo, 2004b). For instance, pilot experiments in which hatchery-reared juvenile sea urchins are experimentally seeded have been conducted independently in management areas for benthic resources; however, the outcomes have been uncertain (Jerez and Figueroa, 2007). Several studies have attempted to develop techniques for *L. albus* culture, including larval and juvenile production (Bustos et al., 1991; Cárcamo, 2004b; Cárcamo et al., 2005; González et al., 1987; Zamora and Stotz, 1994) and enhancement of gonad quality (Cárcamo, 2004a; Lawrence et al., 1997; Olave et al., 2001). However, one bottleneck to development of the urchin culture that has been identified in a number of countries is the long period required to rear urchins to market size (Bustos et al., 1991; Lawrence et al., 2001).

The quantity and quality of food (Fernandez and Boudouresque, 2000; Frantzis and Grémare, 1993; Lowe and Lawrence, 1976), and the feeding frequency (Lawrence et al., 2003) can influence the physiology and growth of sea urchins. *L. albus* is one of the most important benthic herbivores in intertidal and shallow subtidal rocky environments along the Chilean coast (Vásquez, 2007). Some studies have investigated feeding and nutritional ecology in adults of *L. albus* (Bückle et al., 1980; Contreras and Castilla, 1987; González et al., 2008; Vásquez et al., 1984) and in juveniles greater than 20 mm in test diameter (González

E-mail address: francisco.carcamo@ifop.cl.
[1] Tel.: +56 51 2544730; fax: +56 51 2544313.
[2] Review of landings according to official information provided by the Chilean National Fisheries Service (www.sernapesca.cl).

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et al., 1993, 2008). However, studies examining feeding and growth in early juveniles are scarce (Bustos et al., 1991; Zamora and Stotz, 1994), and there are no published studies on the effect of different diets and feeding frequency on early juveniles of *L. albus* (i.e., individuals lower than 10 mm). Therefore, an investigation of the diets and nutritional requirements needed to maximize somatic growth in juvenile sea urchins to achieve optimal growth rates has become a necessity to promote both culture (Daggett et al., 2005; Lawrence and Lawrence, 2004; Pearce et al., 2005) and restocking programs.

The aim of this research was to investigate the effects of different natural foods and feeding frequencies on the growth and feeding of *L. albus* juveniles, including a discussion on the implications of these results for the development of aquaculture and restocking programs.

2. Materials and methods

2.1. Sea urchin juveniles

The sea urchin juveniles used in this research were hatchery-reared individuals. The cultures were maintained at the Hueihue Mariculture Center on the island of Chiloé, Chile (41°53′ S; 73°30′ W). Larval and post-settled sea urchin culture was performed based on the methods described by Bustos et al. (1991) and Cárcamo (2004b). Individuals with a test diameter of ca. 5 mm belonging to a single 5-month-old cohort were used. Before the experiments, their diet consisted mainly of benthic diatoms from the genera *Navicula* and *Nitzschia* (Pennales, Bacillariophyceae) that colonized glass fiber plates arranged in tanks for mass culture (Cárcamo, 2004b).

2.2. Experimental systems

Sea urchin juveniles were maintained in individual cylindrical plastic containers (50 mm diameter and 100 mm height) with a 1-mm-mesh bottom to keep the individuals and food in and allow wastes to pass through. The containers were arranged in a 200-L rectangular tank, and 0.5-μm-filtered seawater was constantly provided. Prior to the experiments, the sea urchins were starved and held for 2 weeks in experimental systems to allow acclimation to laboratory conditions, to clear the gut of ingested food and to standardize their nutritional condition (Minor and Scheibling, 1997).

2.3. Experiment 1: effect of food type on the growth and ingestion rate of sea urchin juveniles

The first experiment was conducted from the 11th of May to the 10th of August, 2008 (90 days). A total of 72 sea urchin juveniles with test diameters from 4.1 to 5.9 mm were divided into eight food treatment groups with nine individuals (replicates) per group. Seven natural foods were tested (six algal diets and one sponge diet) as well as a control starvation treatment. Algal diets corresponded to the fresh fronds of the Rhodophyta: *Gracilaria chilensis*, *Sarcothalia crispata*, and *Porphyra* sp.; the Chlorophyta: *Ulva* sp.; the Phaeophyta: *Macrocystis pyrifera*; and a mixed diet composed of *M. pyrifera*, *Ulva* sp. and *S. crispata* (50%, 25% and 25%, respectively). The sponge diet was the sponge *Iophon tubiforme*, which is commonly found as biofouling agent in mussel and salmon farms on Chiloé Island (P.F. Cárcamo, personal observation). The sea urchins were fed *ad libitum*, with uneaten food removed and fresh food added every week. Control individuals were not given food during the experiment. Containers with sea urchins were randomly distributed in the tank. Additionally, three replicates of each food treatment were placed in containers without sea urchins to control autogenic changes in mass. Seawater was provided at a flow rate of 140 ± 8 ml/min per container, the water temperature was 10.5 ± 0.5 °C, the salinity was 32.0 ± 0.5 ppm, and a light regime of 12:12 (light: dark) was applied (Hammer et al., 2004; Lawrence et al., 2003).

2.4. Experiment 2: effect of feeding frequency on the growth and ingestion rate of sea urchin juveniles

The second experiment was conducted from the 14th of May to the 13th of August 2008 (90 days). A total of 27 sea urchin juveniles with test diameters from 5.2 to 6.1 mm were divided into three feeding treatment groups with 9 individuals (replicates) per group. Three feeding regimes were tested: *S. crispata* available *ad libitum* throughout the experiment (fed continuously or 100% of the time), *S. crispata* available *ad libitum* for 2 days every 5 days (fed for 2 days/starved for 3 days or 40% of the time), and *S. crispata* available *ad libitum* 2 days every 10 days (fed for 2 days/starved for 8 days or 10% of the time). The control treatment corresponded to individuals kept without food. Containers with sea urchins were randomly distributed in the tank. Seawater was provided at a flow rate of 145 ± 9 ml/min per container, the water temperature was 10.2 ± 0.4 °C, the salinity was 32.0 ± 0.5 ppm, and a light regime of 12:12 (light:dark) was applied.

2.5. Measurements and statistical analyses

In both experiments, the growth was estimated monthly by measuring the test diameter (Nikon V-12 profile projector, ±0.01 mm) and the wet body weight (±0.001 g). Daily ingestion rates were calculated by subtracting the amount of uneaten wet food from the amount of wet food provided. The uneaten food was dried at 60 °C for 3 days and weighed to calculate the amount of dry food eaten. The organic content in foods was determined by ashing 5 samples for 4 h in a muffle furnace at 500 °C. The food conversion ratio (FCR) for each individual was calculated as total food intake (g dry weight/urchin)/total weight gain (g wet weight/urchin) (Hammer et al., 2004; Heflin et al., 2012).

The growth (diameter and weight) and ingestion rates were analyzed using repeated-measures ANOVA with food as a factor and experiment time as a repeated factor. A one-way ANOVA was used to test for differences in test diameter and wet weight among sea urchins in each of the treatments at the beginning and end of both experiments. Tukey's post hoc test was used to identify the treatment means that differed significantly (Zar, 1996). The homogeneity of variances was evaluated using Levene's test, and the normality of the data was evaluated using the Kolmogorov–Smirnov test.

If the data on total increase in test diameter and wet weight, the total wet and dry food ingested, and the FCR failed to achieve normality and/or homogeneity of variances and could not be transformed, then a Kruskal–Wallis non-parametric one-way ANOVA on ranks followed by Dunn's post hoc test was used to determine differences among the treatments (Zar, 1996). All data are presented as the mean ± standard error (SE).

3. Results

3.1. Experiment 1

The water content and organic matter varied among the six food treatments (Table 1). *M. pyrifera* had the highest water content, and the sponge *I. tubiforme* had the lowest value (89.5% ± 0.29% and 76.1% ± 1.10%, respectively). For the organic matter, *Porphyra* sp. had the highest value and the sponge *I. tubiforme* had the lowest value (77.5% ± 0.90% and 24.3% ± 1.26%, respectively).

The survival rates were 100% for all the treatments. There were no significant differences in the initial size (test diameter and wet body weight) among treatments (one-way ANOVA, $P > 0.05$, Figs. 1 and 2). Repeated-measures ANOVA showed a significant effect of food type, time, and the interaction between food and time on test diameter, wet body weight, and ingestion rates ($P < 0.05$ for factors and interaction in all dependent variables). The final test diameter (at day 90) varied significantly among foods (one-way ANOVA, $P < 0.05$, Fig. 1 and Supplementary Fig. S1). Sea urchins fed with *Porphyra* sp. and *G. chilensis* had