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Culture potential of the pearl oyster (*Pinctada imbricata*) from the Caribbean.

II. Spat collection, and growth and mortality in culture systems

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Abstract

Temporal variation in abundance of larvae and spat of the pearl oyster *Pinctada imbricata* was studied at several locations on the Colombian coast from May 1997 to June 1998. Larvae were sampled with bongo nets and spat were harvested from collectors at monthly intervals. Abundances of predators (*Cymatium* gastropods, and portunid, xanthid and majiid crabs) were also recorded. A relationship between salinity, particulate organic matter and larvae abundance was observed, leading to peaks in abundance of spat on collectors some weeks later. Average catch rates of 10 spat collector⁻¹ month⁻¹, using collectors made of cheap easily accessible materials, indicate that availability of *P. imbricata* is sufficient to initiate and support aquaculture of this species. Growth and mortality rates of juveniles in three different culture systems at two densities (20% and 30%, i.e. percentage of available area covered by juveniles) showed that density within the same culture system had no effect on growth, but that growth differed significantly among the three culture systems. Growth in ''bag'' systems was lower than in boxes whereas growth in ''suspended'' and ''bottom'' boxes was similar and comparable to the growth of a natural population. The suspended boxes are the easiest to handle because they do not require SCUBA diving and so these systems are recommended. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pinctada imbricata; Aquaculture; Spat collection; Growth rates; Mortality rates; Predators; Caribbean

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1. Introduction

Aquaculture of marine bivalves worldwide has a long tradition and, in developing countries of the tropics, has increased considerably during the last 10 years. Despite the fact that the Caribbean and Pacific coast of Colombia offer excellent conditions, to date no commercial aquaculture of marine bivalves exists in this country. As part of a larger project to evaluate the potential of bivalve species from the Colombian Caribbean for aquaculture the pearl oyster *Pinctada imbricata* (see Urban, 2000 regarding the taxonomic status of *P. imbricata*) was studied. This species is commonly distributed in the tropical Atlantic and forms dense populations in Guajira Province, Colombia (12°10′N, 72°20′W and 12°00′N, 72°10′W, Urban, 2000).

For a complete evaluation of the culture potential of a bivalve species, it is necessary to assess their biology, their performance in culture systems, and their growth and survival under laboratory conditions. The biology (gametogenic activity, growth, mortality and production of P. imbricata from the Guajira Province) has been dealt in Urban, 2000. This study deals with rates of larvae and spat collection and performance of juveniles in culture systems.

The objectives of this paper were to determine temporal variability of larvae and spat abundance, and the abundance of principal predators settling on spat collectors, with a view to assessing the potential for aquaculture of *P. imbricata*. In addition, abundance was compared with abiotic factors: temperature, salinity, particulate organic and inorganic matter. Growth and mortality rates of juvenile *P. imbricata* in different culture systems, and variation of predator abundance, were also estimated and compared with abiotic factors.

2. Material and methods

2.1. Study area

Collection of spat and growth experiments on juveniles in culture systems were carried out in the Tayrona National Nature Park, close to the city of Santa Marta, Colombia (11°20′N, 74°10′W, Fig. 1). Spat collection was conducted in Chengue Bay and the growth experiments in Gayraca Bay. Tayrona Park consists of several small bays offering a great variety of substrata (patches of seagrass, coral reefs, sandy–silty substrates, and mixtures of coral and sand bordered by mangroves). For a detailed description of the study area, see Garzón-Ferreira and Cano (1991). Annual water temperatures varies between 26°C and 30°C and the mean salinity is 36‰. Two climate periods can be distinguished: the *dry season* from December to April, followed by the *wet season* between May and November. Another important characteristic is a local upwelling occurring towards the end of the wet season, especially in September/October. This phenomenon is evident by a sharp increase in salinity (from 34‰ to 37‰) and a drop in the water temperature (from 30°C to 26°C). The upwelling contributes a substantial part of the nutrients for primary production (Blanco, 1988).

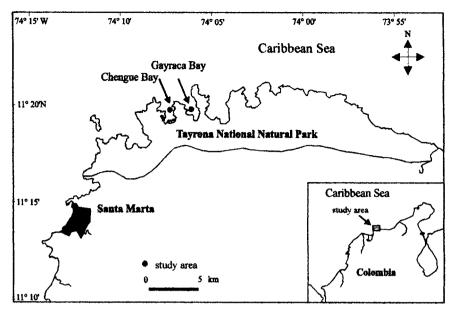
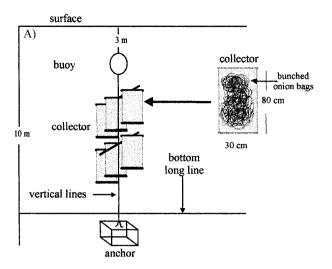


Fig. 1. Locations of spat collection (Chengue Bay) and growth experiments (Gayraca Bay) in the study area at Tayrona National Nature Park.

2.2. Abundance of larvae and spat

Monthly zooplankton samples were taken in Chengue Bay between 9:00 and 11:00 a.m. with a Bongo net (\varnothing 35 cm, 150 μ m). Water volume passing through the net was estimated with a flowmeter (Hidro-Bio Kiel, mod. 438115). Samples were preserved in 70% alcohol and all bivalve larvae of the genus *Pinctada* were identified and counted. In order to identify *Pinctada* larvae, ''morphotypes'' were obtained from laboratory-raised larvae, which were then compared with bivalve larvae obtained from zooplankton samples. Abundance of larvae per m³ was calculated each month.

The abundance of spat, and potential predators, was determined by deploying collectors comprised of ''onion bags'' of plastic mesh (80×30 cm, mesh size 0.8 cm), protected by a propylene net-bag (Fig. 2A). Collectors were tied to a bottom long line at different depths (5, 10 and 15 m) and were left for 8 weeks until recovered. Mean abundance per collector was estimated each month. The same collectors were also used for an intense sampling program for spat during which 240 collectors were deployed in March 1997 and recovered 8 weeks later. These collectors yielded 4900 juveniles of P. imbricata ranging from 0.8 to 1.2 mm shell length. Juveniles were transferred to a 500 1 tank (ultraviolet-irradiated, 1 μ m filtered seawater, 27 °C, gently aerated) and were fed on a 1:1 mixture of Isochrysis galvana and Chaetoceros gracilis of 100,000 cells ml⁻¹. Water was completely changed every 2 days. Juveniles were maintained in the laboratory under these conditions for 4 weeks until they reached an average shell length of 10.6 mm (± 2.4 SD), then they were used for the growth and mortality studies (see below).



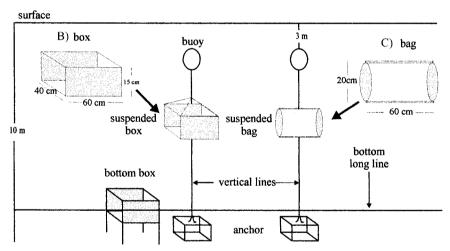


Fig. 2. Culture systems used for culture experiments. (A) Collectors for spat collection. (B) Boxes and (C) bags, intermediate growth culture systems.

Sea bottom temperature and salinity were recorded at monthly intervals. Each month also water and seston samples were taken at a depth of 3 m with a pump.

Water samples were filtered using Whatman Glass Microfibre filters, which were dried for 24 h at 70° C. Particulate inorganic matter (PIM) and particulate organic matter (POM) was calculated after heating filters at 500° C for 4 h.

2.3. Growth and mortality in culture systems

Growth and mortality of P. imbricata was compared in three different culture systems ("bags", "suspended boxes" and "bottom boxes") at two densities (= 20%

and 30%, i.e. the percentage of the available area covered by juveniles). Plastic boxes $(40\times60\times15~{\rm cm})$, covered by propylene net $(0.7~{\rm cm}$ mesh size) were used as suspended and bottom culture systems (Fig. 2B). Bags consisted of propylene net $(120\times40~{\rm cm},~0.7~{\rm cm}$ mesh size). Both ends were glued together, forming a compartment, where the individuals were placed (Fig. 2C). All suspended culture systems were connected to vertical lines and to a bottom long line (Fig. 2B,C). Suspended systems were kept in the water column at least 5 m above the bottom and 5 m below the surface, while bottom systems (boxes) were installed on metal frames, 15 cm above the bottom. The densities of juvenile oysters in the culture systems were maintained constant in the following way: as P. imbricata juveniles form clumps in the culture systems, at each monthly inspection they were separated into individuals. After measuring and counting, individuals to be removed were chosen randomly.

Thus, the experimental design consisted of five ''treatments'', with three replicates each: (1) bags 20% density, (2) bags 30% density, (3) suspended boxes 20% density, (4) suspended boxes 30% density and (5) bottom boxes 20% density. At the end of the experiment variation in mean growth and survival among treatments was compared with a one way ANOVA and the Tukey HSD multiple comparisons test. Growth was always recorded measuring the antero-posterior axis.

From July 1997 to June 1998 culture systems were recovered every 2 weeks and dead individuals and potential predators (gastropods and crustaceans) were removed and counted.

2.4. Growth analysis

Mean monthly growth rates were estimated and compared with abiotic factors. To obtain age at length data, the ''mean birthday'' of the juveniles was defined as 4 weeks before collectors were recovered (i.e. 50% of the period they were in the water = 14th of April 1997). To compare growth between the culture systems (and with the natural population from Guajira Province (see Urban, 2000), growth parameters of the von Bertalanffy model were estimated and the von Bertalanffy growth function (von Bertalanffy, 1938) was fitted to age-length data using an iterative non-linear least-square method (SIMPLEX algorithm, Press et al., 1986):

$$L_{t} = L_{\infty} \cdot \left(1 - e^{(-K(t - t_{0}))}\right) \tag{1}$$

where L_{∞} is the asymptotic length [mm], K the growth constant [yr⁻¹], t the age [yr] and t_0 the age at zero length. As pointed out by Urban, 2000, L_{∞} is under- or overestimated if growth data do not cover most of the species' growth range (Ralph and Maxwell, 1977; Knight, 1968; Theisen, 1973; Urban and Mercuri, 1998). This applies to the data set used in this study because growth of a juvenile cohort was followed for only 1 year where old (i.e. large) individuals were absent. Thus, all growth routines were estimated with fixed asymptotic length, taken from the natural population (Urban, 2000). This procedure also enabled direct growth comparison of cultured and wild oysters.

The three growth parameters L_{∞} , K and t_0 of the von Bertalanffy growth function are necessary to describe a growth curve and the two important ones, L_{∞} and K are inversely related.

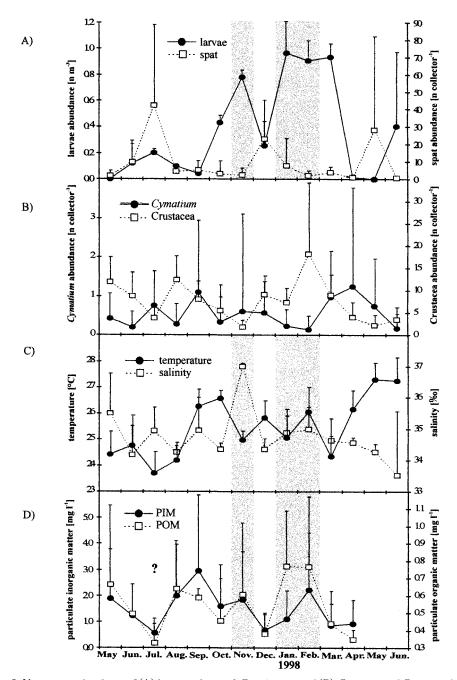


Fig. 3. Variation in abundance of (A) larvae and spat of *P. imbricata*, and (B) *Cymatium* and Crustacea from Chengue Bay, Colombia between May 1997 and June 1998. Variation in water temperature and salinity (C) and POM and PIM (D) is also shown. Vertical bars indicate standard deviations of the means. Shadowed bars indicate peaks in salinity or POM that were correlated with larval abundance.

Table 1

(a) Spearman correlation matrix of the abiotic variables temperature, salinity and POM; the abundance of larvae and spat of *P. imbricata* and the predators *Cymatium* and Crustacea. Values > 0.5 are printed boldface

	Temperature	Salinity	POM	Larvae	Spat	Cymatium
Salinity	-0.436					
POM	-0.214	0.618				
Larvae	-0.607	0.473	0.786			
Spat	-0.393	-0.418	0.107	0.321		
Cymatium	-0.180	-0.284	-0.847	-0.505	-0.144	
Crustacea	-0.036	-0.182	0.143	0.286	0.357	-0.450

(b) Spearman correlation matrix of abiotic variables temperature, salinity and POM, and growth rates of P. imbricata and Cymatium abundance for each culture system. Values > 0.5 are printed boldface

		Temperature	Salinity	POM	Growth rate				
					Bag 20%	Bag 30%	Box 20%	Box 30%	Bottom box 20%
Growth rate	Bag 20%	0.179	-0.873	-0.536					
	Bag 30%	-0.649	0.064	-0.414					
	Box 20%	0.270	-0.688	-0.252					
	Box 30%	-0.270	-0.578	-0.396					
	Bottom box 20%	-0.286	-0.400	-0.393					
Cymatium	Bag 20%	0.218	-0.472	-0.491	0.764	0.303	0.716	0.661	0.764
•	Bag 30%	0.250	-0.873	-0.643	0.929	0.306	0.613	0.829	0.750
	Box 20%	0.414	-0.413	-0.577	0.613	0.182	0.545	0.491	0.631
	Box 30%	0.090	-0.303	-0.396	0.613	0.455	0.400	0.727	0.865
	Bottom box 20%	-0.018	-0.193	-0.144	0.613	0.327	0.618	0.673	0.811

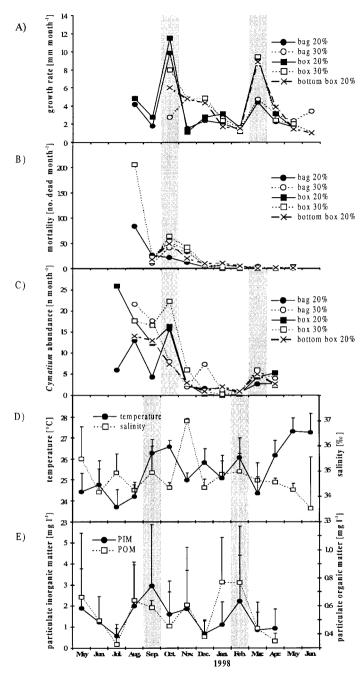


Fig. 4. Variation of (A) growth rates and (B) mortality rates of *Pinctada imbricata* juveniles in three different growth systems. Variation in *Cymatium* abundance (C), water temperature and salinity (D) and particulate organic (POM) and inorganic matter (PIM) (E) is also shown. Vertical bars indicate standard deviations of the means. Shadowed bars indicate October - and March peaks (see text, Fig. 4a–c) as well as temperature - and POM peaks one month before (Fig. 4d and e).

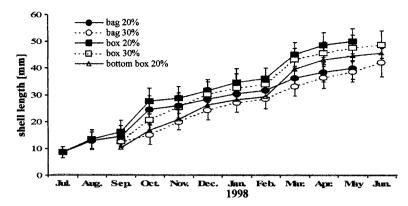


Fig. 5. Mean growth of *P. imbricata* in three culture systems. Vertical bars indicate standard deviations of the means.

Therefore, it is not possible to use only one of them, for example K, to compare different growth sets. Among the growth indices that have been developed, one by Munro and Pauly (1983) was used. Phi prime (Φ') is the "overall growth performance":

$$\Phi' = \text{Log}(K) + 2\text{Log}(L_{\infty}) \tag{2}$$

3. Results

3.1. Larvae, spat and predator abundance in collectors

Two peaks in abundance of larvae were found: November 1997 (0.8 larvae cm⁻³) and January–March 1998 (1 larvae cm⁻³) (Fig. 3A). Spat peaks followed shortly after larvae peaks, although correlation between larvae and spat was low (Table 1a). Several significant correlations of larval abundance with abiotic variables were also observed: larvae/temperature = -0.607, larvae/POM = 0.786 (Table 1a).

Two principal predator groups were observed: Gastropoda and Crustacea. The gastropods were almost exclusively *Cymatium* species (Fam. Ranellidae): *C. pileare, C.*

Table 2 Results of Tukey multiple comparisons test for growth of juveniles of *P. imbricata* raised in three different culture systems. Means and standard deviations of final shell lengths (antero-posterior) after 13 months of growth are also given. Non-significant values are printed boldface

	Means ± SD [mm]	Bag 20%	Bag 30%	Box 20%	Box 30%
Bag 20%	40.1 ± 4.163				
Bag 30%	42.2 ± 5.365	0.036			
Box 20%	50.7 ± 4.721	< 0.001	< 0.001		
Box 30%	49.0 ± 5.172	< 0.001	< 0.001	0.305	
Bottom box 20%	45.7 ± 3.929	< 0.001	< 0.001	< 0.001	< 0.001

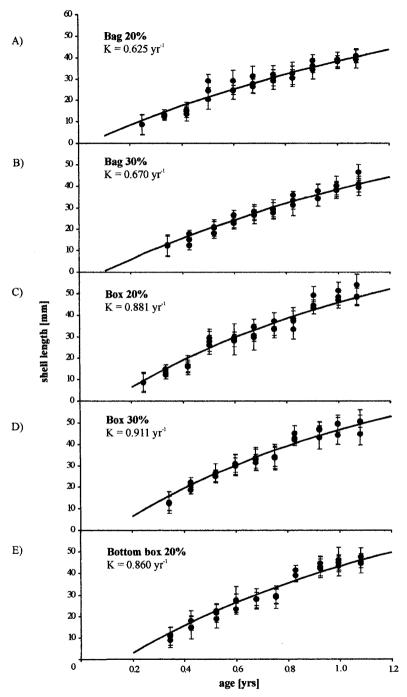


Fig. 6. Growth curves of the von-Bertalanffy-growth-function plotted together with age at length data of P. imbricata raised in different culture systems. Vertical bars indicate standard deviations of the means.

cingulatum, and C. nicrobaricum). The second important group (Crustacea) consisted of three crab families, Portunidae, Xanthidae and Majidae, and the spiny lobster Panulis argus (Fam. Palinuridae) also occurred in low frequencies. The following crab species were identified: Cronius ruber and Charibdis helleri (Portunidae); Pilummnus floridianus, P. dasypodus (Xanthidae); Stenorhynchus seticornis, Macrocoeloma sp., Mithrax pleuracanthus, Podocela gracilipes and Microphrys antillensis (Majidae).

Abundance of *Cymatium* and crustacean predators followed a different pattern to those of the larvae and spat (Fig. 3B). The only significant correlation with an abiotic variable was found between *Cymatium* and POM (= -0.847, Table 1a).

During the study period, temperature varied between 23.5° C and 27.5° C, salinity between 33.5% and 37% and POM between $0.3 \text{ mg } 1^{-1}$ and $0.8 \text{ mg } 1^{-1}$ (Fig. 3C,D).

3.2. Growth and mortality rates of juveniles in culture systems

Fig. 4 shows growth and mortality of P. imbricata, and the abundance of attached Cymatium juveniles in the different culture system. Growth in all culture systems showed two clearly marked peaks: one in October 1997 and another in March 1998. Mean mortality decreased from high levels during the first four months to less than five individuals per replicate per month for the remainder of the study. Some significant correlations occurred between growth (but not equally in all culture systems) and salinity (Table 1b): bag 20%/salinity = -0.873, box 20%/salinity = -0.688, box 30%/salinity = -0.578. The similarity in variance for the growth rate, and the Cymatium abundance curves, is reflected generally by significant positive correlation values, with exception of the growth rates in bags 30% (Table 1b). However, high growth rate coincides with high Cymatium abundance only for October.

The mean growth of P. imbricata in all culture systems is plotted in Fig. 5. The Tukey Multiple Comparisons from the one-way ANOVA (Table 2) of the final lengths after 13 months gave significant differences between the three culture systems (P < 0.001), but not between densities of the same culture system: bag 20%/bag 30%, P = 0.036 and box 20% /box 30%, P = 0.305. Fig. 6 (A–E) shows the age-length data and the estimated growth-curves. The corresponding estimated growth parameters (L_{∞} , K and t_0) as well as the growth performance index Φ' are listed in Table 3.

D cooth	0.1	0.005	0.000	5.000	0.044
Culture system	L_{∞}	K	t_0	MSE ^a	Φ'
Growth parameters	and growth perform	ance index (Φ')) of <i>P. imbrica</i>	ata in different cul	ture systems
Table 3					

Culture system	L_{∞}	K	t_0	MSE ^a	Φ'	
Bag 20% ^b	84	0.625	0.030	5.022	3.644	
Bag 30% ^b	84	0.670	0.088	4.722	3.675	
Box 20% ^b	84	0.881	0.106	9.797	3.794	
Box 30% ^b	84	0.911	0.110	9.046	3.808	
Bottom box 20% ^b	84	0.860	0.158	10.884	3.783	
Natural population ^c	63.9	1.605	_	8.449	3.816	
Natural population ^b	84	0.939	_	9.070	3.821	

^aMean Square Error.

^bAnalysis with fixed I_{∞} (= 84 mm).

^cAnalysis with "free" L_{∞} (= 63.9 mm) taken from Urban, 2000.

4. Discussion

This study indicates that the abundance of P. imbricata in Colombia depends on a combination of abiotic factors. In particular, peaks in the abundance of larvae coincide with increases in salinity and POM. According to Blanco (1988), peaks in salinity and POM are caused by upwelling events, especially in September/October. Apparently, upwelling favours the survival of larvae, which gives rise to higher levels of spatfall. Barnes (1957) stated that synchronization of spawning in marine epibenthic communities is tuned principally by phytoplankton peaks and only indirectly caused by temperature. Friedman and Bell (1996) and Friedman et al. (1998) studied abundance of P. margaritifera and P. maculata on different collector types from the Solomon Islands. They state that heavy rainfall increased particulate matter and thus fouling rates, which adversely affected the survival of spat on collectors. Knuckey (1995) studied spat collection of P. maxima in the Timor Sea (Australia) and observed a temperature increase as well as an increase of nutrients washed into the sea during monsoon rains. The increase of productivity favored spat settlement. In that study, particulate matter increased and salinity decreased presumable due to run-off. Similar to Knuckey (1995), the abiotic changes described in this study, have positive effects on larvae and spat abundance. It is in accordance with Southgate and Lee (1998) that salinity has to be considered as a major spawning cue for tropical bivalves.

A *P. imbricata* population from Guajira Province showed a long phase of high reproductive output from January to June, and a minor spawning peak in October (Urban, 2000). These results match those of the present study: the November larvae peak corresponds to the October spawning activities and the January/February larvae peak to the longer reproductive phase between January and June.

It is a well known fact that natural predators attach themselves as larval stages to bivalve culture systems and later cause large mortality rates (Friedman and Bell, 1996). Cymatium and the same crab families as observed in this study were reported by Friedman et al. (1998) as principal predators found on their collectors, causing high mortality rates on *Pinctada* spat. In our studies the greatest mortality occurred at the time when predators were abundant and the spat were small. This was most likely because the growing individuals became stronger and less vulnerable to the small and recruiting predators (which were removed every two weeks). An exception was October 1997, when a *Cymatium* peak was observed, causing high mortality rates in the same month. The second *Cymatium* peak in March did not cause high mortality of the *Pinctada* juveniles, presumable because the juveniles had outgrown the size range vulnerable to predation.

POM increased in August/September 1997 and January/February 1998, and in both cases was followed by an increase in Cymatium abundance and growth rates of P. imbricata juveniles. High mortality rates were observed in the culture systems 1 month later, in October 1997. It is reasonable to conclude that this POM increase favored settlement and survival of Cymatium, as well as the growth rate of P. imbricata.

Monthly growth rates of P. imbricata varied between 11.5 (maximum in October 1997) and 1.0 mm month⁻¹ (minimum in June 1998) with an average growth rate of 3.7 mm month⁻¹ (of all culture systems). These results are very close to those reported for

P. maxima by Knuckey (1995) (maximum = $10.4 \text{ mm month}^{-1}$, average = $5.8 \text{ mm month}^{-1}$) and Rose and Baker (1994) (7–9 mm month⁻¹).

Growth of P. imbricata differed significantly among the intermediate culture systems. However, regarding the effect of the density, no differences were found for the two densities used in the same culture system.

The growth curves (Fig. 6) and the corresponding von Bertalanffy parameters (Table 3) were estimated in order to compare growth performance between the culture systems as well as with a natural population. The Φ' values of Table 3 indicate low growth rates for the bag-culture systems, and similar (higher) growth rates in the other culture systems and in the natural population. Gaytan-Mondragon et al. (1993) compared growth of P. mazatlantica from Mexico in different culture systems (i.e. suspended pearl nets, lantern nets and pocket nets and bottom cages). Contrary to these results, they observed no significant growth differences (ANOVA) between the culture systems and a 24% greater survival in bottom cages. They recommend use of bottom cages as the most suitable culture system, owing to the reduced fouling and, therefore, reduced maintenance of this system. In this study, the depth difference between bottom boxes and suspended boxes was only 5 m. This was probably too low to lead to different fouling rates, which affect growth as in the study of Gaytan-Mondragon et al. (1993).

5. Conclusions

With the experimental collectors used in this study, up to 45 spat collector $^{-1}$ month $^{-1}$ (average 10 spat collector $^{-1}$ month $^{-1}$) were obtained. Collectors were principally made of onion nets, a recycled cheap material, which is easily accessible at local markets. The design is especially useful for local fishing communities with few resources and which cannot afford sophisticated equipment. The results also show that the natural availability of P. imbricata spat is sufficient to initiate and support aquaculture.

Bags are the least expensive culture systems and can be manipulated more easily on the long lines than boxes. However, bags have a negative effect on growth as the oysters tend to crowd or clump in the bottom folds of the mesh. Growth and mortality of P. *imbricata* in bottom boxes was not significantly lower than in suspended boxes. However, as bottom boxes require SCUBA diving to handle them, suspended boxes are more suitable systems for intermediate culture. In conclusion, suspended boxes with 30% density were the most successful of all culture systems tested in this study. The fact that growth in suspended boxes and in the natural population was almost similar indicates that the materials and the mesh size used are adequate. A commercial size of >50 mm shell length can be reached within 1 year. Further studies using higher stocking densities (i.e. 40% +) are needed to establish the optimal number of spat in the culture systems.

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