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Note

Optimizing food distribution in closed-circuit cultivation of edible sea urchins (*Paracentrotus lividus*: Echinoidea)

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Abstract – In the framework of echinoid cultivation, whose objective is to succeed in continuously producing large amounts of edible sea urchins (*Paracentrotus lividus*) under controlled conditions (aquaculture), gonadal growth is to be optimized. Among the various parameters influencing the production of roe, the quantity of food distributed was tested for optimization. After a 1-month fast, echinoids were fed artificial food pellets (enriched in soybean and fish proteins) for different periods of time over 48 h, the food thus being available ad libitum for 8, 16, 24, 32, 40, and 48 h; the cycles were repeated for a month. The results show that the quantity of food intake and the gonad index peak after about 35 h of food availability. This suggests food should be distributed discontinuously for optimal gonad production and minimal waste. © Ifremer/Elsevier, Paris

Aquaculture / food ration / gonad growth / artificial diet / sea urchin

1. INTRODUCTION

In the last decades, there has been a continuous increasing demand for sea urchin roe. As a consequence, in European coastal waters, the edible sea urchin *Paracentrotus lividus* has been overfished [1, 17, 19, 22]. Aquaculture seems to be the ideal alternative to supply the market. In echiniculture, the major objective is to obtain sea urchin roe all year long. Therefore, the growth of the gonad has to be optimized. The food used in the present study is a prepared diet that has been proven efficient for gonadal production in *Paracentrotus lividus* [11] and other species [6, 8–10]. Thus, our research focused on measuring the optimal period of food distribution so as to obtain the largest gonadal production rate with a minimal waste (both faeces and leftovers).

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2. MATERIAL AND METHODS

2.1. Material and culture procedure

Individuals from a single cohort of *Paracentrotus lividus* (diameter 25 ± 2 mm) raised from seed at the 'Centre régional d'études côtières' (CREC, Luc-sur-Mer, France) echiniculture facilities were used. Prior to the experiment, the sea urchins were starved for a month to make sure the gonads were empty, as they are a storage organ. During the experiment, the individuals were kept in specific rearing structures at 16 ± 1 °C and under a 12/12-h photoperiod. One batch (each batch consisting of 4 groups of 9 individuals) was measured for initial references. One other batch was left without food throughout the experiment to serve as a control. The other batches were fed ad libitum for

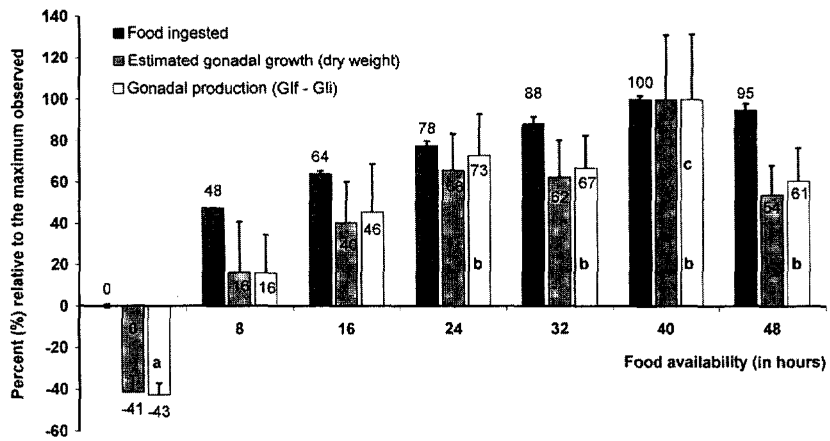


Figure 1. Feeding, gonadal growth and gonad production relative to food availability. The values are expressed in percent relative to the maximum observed (transformed from original data in g). The results of the Mann-Whitney U test for the effect of food availability on gonadal production shows that (a) the starved batch (0 h) is significantly different from all others ($P = 0.021$); (b) the batch with 8-h food availability is similar to the one with 16-h food availability but significantly different from all others ($0.021 < P < 0.083$); (c) the batch with 40-h food availability is significantly different from the ones with 8-h and 16-h food availability ($P = 0.043$ and $P = 0.083$ respectively). Bar: standard error.

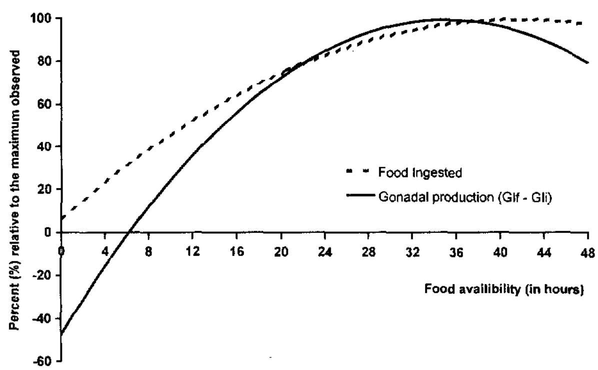


Figure 2. Polynomial regression curves for food intake and gonadal production (*GI*). Sea urchins must feed for at least 7 h every two days to start any gonad production. Feeding increases quite proportionally until it reaches a peak. Maximum food ingested is when food is available for 42/48 h. The maximum *GI* increase is when food is available for 35/48 h.

periods of 48, 40, 32, 24, 16 and 8 h during each 48-h time period. After each period, the food remaining was removed, and replaced at the appropriate time, with a precise quantity of new food. The experiment lasted 1 month (30 days).

2.2. Food used and methods

The food used was artificial extruded food containing soybean protein, in the form of cylindrical pellets 8 x 15 mm. This food contains mainly wheat, soybean and minerals (for exact composition see [10]). The immersed weight of the individuals was measured by group for each batch initially, prior to the first feeding. Measuring the immersed weight allows the assessment of the apparent weight of the calcareous skeleton; the other organs of the sea urchin (i.e. gonads, digestive tract and coelomic fluid) have about the same density as sea water. The measure is standardized according to salinity and temperature. The advantage of this procedure is the minimization of the error in the estimation of the initial weight of the animals used in the experiment.

Before each feeding, the food was weighed and the leftovers were also weighed after drying for 48 h in an incubator at 70 °C. At the end of the experiment, all the individuals were dissected, one gonad was fixed in Bouin's fluid for histology, then soma and gonads were dried and weighed. The gonad weight was corrected for the one-fifth portion removed.

We estimated gonadal growth by assessing the difference between the final gonadal index (GI_f) and the gonadal index of the initial batch (GI_i), to eliminate the bias caused by variation of somatic weight, as follows: with *Gw* and *Sw* being the dry weight of the gonads and the soma respectively:

$$\Delta GI = GI_f - GI_i \text{ with } GI = \frac{Gw}{Sw + Gw} \times 100$$

The Mann-Whitney U test was used to assess the effect of the quantity of feeding on the gonadal index. The total ingested food (*F*) was calculated as follows:

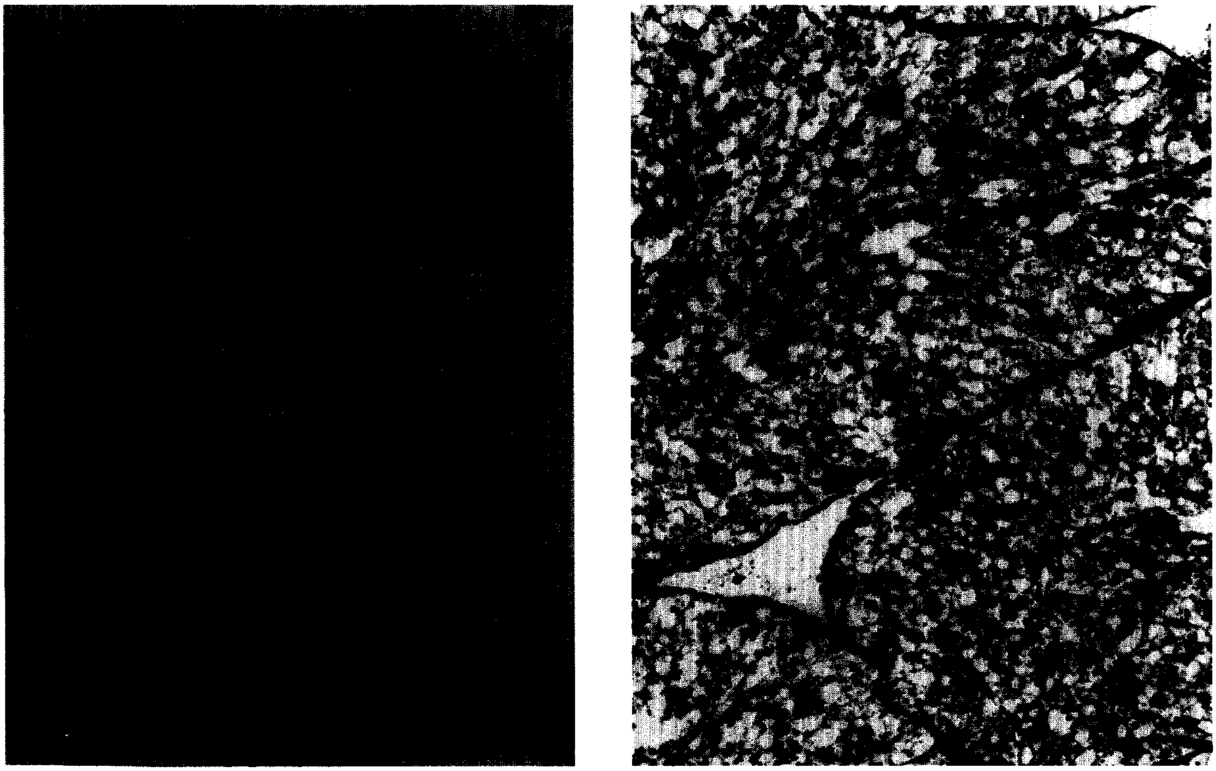
$$F = (\Sigma P_i - \Sigma L_i) \times k$$

with *P* the portions distributed, *L* the leftovers, and *k* a correction factor that takes into account the partial degradation of the food in the water.

The maturity index (*MI*) was established following an 8-stage scale [20], and the effect of food availability was tested by Watson's U^2 test for circular data.

3. RESULTS

The sea urchins did not reach maturity during our experiment and hence, did not spawn so the results of *GI* and gonad production are not biased. As there are no differences between sexes (same gametogenic pace and *GI* values, pers. obs.), the results were pooled.



(a) (b)

Figure 3. (a) Gonadal aspect after starvation; the lumen of the acini is empty; (b) gonad after a month of feeding, intense network of nutritive cells which have filled with nutrients can be seen; (X 100, Masson's trichrome).

3.1. Feeding

The sea urchins fed regularly from the start of the experiment. *Figure 1* summarizes the results. The values are represented as the percent relative to the maximum observed so that the feeding, gonad production and gonad index can be readily compared as they are not on the same scale. As the gonad index (final data) is biased by the growth or regression (negative growth) of the soma, only the gonadal production was taken into account *(figure 1)*.

There are significant differences in gonadal production dependent on the duration of food availability. The starved individuals' gonads regress. The individuals which have food available for 40 h have the largest gonadal production (Mann-Whitney U, $P < 0.1$). The total ingested food and the gonad production are logically correlated (Pearson, $r^2_s = 0.872$).

Figure 2 shows the quadratic regression curve fitting the data (with a r^2 of 0.744 for the gonadal production and 0.969 for total food ingested, both highly significant, $P < 0.0001$). The curve allows the prediction that the maximal gonadal production corresponds to 35 h of food availability. The time corresponding to the highest rate of food ingestion is 42 h. The curve also shows that the sea urchins must have at least 7 h of feeding time every 2 days to be able to develop gonads. Under that threshold, the animals use up their reserve material and the weight of the gonads actually continues to decrease.

3.2. Gonad and maturity indices

The gonad index follows the same tendency as the other data but the effect of food quantity is not as manifest and significant. The values are comprised between 1.41 % for individuals with 8-h food availability and 2.23 % for individuals with 48-h food availability, with 0.32 % for the control batch. One can note that the gonad index is biased by the variation of the weight of the soma during the experiment.

The maturity index shows little variation ($1.47 < MI < 1.88$) although the value for 16 h of food availability (1.88) is significantly higher than the others (Watson's U^2, $P < 0.05$). The aspect of the gonads change from the initial state; their reserve tissue shows somatic cells filled with reserve material *(figure 3)*.

4. DISCUSSION AND CONCLUSION

Recovering leftovers every other day allowed us to notice that feeding was relatively constant. This agrees with the results on *Lytechinus variegatus* where frequency of feeding had no effect upon the feeding rate [7]. However, a more recent study [15] showed that the feeding rate of *Strongylocentrotus droebachiensis* was higher for individuals fed ad libitum than for those being rationed. Even if the feeding is approximately constant, the total ingested food (*F*) follows a quadratic curve with a maximal value for 42 h of food availability, meaning that the sea urchins reach their maximum degree of feeding without having food accessible at all times. This could correspond to the approximate minimal gut retention time, which is estimated at 36–48 h [5] and 30–50 h [16]. Thus, in this experiment, the feeding does not seem to be periodic since food is consumed at all times. This disagrees with the findings of Caltarigone et al. [4] and Minor and Schliebling [15] who found that *Paracentrotus lividus* displayed cyclic feeding, with a duration lasting several days and which differed from individual to individual.

The gonadal production is highest for 35 h of food availability meaning that food conversion rate and/or nutrient storage are not directly proportionate to the quantity of food ingested as far as the gonads are concerned. After starvation, the sea urchins first tend to restore their gut wall [2] and second to store nutrients in the gonads to survive further food shortage and possibly to produce gametes. This might explain why the maximal gonadal production is reached before the maximal feeding rate. When abundant, or over a certain threshold, nutrients are allocated both to the soma and the gonads for growth and gametogenesis (see [12, 17] among others and [13] for *P. lividus*).

The results for the gametogenic aspect are not significant. This is most probably due to the duration of the experiment. The sea urchins need a certain period of time to refill their gonads after starvation. In addition, the gonads have to contain a certain amount of nutritive material to make the production of new cohorts of gametes possible [3, 17, 20]. The gametes are thus found here in a recovery stage, presenting a typically heavy network of nutritive phagocytes filled with nutrients. We could not identify a sexual disparity in the growth of gonads as described previously [21] for *Strongylocentrotus droebachiensis*.

Our results allow us to conclude that in aquaculture, the sea urchins need not be fed continuously to produce large gonads. Thus, it is useless to feed them in excess. The ideal quantity of food can be estimated in order to prevent any waste and require less cleaning of the rearing structures. They also tell us that this basic method, provided the type of food is well assimilated and that other parameters are optimized, is an efficient way of producing large gonads in rearing conditions.

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