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LAND-BASED, CLOSED-CYCLE ECHINICULTURE OF *PARACENTROTUS LIVIDUS* (LAMARCK) (ECHINOIDEA: ECHINODERMATA): A LONG-TERM EXPERIMENT AT A PILOT SCALE

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ABSTRACT Today, most sea urchins fisheries worldwide must deal with overexploitation. Better management of exploited field populations and/or aquaculture is increasingly considered necessary to sustain sea urchin production in the future. In this context, we evaluate the potential of land-based, closed-cycle echiniculture. A long-term experiment with the edible sea urchin *Paracentrotus lividus* has been done on a pilot scale. The process allows total independence from natural resources, because the entire biological cycle of the echinoids is under control (closed-cycle echiniculture), and all activities are performed on land. Furthermore, a method has been set up to control the reproductive cycle with the aim to produce marketable individuals all year long. Performances obtained on each stage of the rearing process are quantified and analyzed. Overall, the results of this experiment are promising; however, some problems remain to be solved before we can claim profitability. The most important finding is that land-based, closed-cycle echiniculture is a potential viable supplement to fisheries to sustain worldwide sea urchin roe production.

KEY WORDS: Sea urchin, *Paracentrotus lividus*, aquaculture, larval culture, metamorphosis, growth, roe enhancement

INTRODUCTION

Depending upon their respective gastronomic cultures, people consider sea urchin gonads (both male and female gonads are collectively referred to as roe) as either a fine and delicate seafood or as absolutely inedible. However, its economic value is well established given the price consumers are willing to pay. The wholesale price of live sea urchins in France ranges from 30 to 120 FF/kg (price range in the 1990s at Rungis, Paris) and fresh roe in Japan from 6,000 to 14,000 ¥/kg (price in Japan in the 1990s, see Hagen 1996). Both market prices are roughly equivalent in terms of fresh roe, making sea urchin roe one of the most valuable seafoods in the world. In both markets, the lowest prices are those of imported sea urchins, which are considered to be of poorer quality.

The most important market, Japan, imports approximately five thousand tons of sea urchin gonads per year, the equivalent of 40 to 50 thousand tons of live sea urchins (Hagen 1996). According to the same author, the Japanese consume approximately 60,000 tons of whole sea urchins per year. The second largest consumer is France, with an annual consumption of approximately 1,000 tons of whole sea urchins (Le Gall 1990).

Increasing demand for sea urchin roe and a steady rise in price have led to worldwide intensification of sea urchin fisheries (Conand and Sloan 1989, Le Gall 1990, Saito 1992), which has now (1998) probably reached its maximum. This production cannot be sustained at current levels, because the declining productivity of overexploited existing stocks can no longer be compensated by harvest of new stocks, as was possible over the last three decades (most exploitable natural populations have already been fished). In Japan, this decline occurred despite the development and implementation of extensive domestic fishery enhancement techniques, which include the annual release of 60 million juvenile sea urchins into the wild (Saito 1992, Hagen 1996). Consequently, the worldwide supply of high quality sea urchin roe will be unable to meet market demand unless commercial sea urchin aquaculture develops to partially replace the steady decrease in natural captures.

Aquaculture of echinoderms, including sea urchins and sea cucumbers, is known as echinoculture (Le Gall and Bucaille 1989, Le Gall 1990, Hagen 1996). We prefer to use the term echiniculture to describe sea urchin aquaculture exclusively (Echinoidea); thus, it is more accurate in this context. This activity is not yet fully developed. Maintenance or rearing of sea urchins in the laboratory has been successfully performed for different species (Hinegardner 1969, Fridberger et al. 1979, Cellario and Fenaux 1990). Several different processes are being experimented on a larger scale, ranging from sea urchin ranching (cultivation in the field, see Fernandez and Caltagirone 1994, Fernandez 1996), to land-based systems (Le Gall and Bucaille 1989, Le Gall 1990, Fernandez 1996) or polyculture (sea urchins cultivated in cages with fish, see Kelly et al. 1998). Nevertheless, considering the limited carrying capacity of natural sites that are already largely exploited by fisheries, only land-based or cage techniques will help to sustain worldwide sea urchin roe production. Similarly, only cultivation processes totally independent of natural stocks; that is, by controlling the complete life cycle of the echinoid, will lower the pressure imposed by fisheries upon natural populations. In this context, this paper presents a 7-year experimental rearing method to produce the edible sea urchin *P. lividus* on a pilot scale, and discusses the biological and technological issues that emerged from this cultivation method.

MATERIAL AND METHODS

The aim of land-based, closed-cycle echiniculture is to get maximum control over each phase of the sea urchin's life cycle by controlling major environmental parameters (temperature, photoperiod, water quality, quality and quantity of food). A land-based system has advantages over rearing methods performed directly in the sea. The greatest of these is the ability to control the whole life cycle of the animal (closed cycle), thus the sea urchin never depends, at any stage, on a supply of animals originating from the field.

The method used here is adapted from Le Gall (Le Gall and Bucaille 1989, Le Gall 1990) with some fine-tuning and modifications that allow a routine output of sea urchins on a pilot scale. An experimental facility was set up at the Centre de Recherche et d'Etude Côtière (CREC, Normandy, France) in which several generations of sea urchins were reared according to a thoroughly defined experimental procedure.

Pilot Echiniculture Facility

The experimental facility includes a hatchery (30 m^3) and a cultivation room (160 m^3). The hatchery is equipped with 11 200-L larval rearing tanks (see below) and a system for phytoplankton production (classical devices for large-scale production).

The cultivation room is insulated, thermoregulated at 22°C ± 1°C, correctly aerated, and exposed to a 12h/12h photoperiod. It is equipped with 10 autonomous rearing structures with either three or six superposed 4-m long and 60-cm wide ponds called toboggans. Each set of toboggans hangs over a reserve/settling tank of the same length, 80 cm wide and 80 cm deep. The water depth in the toboggans varies between 5 and 10 cm. A centrifugal pump transfers water from the reserve tank to the top level, with a flow of 8 to 10 m^3/h (4 to 5 m^3/h for the pregrowth structure, see below). The water then recirculates by gravity from one level to the other (each toboggan has a gentle slope to help water run into it and is connected to the previous and the next one at its opposite ends, see Fig. 1). This device, specifically designed for sea urchin cultivation, optimizes both the surface available for the postmetamorphic individuals and the water current around them. It also facilitates access to the animals and their visual control. The 10 rearing structures are organized as follows:

(1) One pregrowth structure of 3 toboggans with a capacity of 1,500 L of circulating water thermoregulated at 20°C ± 1°C. The water is renewed at a rate of 150% per day. This structure can hold a biomass between 0.2 and 1 kg/m^2 of toboggans.

(2) Two growth structures made of six toboggans each. The capacity of each structure is 3,000 L of circulating water thermoregulated at 18°C ± 1°C and with a water renewal ranged between 100 and 600% per day, depending upon the density of sea urchins present in the structures. These structures can hold a maximum biomass of 7 kg of sea urchins per m^2 without supplemental water filtration.

(3) Seven experimental/conditioning structures of three toboggans each with a capacity of 1,500 L of circulating water. These are isolated from one another so they can be thermoregulated individually from 10°C to 25°C, and each has up to six different photoperiods (a dark separation divides the toboggans in their center). An electronic system allows the transition of light to darkness and vice versa, thus simulating dawn and dusk. The rate of water renewal can be fixed between 50 and 600% per day. Biomass varies following criteria imposed by the experiments.

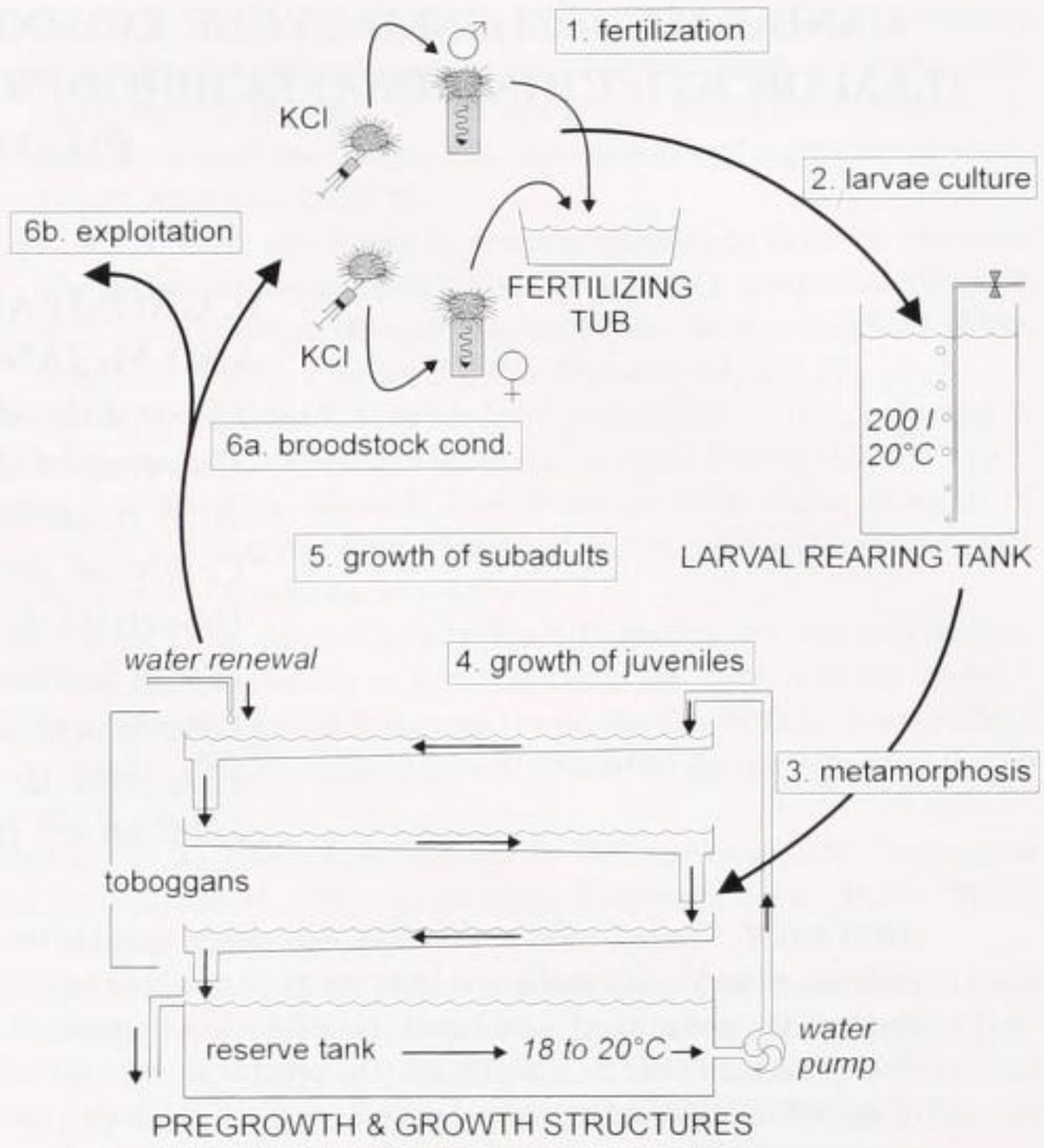

Figure 1. Overview of the closed-cycle process and devices used to produce sea urchins on land at a pilot scale.

Additional devices are grouped in a technical room containing a central thermoregulation system (a thermorefrigerating pump providing either cold or hot water to the heat exchangers that equip the rearing structures), a water pumping and filtration system, an emergency electric generator, and a central alarm. The water is pumped directly from the sea at high tide and is stored in a reservoir of 60 m^2. It is filtered before being used (30-μm mesh cartridge mechanical filtration, followed by a 14 m^3 biological filter and two settling tanks of 8 m^3 each).

Origin of the Animals

The species cultivated is *Paracentrotus lividus* Lamarck (1816). This species is found all along the European coast from the northern Atlantic Irish coast to the Mediterranean Sea. All individuals used come from a single population located in Morgat, Brittany, France. Some were directly collected in the small tide pools that spread all along the rocky shores of Douarnenez Bay (emerged only during high coefficient tides). The remaining animals come from artificial fertilizations in the laboratory and were grown in the structures [cross fertilizations of first (F1) or second (F2) generation of sea urchins collected in the field]. By so doing, the age and the parental origin of the F1 and F2 sea urchins are known precisely.

Rearing Method

Aiming at closely matching the echinoid requirements along their life history and minimizing technical constraints, the dissociation of the whole rearing cycle into seven stages is essential (Fig. 1). These stages are: (1) fertilization; (2) larval culture; (3) metamorphosis; (4) growth of juveniles; (5) growth of subadults;

and (6) growth of adults, which is further divided into (6a) conditioning for marketing of roe (exploitation); and (6b) providing gametes (broodstock).

Stage 1

Fertilization is performed using gametes issued from healthy animals that restored their gamete potential as described below in broodstock conditioning (stage 6b). The gametes are obtained by stimulating the parents to spawn with 0.5 N KCl (injection of 50 μL per g of body weight through the peristomial membrane). The gametes of each individual are collected in a small jar of 50 mL in 20°C filtered natural seawater (on a 1 μm cartridge filter, referred to hereafter as "larval rearing water").

When the spawning is over, the volume of the gametes is evaluated. The ova of a single female are transferred in a fertilization tub; that is, a shallow polyethylene container. The volume is brought to 800 mL with the same water. One fifth of the spermatozoa of a single male is added to the ova. The mixture is kept at 20 ± 1°C during 4 h, and the tub is gently stirred three or four times during that period. After that, the success of the fertilization is checked, and the fertilized eggs are counted (most often over 90% of the eggs are fertilized).

Stage 2

Rearing of the larvae is done in a 200-L polyethylene cylindrical tank where larval rearing water is introduced 24 h beforehand and stabilized at 20 ± 1°C. The embryos (in the gastrula stage) are introduced at a concentration of 250 per liter. This density is low enough to allow the entire rearing of the larvae to be conducted without renewing the water. The food (*Phaeodactylum tricornutum* Bohlin issued from cultivation in Erdschreiber medium) is introduced from the third day postfertilization (acquisition of larval exotrophy). The larvae are fed once a day with 600 mL of bloom algal cultivation (concentration around 10×10^6 cells/mL). The whole is kept in dim light with a 12h/12h photoperiod and is gently mixed and aerated by central bubbling.

Stage 3

From the sixteenth day onward, competence to *metamorphosis* is checked daily (Standard Competence Test or SCT, adapted from Gosselin and Jangoux 1996). One hundred larvae are transferred in a clean SCT sieve (a 10-cm high, 20 cm^2 sieve with a bottom mesh of 250 μm placed 1.5 cm above the water floor). This SCT sieve is placed in the pregrowth structure in the presence of a metamorphosis stimulating factor (living *Corallina elongata*, Ellis and Solander, freshly collected from the field). The percentage of metamorphosed larvae is determined 24 h later. If this value lies around 80%, the whole batch is transferred in the pregrowth structure aiming at its fixation on one or two metamorphosis sieves (similar to SCT sieves but each covering 1,800 cm^2). Batches containing large amounts of larvae exhibiting bad development, abnormalities, or too low metamorphosis ratios are discarded.

Stage 4

Growth of juveniles. The postmetamorphic period begins with a short endotrophic stage. During this period, the postmetamorphic individuals, also called postlarvae, reorganize their digestive tract (Gosselin and Jangoux 1998). The mouth and anus of the future juvenile are not yet pierced. This postlarval stage lasts for up to 8 days, after which the echinoids become exotrophic juveniles. One or two days before development of exotrophy, 100 g fresh weight of *Enteromorpha linza* (L.) Agardh collected in the field are distributed in each sieve. From this moment onward, the same food quantity is given every time it is completely consumed. Some *Gammarus locusta* L. are also introduced to clean the sieves of decomposing parts of the algae.

The juveniles are left in these sieves until the mean individual size in the batch reaches 2 mm. The entire batch is then transferred in 500 μm mesh pregrowth sieves. A homogeneous bed of *E. linza* is maintained in the sieves. The bottoms of the sieves are cleaned every week using filtered seawater. Because the growth of the juveniles is not homogeneous (Grosjean et al. 1996), the animals are graded each month, and those with a diameter greater than 5 mm are transferred into a 1-mm mesh pregrowth sieve. The *E. linza* diet is maintained, and the sieves remain in the same pregrowth structure.

Stage 5

Growth of subadults. Each month, sorting of size is done to collect all individuals over 10 mm. The individuals whose size exceeds 10 mm but is below the minimum market size of around 40 mm for *P. lividus* are defined as subadults. They are potentially mature but not large enough for market. Consequently, their somatic growth performances must be promoted, while their gonadal growth should be kept as low as possible to optimize food allocation to the soma.

Subadults are placed in rectangular rearing baskets, with all sides made of 5-mm mesh. These rearing baskets are placed 1.5 cm above the bottom of the toboggans and are just slightly narrower. This is important to allow good water circulation around and inside them, and good elimination of solid wastes produced by the sea urchins. Their surface ranges between 1,200 and 2,400 cm^2. When the size of the animals increases above 15 mm in test diameter, they are transferred in the same type of rearing baskets, but with 10-mm mesh, which allows even better water circulation.

Subadults, inside their rearing baskets, are transferred to a growth structure. From this time onward, and twice a week, they are fed *ad libitum* with fresh kelp, *Laminaria digitata* (Hudson) Lamouroux. Cleaning of the baskets and toboggans is also done twice weekly. Dead or dying animals are removed daily. Each month, sorting by size is done to separate the batches into different size categories from 5 to 5 mm. The entire cultivation is kept in 12h/12h photoperiod.

Stage 6a

Conditioning adults for market. When the sea urchins reach 40 mm, they are prepared to get marketable gonads in conditioning structures. For commercialization, it is of the utmost importance that the echinoids' gonadal cycle is synchronous, presents the right stage of maturity (reproductive stages 4 and 5, growing and premature, according to Spirlet et al. 1998a), and is of acceptable texture (firm and not leaking), size (as large as possible), good color (yellow-orange to bright orange), and taste. *P. lividus* has an annual reproductive cycle that tends to fade in constant artificial conditions: lacking the "usual" stressors (low temperature, lighting variation, lower quality or lack of food during winter), the echinoids tend to bypass the growth phase of the gonads and have permanent gametogenesis, giving rise to flabby gonads with few nutritive phagocytes. Such gonads are unacceptable in the market. To counteract this, the echinoids are starved at a temperature of 12

to 14°C and at a 12h/12h photoperiod. This leads to consumption of the possible content of the gonads, which also act as storage organs, in order for the animals to get in phase regarding their reproductive cycle (reproductive stages 1 to 3, spent and recovering, Spirlet et al. 1998a). When the content of the gonads is fully consumed; that is, between 1 and 2 months later, depending on their initial state, sea urchins are fed *ad libitum* with either *Laminaria digitata* or an appropriate artificial food rich in proteins (Klinger et al. 1994, Klinger et al. 1997, Klinger et al. 1998, Williams and Harris 1998) at a higher temperature (at least 16°C). The duration of this feeding stage is dictated by the maturation of the gonads and lasts for 6 weeks to 3 months, mainly depending on the food quality. Usually, both the size and the maturation stage simultaneously reach acceptable values, and gonads are ready for the market at the end of this starving–feeding treatment (see results).

Stage 6b

Conditioning broodstock. Maintaining mature broodstock of *P. lividus* all year long is done by keeping individuals at high temperature (between 18°C and 20°C) and under either a fixed photoperiod of 12h/12h (directly in the growth structures) or, better, in total darkness (in a conditioning structure), leading to the disruption of their reproductive cycle. In these conditions, food is the most important factor to get large quantities of good quality gametes. Feeding adults *ad libitum* with fresh *Laminaria digitata* ensures both the quality and the quantity of sexual output. The quality of gametes is often a little bit lower from December till February, although still usable most of the time.

Measurements of Reared Sea Urchins

Essentially two criteria are used to quantify the performances of the rearing method: (1) the survival rate with time; and (2) the growth rate; that is, the change of test diameter of the urchins with time (gonadal size and quality are taken into account only after the minimal market size has been reached). The first is determined by counting survivals in a single batch (issued from a single fertilization and a single larval rearing tank) at various times. The counting of eggs, embryos, and larvae is performed on at least five samples of the homogenized batch (the volume chosen to count each time is at least one hundred individuals), and the total amount is estimated by extrapolating the mean concentration found to the whole volume. The survival rate of competent larvae, postlarvae, and juveniles is determined by rearing subsamples of 50 to 100 individuals in SCT sieves. Several replicates (at least five) are sacrificed and counted at each time. All subadults and adults of a batch are counted and measured every 3 months (typically between a few hundred to a few thousand individuals in each batch) during size sorting. Measurements of subadults and adults do not induce additional stress or mortality other than those occurring during the normal size grading operation (no additional manipulations). Mortality caused by manipulations could thus be attributed to the rearing method itself.

Size is evaluated by means of the diameter, which is measured to the ambitus of the test (its largest part) considered without spines. To prevent errors caused by a possible slightly oval shape, we measure two perpendicular diameters, both to the ambitus, and only the average is considered. The diameter of juveniles, after fixing them (glutaraldehyde 3%), is measured on digitized microphotographs using a specific image analysis software (Grosjean et al. 1996). The diameter of subadults and adults is measured with a sliding caliper. Fresh weight, used to evaluate biomass, is measured after draining residual water on absorbent paper for 5 minutes.

The relative size of the gonads is quantified by means of the fresh and dry weight gonadal indices (GI, also called gonadosomatic indices). These indices are defined as the ratio between the fresh (or dry) weight of the gonads and the total fresh (or dry) weight of the urchins. First, fresh weight of the urchins is determined after drying them for 5 minutes on absorbent paper. The animals are then dissected, and the five gonads are extracted and weighed. One gonad is fixed in Bouin's fluid for further determination of its gametogenic stage (see below). The remaining four gonads are weighed again, and the difference is computed to allow correction of the dry weight for the missing gonad. The remaining gonads and the soma are then dried at 70°C during 48 h (constant weight) before being separately weighed. Dry weight gonad index is more accurate but has been found to be less representative of the "filling" of the sea urchins (how much space the gonads occupy inside the coelomic cavity), especially when comparing various maturity stages and/or various diets (unpublished results). Both indices are provided to allow comparisons.

The maturity stage is determined on histological sections of the fixed gonad following an 8-stage scale defined by Spirlet et al. (1998a). The maturity index (MI) corresponds to the arithmetic mean of all the observed maturity stages. Male and female data are pooled for both the GI and the MI, because differences between sexes are not significant (Spirlet et al. 1998a, Spirlet et al. 1998b).

RESULTS

Table 1 shows the age, density, and survival rate for each stage described in rearing conditions. These data come from 29 fertilizations studied over several years taking into account, among other things, seasonal variations. The survival rate for larvae is about 56%. Competence is reached most often in 18 days (mode and median value), with an average value of 19.5 days, a minimal time of 16 days and a maximal time of 25 days. The mean metamorphosis rate is 80.4% when larvae are competent. This rate was reached in almost two-thirds of the fertilizations that attained the competent stage (nonsymmetrical distribution). Thirty percent of the larvae were discarded, either because of incomplete development or too low a metamorphosis rate. The remaining larvae were used for studies on postlarval or juvenile stages (and, thus, sacrificed whenever measured) or were reared to the adult stage. Overall, the survival rate is homogeneous from one fertilization to the other and for all stages, except during and after the acquisition of exotrophy (transition from the postlarval to the juvenile stage): the average rate is 54.5%, but extremes are close to 0 and 100% (13% and 94.5%, respectively). Whatever the success of this transition, the most critical period for survival is the juvenile stage, with a very low survival rate of 5%. Most mortality occurs during the few first months of the juvenile's life (and even probably during the few first weeks), with a progressive decrease around 8 to 9 months of age.

Figure 2 shows both the survival rate and the size distribution over time of a batch followed for 7 years, far beyond the minimal marketable size and age. For the sake of clarity, only data taken every 6 months are presented, although measurements were made every 3 months beginning at 6 months of age. The trends observed on this single cohort are representative of the way animals grow in

TABLE 1.

Age, density, number, and survival rate of sea urchins at each rearing stage.

Rearing Stage	Developmental Stage	No. Replicated Fertilizations	Age (Min/Median/Max)	Mean Density (No. Ind./Vol. or/Surf. Unit)	Mean No. Individuals in 1 Batch	Survival from Previous Stage (%) Mean ± SD	Mean Global Survival Rate (%)
1	Embryos	29[a]	4 h	250/l	50,000	——	100
2	Competent larvae	29[a]	16 day/18 day/25 day	141/l	28,200	56.4 ± 11.6	56.4
3	Postlarvae	18[b]	idem + 1 day	$6.5 \times 10^4/m^2$	22,700	80.4 ± 14.4	45.3
4	Juveniles	9[b]	idem + 10 day	$3.5 \times 10^4/m^2$	12,400	54.5 ± 26.8	24.7
5	Subadults	6[c]	ca. 9 months	$4,000/m^{2d}$	600	4.9 ± 1.5	1.2
6a&b	Adults	5[c]	1.7 y/2.6 y/3.5 y	$250/m^{2d}$	310	51.5 ± 3.0	0.6

[a] Total number of larval rearing tanks: 103, from which 72 have produced enough usable competent larvae.
[b] In the pregrowth structure. 5 to 15 replicates are measured at key times for each fertilization (see Material and Methods).
[c] In the growth or conditioning structures. Each batch is issued from a single larval rearing tank and is followed over 2 to 7 years.
[d] Densities in the rearing structures are adjusted during sorting operations according to both the individual size and the survival rate.

cultivation, as confirmed by the five other independent batches measured over 2 to 4 years (for an illustrated example of another batch, see Grosjean et al. 1996).

Mortality (represented on the backwall of the 3-D box in Fig. 2) remains very high until about 9 months of age in the pregrowth structure. In the figured case, from around 12,400 juveniles issued from one rearing tank only 725 individuals where counted after 6 months and 507 remained after another 3 months. Mortality dropped after this critical period, and 491 individuals were still alive 3 months later (1 year of age). This corresponds, respectively, to a mortality of 94% (between the acquisition of exotrophy by the juvenile to 6 months old), 30% (during the next 3 months), and 3% (after the following 3 months). The mortality rate of subadults stabilizes around 5.4% per trimester until 6 years of age, but ranges from 0.9% per trimester to 12.7% per trimester. Most of this variation is correlated with season: mortality is higher during winter; whereas, summer mortality nearly reaches 0%. Most of winter mortality occurs in waves that start unpredictably and last for 2 to 3 days.

Juvenile's individual growth in test diameter is slow. It accelerates for subadults but then scatters for intermediate sizes (15 to 35 mm), even inside a presumably homogeneous batch. This scattering often results in bimodal or trimodal size distributions (see Fig. 2 for an example and Grosjean et al. 1996 for an analysis). When echinoids approach asymptotic size, their growth rate drops. Hence, the leading group is eventually caught up by the trailers around or slightly above the minimal market size. This minimal market size is attained between 1.7 and 3.5 years old (respectively, 10% and 90% of the individuals are larger than 40 mm), with a median value of 2.6 years.

Biomass variations (Fig. 3, same batch as in Fig. 2) are correlated to both the survival rate and the growth speed of reared echinoids. The higher mortality observed in winter overrides growth speed, and biomass tends to decrease slightly. Summer biomass is highest during the third and the fourth years in this case. The first peak of biomass (around 3.5 years old in the figured case, between 2.8 and 3.5 years old for the other batches, depending on the season) correspond to reaching the minimal market size by more than 90% of the individuals and seems to be the best time to commercialize them after conditioning their gonads (stage 6a) from a strict biological point of view. At that time, between 35 and 40 kg of fresh weight sea urchins are produced in a single batch. This represents an over-all yield per surface unit of the growth structures of 4 to 7 kg/m^2 of toboggans/year. To obtain this result, roughly 400 kg of kelp was provided to the sea urchins. Thus, over-all food conversion efficiency lies around 10%.

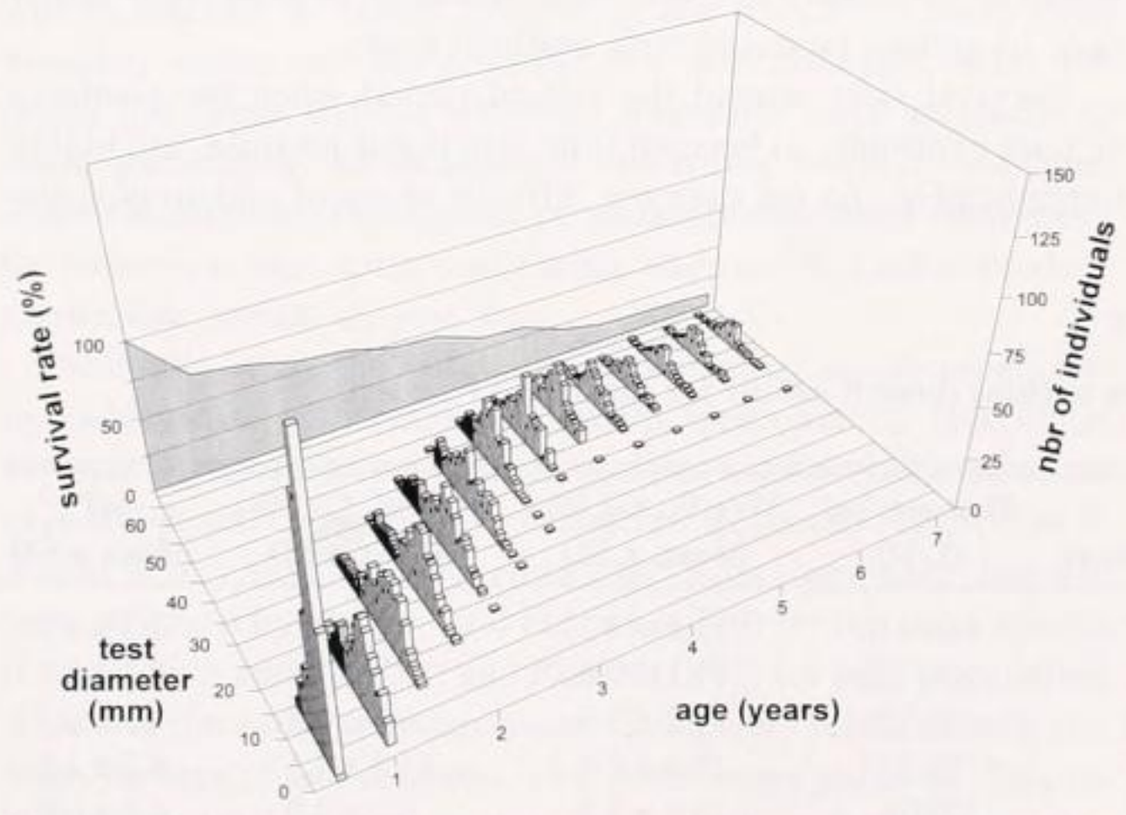

Figure 2. Changes with time in the size distribution and survival rate of one fertilization issued from a single larval rearing tank and followed over 7 years. Note the leading group that singles out (represented by dark bars in the histograms).

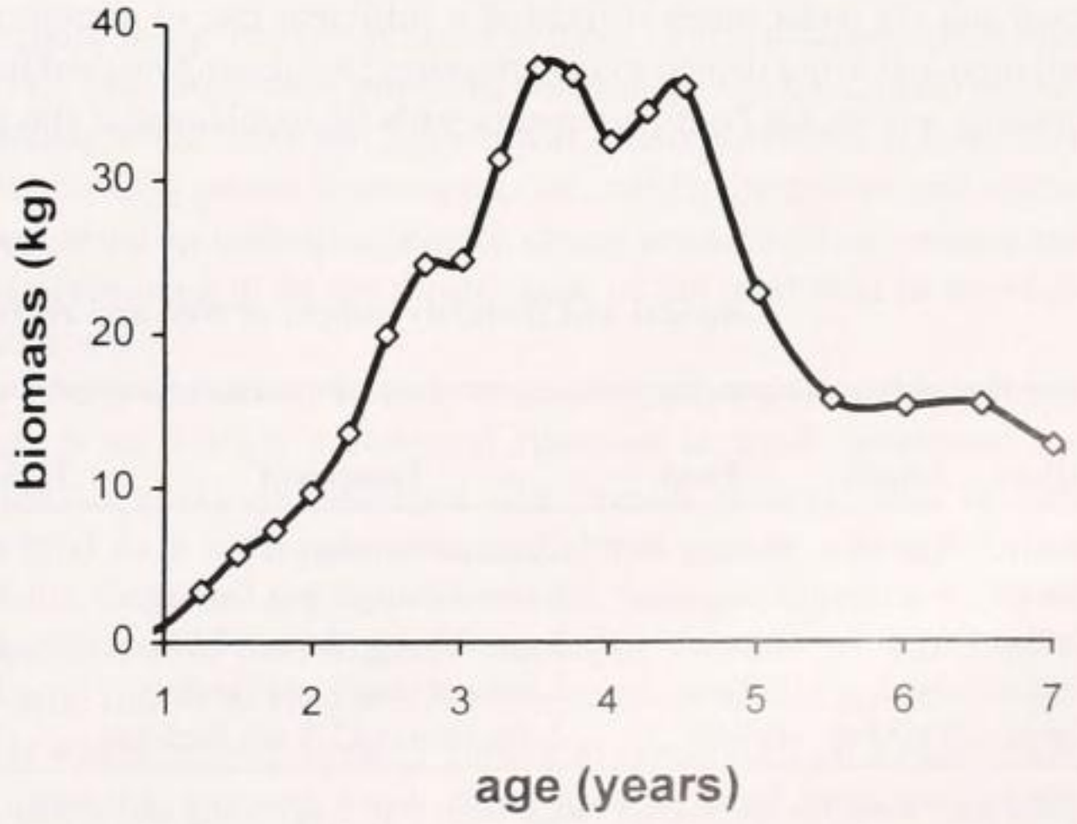

Figure 3. Change with time in the biomass of a reared cohort of sea urchins (the same batch as shown in Fig. 2).

Table 2 presents some results obtained after conditioning the sea urchins for market with the starving–feeding method. To allow comparisons, GI and MI of field echinoids issued from Brittany are also provided. In the field, best GI was observed in March and reached a mean value of 11.6% in fresh weight. Sea urchins conditioned in cultivation show similar GI and MI. The feeding period must be extended to 3 months when using *L. digitata;* whereas, 2 months are sufficient with the artificial diet to obtain the same results. Feeding 3 months with the pellets leads to a remarkable mean GI of 17.5% in fresh weight. Such a GI has never been observed in the field and corresponds to complete filling of the coelomic cavity with the gonads, the digestive tract, almost empty, being compressed against the body wall. However, the MI is too high, and the gonads contain too many gametes to satisfy market criteria. Furthermore, the color obtained with the pellets is too pale (white to beige), and the taste does not match wild roe; whereas, gonads produced with *L. digitata* are of good quality. An out-of-season conditioning was initiated in July with a long-day photoperiod (17h/7h). Very large gonads (GI around 14%) with an adequate MI were obtained in October, after only 6 weeks of feeding with the artificial food. Hence, the starving–feeding method could be used to produce marketable gonads all year long.

DISCUSSION

Both the increasing demand for roe and systematic overexploitation of wild populations support the need for sea urchin cultivation independent of field resources. The method presented here is one design of a rearing process that satisfies this criterion. It seems to be successful at any life stages of *P. lividus* on a pilot scale.

Obtaining gametes of *P. lividus* in large amounts is an easy task, as is the rearing of its larvae with the proposed method (rudimentary devices, low maintenance and feeding with one of the easiest microalgae to grow: *Phaeodactylum tricornutum*). Metamorphosis is a little bit more critical but can be achieved with care and use of a good inductant (fresh coralline algae). Rearing of juveniles, subadults, and adults is feasible if five major constraints are simultaneously respected. A specific design of the rearing structures and baskets provides (1) correct water flow around the echinoids (for gas exchanges and removal of solid wastes) and (2) sufficient bottom surface on which those benthic animals can settle (stacked toboggans). The maintenance of good water quality is ensured by (3) the adaptation of the sea urchin density at each life stage and (4) water renewal fixed at a sufficient rate to minimize pollution and avoid depletion in carbonates (see rearing method for tolerable values for both parameters without supplemental filtration). Finally, (5) providing adequate food *ad libitum* promotes somatic and gonadal growth. Further regulation of resources allocation is possible by diet (starving–feeding method), temperature, and photoperiod conditions, leading to good quality of the final product—the roe—, which could be obtained all year long.

If all these five conditions are met, *P. lividus* behaves fairly well in cultivation and seems highly resistant to diseases. The only cases of disease observed (mainly necrosis on the test or spines) were attributed to opportunistic bacterial or fungal infections attributable to poor rearing conditions; that is, when one or several of these five parameters were poorly controlled. Cannibalism was also observed when the quality of food was low or when carbonates concentration or pH dropped ("foraging" behavior to compensate for the lack in calcium carbonates?) or on dying animals, but never on healthy individuals maintained in good condition.

Growth is perfectly asymptotic, and the maximal size of 45 to 65 mm (individual variation) is reached around 3.5 to 4 years old in the rearing conditions described. This size is similar to that observed among the field population of Morgat, from which reared sea urchins descend directly or indirectly. In Brittany, the most precise estimation of size at age for wild populations of *P. lividus* was performed by Allain (1978) by analysis of the growth bands in the skeleton. According to this author, wild sea urchins reach the size of 40 to 50 mm in 4 years, which is a little longer than in the present rearing conditions (between 2 and 3.5 years). The gain could probably be attributed to the food distributed *ad libitum* all year long and to the water temperature (heating of the water in the winter) as already suggested by Le Gall (1990).

The success of the present method leads to optimistic forecasting for the future of echiniculture. However, we should probably expect slightly different results with large-scale, intensive cultivation. With the experience acquired during this long-term trial and some informal observations performed at a larger scale, we can predict some problems that could potentially arise when scaling up or when considering profit. These problems can be ranged into four different categories: (1) loss of profit because of high and/or uncontrolled mortality of juveniles and subadults; (2) unevenly distributed growth rates caused by intraspecific competition; (3) lack of carbonates and accumulation of CO_2 because of skeletogenesis in intensive closed-circuit systems; and (4) problems linked to the quality of food, water pollution, or poor color and/or taste of gonads produced with artificial diets.

Survival rates around the critical period when the postlarva acquire exotrophy to become fully functional juveniles are highly unpredictable. To get over the difficult phase of endotrophy, the

TABLE 2.

Gonadal and maturity indices of wild and reared sea urchins (pooled results for males and females).

Origin	Month	Food	Treatment	Temperature	Photoperiod (L/D)	Wet W. GI (%) Mean ± SD	Dry W. GI (%) Mean ± SD	MI[a] Mean ± SD
Field	March	Natural diet	Collected in Morgat[b]	10°C	13h/11h	11.6 ± 4.2	7.1 ± 2.6	4.3 ± 0.5
Cultiv.	June	*L. digitata*	2 mo starving/3 mo feeding	16°C	12h/12h	11.1 ± 2.6	7.3 ± 1.7	4.4 ± 0.5
Cultiv.	May	Pellets[c]	2 mo starving/2 mo feeding	16°C	12h/12h	11.2 ± 3.3	6.7 ± 2.1	4.2 ± 1.3
Cultiv.	June	Pellets[c]	2 mo starving/3 mo feeding	16°C	12h/12h	17.5 ± 2.4	11.3 ± 1.5	6.2 ± 1.0
Cultiv.	October	Pellets[c]	2 mo starving/1.5 mo feeding	16°C	17h/7h	13.9 ± 1.5	9.7 ± 1.0	4.8 ± 0.9

[a] Best MI values for the market range from 4 to 5 (growing and premature reproductive stages).

[b] Mean values obtained on samplings during 3 consecutive years.

[c] For the composition of this food, see Williams and Harris 1998 (Table 1, "new diet").

larvae must store enough reserves before undergoing metamorphosis. In addition, the early juveniles must promptly find suitable food when their digestive tract becomes functional. It seems that one or both parameters are not always optimal in rearing conditions. In any way, with a mean 55% success rate, we obtain over 12,000 viable juveniles per 200-L tank, which is enough for our use, but can probably be improved. Indeed, gametes are not limited: a female of 40-mm diameter usually produces around 5 to 7 millions of eggs. Thus, the 50,000 embryos introduced in one larval rearing tank represent only about 1% of a whole spawn (about 0.2% of the sperm produced by a single male). Hence, only a few dozen mature adults are necessary to produce enough gametes for mass production of larvae.

However, after the critical phase of exotrophic acquisition, the mortality of juveniles remains very high until they reach about 10 mm in test diameter. To minimize this, juveniles are reared in specific structures referred to as pregrowth structures where biomass is kept at a low level and where water quality is of prime importance. Moreover, quality of the immediate environment of juveniles is improved by use of a good "water-resistant" diet (*Enteromorpha linza*) and by means of cleaners (*Gammarus locusta*). In any case, the space occupied in the pregrowth structure by juveniles and the total care they need remain much lower as compared to subadults and adults (compare densities in Table 1). This minimizes the cost of losing many juveniles from the point of view of the total productivity of the cultivation.

More insidious is the effect of winter mortality of subadults and adults. Its cumulative value is ten times lower than juvenile mortality, but its cost is much higher, because it concerns individuals occupying a significant space in the growth structures and having already consumed a significant amount of food (drop of the overall yield per surface unit and food conversion efficiency). However, the cause of this seasonal variability cannot be explained. It could be because of lower quality of food (fresh kelp with a seasonal variation in their composition, Gayral and Cosson 1973, Abe et al. 1983) or to any pollution of the water probably induced by the food itself (bad quality food is less ingested and decomposes more easily), or to another undetermined cause. For the moment, waves of mass mortality have not been correlated with either temperature variability of the natural seawater, meteorological conditions (atmospheric pressure, rain), or feeding. However, any correlation will be difficult to assess, because of the scarcity of these mass mortality waves and the probable, but not quantified, delay between the stress and the observed mortality. Total productivity could undoubtedly be enhanced if this winter mortality was lowered or eliminated. To suppress or minimize the winter decrease in the biomass is also worth considering when one intends to produce marketable gonads all year long.

Mortality is not the only problem inhibiting steady productivity: widespread distribution of growth speed among individuals expands the time interval when largest and smallest individuals are exploitable and constrains to sort batches frequently. Growth of *P. lividus* is very slow at the juvenile stage. This "lag-phase" has also been observed by Cellario and Fenaux (1990) for the same species in cultivation and by Ebert and Russel (1993) for wild populations of *Strongylocentrotus franciscanus* Stimpson. When growth initiates in term of test diameter, size distribution expands. This individual variability is not genetic but is attributable to a reversible size-based intraspecific competition (Grosjean et al. 1996) that takes place rapidly, even in size-sorted batches, although sorting reduces its effect. Presently, the exact impact of this competition on productivity and the best way to avoid it (if it should be avoided at all) are still unknown.

A third problem that will probably occur when considering further intensification of echiniculture in closed or semiclosed systems is the depletion of dissolved carbonates and the accumulation of CO_2 in seawater. In growing, the sea urchin builds a magnesium-calcite skeleton. This skeleton represents an important fraction of the body weight: between 28% and 31% of the total fresh weight for *P. lividus* (measured on animals issued from the reared strain, after digestion of organic tissues with a 12°Chl bleaching agent under gentle agitation, n = 356). Thus, for each kg of fresh weight produced, about one-third has to be supplied in one or the other form of calcium carbonate. However, *P. lividus* is unable to assimilate efficiently carbonates provided as a solid substrate (calcareous rocks, algae, or cuttlefish bones, for example), because the pH of its digestive tract is too high to dissolve large amounts of solid calcite (between six and eight, for a review see Lawrence 1982, for data concerning *P. lividus* see Claerebout and Jangoux 1985). The main usable source of magnesium/calcium carbonates is thus present under a dissolved form in seawater. If calcium and magnesium ions (respectively 400 mg and 1,350 mg per kg seawater at a salinity of 35‰, Spotte 1991) are not limiting, the quantity of dissolved carbonates available could be consumed very quickly in intensive closed or semiclosed systems (unpublished data). Most of the carbonate alkalinity (about 2.3–2.4 meq/kg seawater, corresponding to 140 mg of HCO_3) remains unavailable for skeletogenesis, the pH dropping too much when sea urchins consume it (carbonate and bicarbonate are the most important chemical components that buffer pH in seawater, Stumm and Morgan 1981). The actual fraction the sea urchins can use is still unknown, but is probably under 10% of the total carbonate alkalinity. To illustrate this, without supplemental chemical filtration and with a usable fraction of 10% of the dissolved carbonates to produce skeleton that final weight represents 30% of the total sea urchin fresh weight, one must provide at least 24,500 m^3 of seawater per ton of sea urchin fresh weight produced. However, this optimistic calculation does not consider mortality that otherwise also exports carbonates.

Precipitation of bicarbonates (the main form of dissolved carbonates in seawater at usual pH) into calcium carbonate is a dismutation reaction that liberates a stoichiometric amount of carbonic acid in the water column. This carbonic acid, together with the CO_2 produced by the respiration of sea urchins, algae, and bacteria in the rearing structures, tends to reach rapidly undesired levels in a large-scale intensive cultivation. We have observed sea urchins whose skeleton growth was totally inhibited in these conditions. CO_2 partial pressure was recorded to be 5 to 9 times higher than usual in seawater (despite strong aeration of the water) and was presumed to be the direct cause of the inhibition of the skeletogenesis.

These limitations force us to choose either a flow-through system or to provide a chemical filtration to level carbonates and carbonic acid concentrations. The present method could be considered as a semi-intensive, semiclosed system where both sea urchin densities and water renewals remain compatible with the equilibrium of the inorganic carbon in seawater without supplemental filtration. However, such a trade-off would not be compatible with a rearing strategy aiming to raise profit on a large scale.

For the moment, fresh algae used as food form part of the natural diet of *P. lividus*. The composition of this food is presumably correct, although it might not be necessarily optimal (Frantzis

and Grémare 1992, González et al. 1993, Fernandez and Boudouresque 1998). The major problem encountered with food is its stability once put in the rearing structures, because this echinoid, being a grazer, ingests it slowly. Uneaten food could easily give rise to undesired pollution. Hence, we recommend the use of a stable diet (*Enteromorpha linza*) in the present rearing method instead of higher quality algae (*Laminaria digitata, L. saccharina* Lamouroux or *Rhodymenia palmata* (L.) Greville, unpublished results) for juveniles. We also avoid using artificial diets at water temperature above 16°C without the presence of an efficient biofilter in the rearing structures.

The use of fresh algae is not always possible or profitable on a large scale (Fernandez 1996). Hence, an artificial diet designed specifically for sea urchins seems necessary for intensified echiniculture and is presently under investigation by several authors (Fernandez and Caltagirone 1994, Klinger et al. 1994, Klinger et al. 1997, Klinger et al. 1998, de Jong-Westman et al. 1995a, de Jong-Westman et al. 1995b, Fernandez 1996). Results obtained so far are encouraging, especially in terms of GI, but the food we were able to test gave unsatisfactory results in terms of color and palatability of roe. Recent testing of semimoist diets on *Strongylocentrotus droebachiensis* Müller (Motnikar et al. 1997) seems to confirm the positive effect of the artificial diet on the gonadosomatic index and the failure to obtain high quality gonads in terms of color and taste. Trials with carotenoids-enriched artificial food to enhance the color do not yet produce high quality gonads (Goebel and Barker 1998). Thus, better formulation of the food is basic to achieve correct taste and color for exploitation.

Finally, we should mention that the rearing method described here is labor intensive. Hence, manpower cost could be too high when considering profit. This would require some adaptation or mechanization of the most time-consuming operations: feeding subadults and adults, cleaning the growth structures, grading the batches or extracting the gonads if sea urchins are not commercialized alive (exportation to Japan). However, these are technical problems that could be solved by the industry.

CONCLUSIONS

This rearing method constitutes a good working basis for design of a closed-cycle, land-based echiniculture. We suggest it could be used as a standard method to evaluate improvement obtained by adaptations or modifications aimed at intensification or profitability of echiniculture. This method could possibly be adapted to other species, allowing better comparisons of the biology of respective species as well as their aquaculture potentials.

Latent remaining problems when scaling up and intensifying cultivation, aiming at raising profit, should not be regarded as unavoidable limitations, but should be considered as challenges to address in further studies. Being a "new" cultivated species, it is not surprising that these obstacles mostly concern less known life stages or "biological features or characteristics" of sea urchins: the transition between the endotrophic postlarva and the exotrophic juvenile, the mechanism of the intraspecific competition, the carbonate budget needed for skeletogenesis, and the biochemical pathways in gametogenesis and in stocking reserve material in the gonads. Thus, it is probable that advances in fundamental biology of echinoderms, and more particularly of echinoids, will suggest solutions to these problems in the future.

It would seem that further development of closed-cycle, land-based sea urchin cultivation is worthwhile and will undoubtedly promote diversification of aquaculture and production of high quality seafood. This will, secondarily, lead to conservation of the natural environment by limiting the fisheries impact on natural populations of sea urchins.

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