A comparative study of the ovarian development in wild and pond-reared shrimp, *Penaeus kerathurus* (Forskål, 1775)

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Abstract

Ovarian maturation was studied in pond-reared and wild-caught specimens of the shrimp *Penaeus kerathurus* in order to evaluate the influence of extensive culture conditions on the reproductive capacity of this species. Monthly samples of five female shrimp were taken from the wild and the pond from late winter (February–March) to mid-summer (July). For each specimen sampled, the body length, body weight and ovarian weight were recorded, and the gonadosomatic index (GSI) calculated. Once dissected out and weighed, the ovaries were processed for histological examination. The size reached by the shrimp reared in the pond was comparable to that recorded in the wild. However, significant differences were found in the GSI and the frequency (expressed as percentage) of postvitellogenic (i.e. vitellogenic plus mature) oocytes, which may be considered as good indicators of the sexual maturation rate. On the basis of the overall gonad histology, five distinct stages have been identified throughout the process of ovarian maturation. A major histological anomaly detected in all pond-reared shrimp examined was the absence of fully mature oocytes, which is due to the inability of late vitellogenic oocytes to synthesize cortical rods. Consequently, stage IV ovaries (characterized by the presence of mature oocytes, i.e., those bearing cortical rods) were not found in captive animals. As the cortical rods are believed to play an important role at early development in penaeid eggs, their absence is probably one major

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constraint resulting in a broodstock population with reduced reproductive potential under culture conditions.

1. Introduction

To date, the efforts devoted to the implementation of a suitable technology for the culture of the shrimp *Penaeus kerathurus* have met important difficulties. Achievement of maturation and spawning of penaeid shrimp in captivity allows the maintenance of permanent broodstock and thus reduces the need for collections from the wild; therefore, the understanding of reproduction is important for the development of a complete culture technology (Primavera, 1985; Quinitio et al., 1993). *P. kerathurus* maintained in captivity have been observed to reach sexual maturity, either in salt ponds (Rodríguez, 1981) or in tanks under controlled diet and water temperature (Luis and Ponte, 1993; Luis, 1993). Nevertheless, full maturation is not accompanied by a comparable reproductive potential in these conditions, since the fertility rate and number of spawned eggs are low (Luis, 1993).

The microscopic anatomy of penaeid ovaries has been described for various species (King, 1948; Duronslet et al., 1975; Yano, 1984, 1988; Bell and Lightner, 1988; Tan-Fermin and Pudadera, 1989; Krol et al., 1992; Quinitio et al., 1993), including *P. kerathurus* (Rodríguez, 1977, 1985). Aside from general histology, quantifiable variables, such as oocyte size frequency and oocyte diameter, have proved good indicators of the ovarian developmental stage in *Penaeus monodon* (Tan-Fermin and Pudadera, 1989). However, comparative histology of the ovary has not been used to assess possible differences in the process of ovarian maturation between wild and captive female shrimp. Thus, previous studies comparing ovarian maturation in wild and captive populations resort to direct observation of morphological features (size and overall appearance of the gonad) (Laubier-Bonichon, 1978; Rodríguez, 1981; Luis and Ponte, 1993; Luis, 1993; Menasveta et al., 1993). The histology of ovarian development of wild-caught and pond-reared *P. kerathurus* shrimp was examined throughout the ongrowing and maturation season (from late winter to mid-summer) to evaluate the influence of extensive culture conditions on female sexual maturation in southern Spain.

2. Materials and methods

2.1. Animals and sampling

Wild specimens of *P. kerathurus* (Forskål, 1775) were captured monthly (from February to July, 1994) by commercial trawlers from the Gulf of Cádiz (off Sanlúcar de Barrameda, southern Spain). Juveniles (weight ranging from 3 to 7 g), destined to be cultured in earthen ponds under extensive conditions, were captured in the channel "Caño de Sancti Petri" (Chiclana de la Frontera, Cádiz) in September 1994. After

thorough selection, 700 apparently healthy specimens were stocked in a 2000 m^2 pond (average depth 1.5 m) located in Chiclana de la Frontera (Cádiz) which had been prepared as described earlier (Rodríguez, 1981). Sea water was pumped from an adjacent 4 hectare pond during the night and filtered through a 1 mm mesh net. The daily renewal rate was between 10% and 20%. Salinity ranged from 35 to 39.5‰ throughout the experimental period, and the temperature records were similar to those reported previously (Rodríguez, 1981), with an average maximum value of 29°C in July and a minimum of 8.5°C in February.

The first sampling of pond-reared shrimp was carried out in March 1994, after which samples were taken monthly until late July, when the reproductive activity of this species is the highest (Rodríguez, 1985). The samples of wild-caught and pond-reared shrimp were transported in aerated containers to the laboratory, where the shrimp were maintained in an open sea water system until use (within 24 h after capture). Five females of each experimental group (13 specimens in the case of wild shrimp captured in February) were sampled. Body length (distance between the tip of the rostrum and the tip of the telson, BL), body weight (BW), ovarian weight (OW), overall appearance of the ovary, and presence/absence of spermatophores in the thelycum were recorded. The gonadosomatic index (GSI) was calculated as percentage of the ovarian weight relative to the BW.

2.2. Histology

Following removal and weighing of the ovaries, fragments of their middle region were fixed for 24 h in 4% formaldehyde in phosphate buffer 0.1 M, pH 7.4. Afterwards, the tissue was dehydrated through increasing concentrations of ethanol and embedded in hydroxyethylmethacrylate. Semi-thin (3 μm thick) sections stained with toluidine blue were examined and photographed in a Leitz light microscope.

To determine the diameter range of the oocytes at the three developmental stages distinguished in this study, the longer diameter of at least 20 cells per stage and ovary section was measured with an eyepiece micrometer (Quinitio et al., 1993). Only oocytes showing nuclei sectioned approximately at the equatorial plane were measured. Slight shrinkage that may take place due to dehydration of the tissue was not considered in the measurements.

The oocyte size frequency (i.e. the relative frequency of the three oocyte categories expressed as percentages) was estimated for all sampled specimens by duplicate counting of the total number of oocytes appearing in the field of view of the microscope when operating with ×25 objective and ×10 eyepiece lenses. In practice, two randomly selected zones of the histological specimen were counted in a similar way to that described by Tan-Fermin and Pudadera (1989). Depending on the histological samples, the total number of oocytes which appeared in the field of the microscope varied between 100 and 500 approximately.

2.3. Quantitative data and statistical analysis

Data on BL, BW, OW, GSI and percentages of oocytes at the different developmental stages are represented as means ± s.e.m. (standard error of the means).

Means of BL, BW, OW, GSI and percentage of vitellogenic oocytes were compared by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test at $P = 0.05$, in order to determine significant seasonal differences in each group of shrimp separately. In addition, to test significant differences between wild and pond-reared shrimp, the data were compared month to month with the same statistical tests. To avoid deviation from normality, the data were arcsine transformed (Zar, 1984).

For statistical treatment of the values of oocyte size frequency, the percentage of mature oocytes, when present (i.e. in wild animals captured in June and July), was added to that of non-mature vitellogenic oocytes, thus including all the oocytes that had started vitellogenesis in a single group under the term of postvitellogenic oocytes. This gives a more realistic idea about the vitellogenic capacity of the ovary in the two shrimp populations.

3. Results

3.1. Biometric data

In the wild population the body size increased gradually from February to the maximum values of June, with a slight decline in July (Table 1). The maximum values of gonad weight and GSI corresponded, in contrast, to July (Fig. 1, Table 1). Cultured shrimp showed maximum size and weight in July (Table 1), whereas OW and GSI
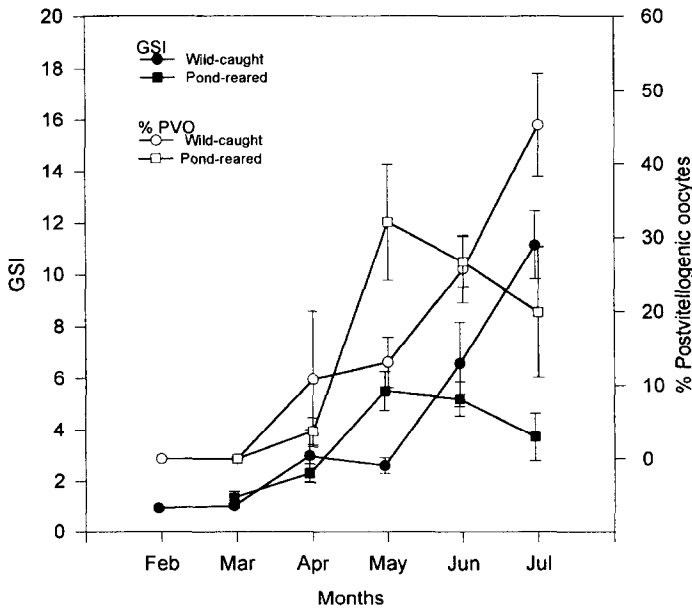

Fig. 1. Monthly variation of GSI and percentage of postvitellogenic oocytes (PVO) in wild-caught and pond-reared *Penaeus kerathurus* shrimp.

Table 1
Monthly variation of body length (BL), body weight (BW), ovarian weight (OW) and gonadosomatic index (GSI) in wild-caught and pond-reared *P. kerathurus* populations.

	Wild-caught shrimp				Pond-reared shrimp			
	BL (mm)	BW (g)	OW (g)	GSI (%)	BL (mm)	BW (g)	OW (g)	GSI (%)
February	110.6 ± 1.3 [a]	9.6 ± 0.3 [a]	0.1 ± 0.0 [a]	1.0 ± 0.0 [a]	-	-	-	-
March	115.2 ± 2.4 [a]	11.1 ± 0.6 [ab]	0.1 ± 0.0 [a]	1.1 ± 0.1 [a]	104.2 ± 3.4 [a,*]	8.3 ± 0.7 [a,*]	0.1 ± 0.0 [a]	1.4 ± 0.2 [a]
April	129.8 ± 5.1 [b]	16.5 ± 2.1 [bc]	0.6 ± 0.3 [a]	3.0 ± 1.0 [a]	134.2 ± 4.4 [b]	18.8 ± 1.7 [b]	0.4 ± 0.1 [b]	2.3 ± 0.4 [ab]
May	136.8 ± 3.6 [bc]	19.2 ± 1.7 [cd]	0.5 ± 0.1 [a]	2.6 ± 0.3 [a]	136.4 ± 3.3 [b]	20.5 ± 2.3 [b]	1.1 ± 0.2 [bc,*]	5.5 ± 0.8 [bc,*]
June	147.2 ± 3.6 [de]	25.6 ± 1.7 [de]	1.7 ± 0.5 [b]	6.6 ± 1.6 [b]	136.2 ± 2.8 [b,*]	18.4 ± 1.0 [b,*]	1.0 ± 0.2 [c]	5.2 ± 0.7 [c]
July	143.4 ± 2.1 [c]	23.9 ± 1.1 [e]	2.8 ± 0.4 [b]	11.3 ± 1.4 [c]	144.4 ± 3.9 [b]	22.6 ± 2.0 [b]	0.8 ± 0.1 [c,*]	3.7 ± 0.9 [c,*]

Data are means ± s.e.m. (n = 5, except for February, where n = 13). s.e.m. = 0.0 implies an s.e.m. of < 0.05. Values within a given column bearing different superscript letters are significantly different (P < 0.05). Asterisks on values within a given row indicate significant differences (P < 0.05) between the two populations in the corresponding month.

showed peaks two months earlier, and then decreased throughout the next two months (Fig. 1).

Within either experimental group, BL, total weight, OW and GSI showed significant differences ($P < 0.05$) with the time of year (Table 1). Comparison between the two populations showed that OW and GSI values were significantly different ($P < 0.05$) for the months of May and July (Table 1), which indicated that ovarian maturation proceeded differently in wild and captive shrimp populations.

3.2. General ovarian histology

The ovarian histology of *P. kerathurus* is typical of that described previously for other penaeids. Based on histological features, five distinct stages have been identified throughout ovarian development:

3.2.1. Stage I (previtellogenic)
Ovaries (Fig. 2a, Fig. 3a) contain only oogonia and previtellogenic oocytes. Two distinct size categories of previtellogenic oocytes can be recognized. The smaller oocytes (17–26 μm in diameter) lie at the centre of the ovary, whereas the larger oocytes (26–65 μm in diameter) appear in follicles located more peripherally.

3.2.2. Stage II (early vitellogenic)
Ovaries (Fig. 2b, Fig. 3b) display oocytes at early vitellogenesis (80–100 μm in diameter). The cytoplasm becomes more acidophilic and undergoes a significant increase in size.

3.2.3. Stage III (late vitellogenic)
Ovaries (Fig. 2c, Fig. 3c,d) contain large oocytes (100–200 μm in diameter) whose cytoplasm is filled with yolk granules. These yolky oocytes are preferentially located in the outer regions of the ovary.

3.2.4. Stage IV (mature)
Ovaries (Fig. 2d) are characterized by the presence of fully mature oocytes that surpass 200 μm in diameter. As a typically distinctive feature, the cytoplasm of mature oocytes show conspicuous cortical rods at the cell periphery.

3.2.5. Stage V (spent or degenerating)
Ovaries (Fig. 2e, Fig. 3e) are distinguished by the presence of atretic (degenerating) oocytes.

3.3. Wild-caught shrimp ovarian histology

In all *P. kerathurus* captured from the wild in February and March (Fig. 2a) the ovaries contained only oogonia and previtellogenic oocytes (stage I). In April and May (Fig. 2b) most ovaries had started vitellogenesis, showing histological features of stage II, with a mean of over 10% vitellogenic oocytes (Table 2). Stage III ovaries were
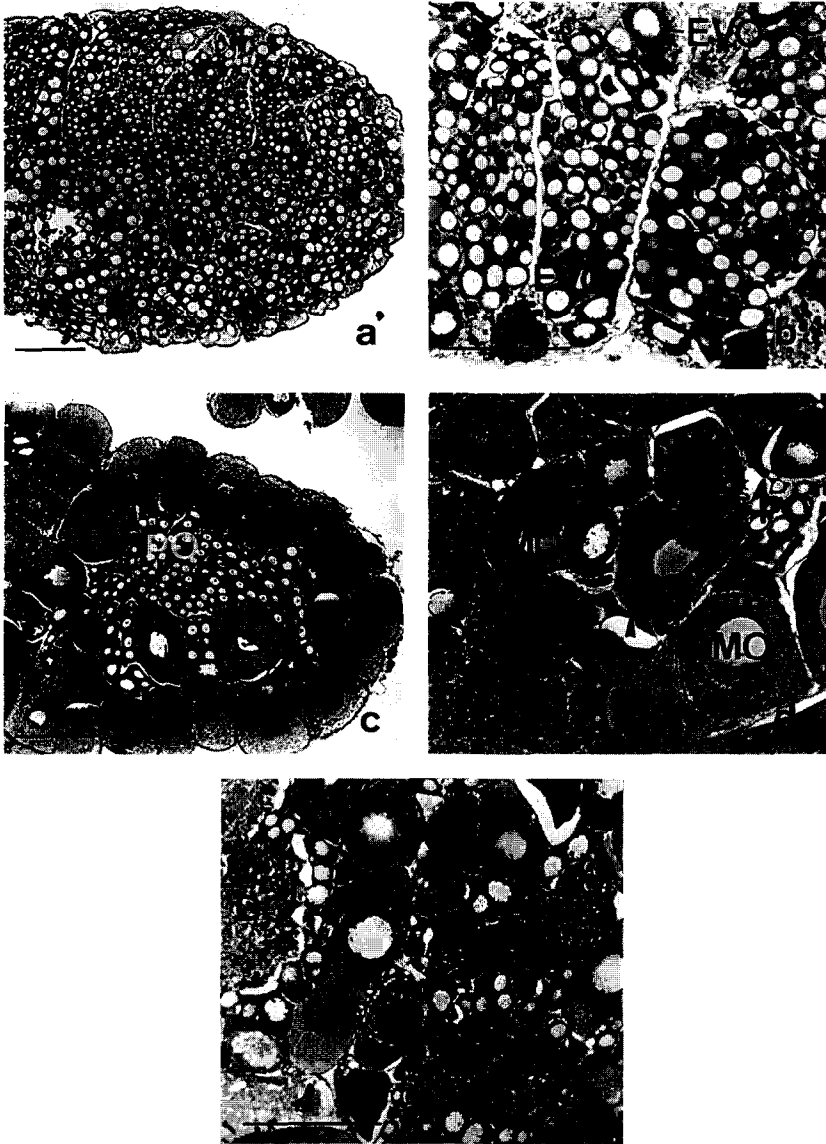
Fig. 2. Sections of wild-caught *Penaeus kerathurus* ovaries stained with toluidine blue. (a) stage I (March); (b) stage II (April); (c) stage III (June); (d) stage IV (July); (e) stage V (July). DO: degenerating oocytes; EVO: early vitellogenic oocytes; LVO: late vitellogenic oocytes; MO: mature oocytes; PO: previtellogenic oocytes. Arrowheads: cortical rods. Scale bars: 200 μm.

predominant in shrimp sampled in June (Fig. 2c), though ovaries containing mature oocytes with cortical rods and others with degenerating oocytes were also present. In this month the overall percentage of vitellogenic oocytes increased to nearly 20%. All
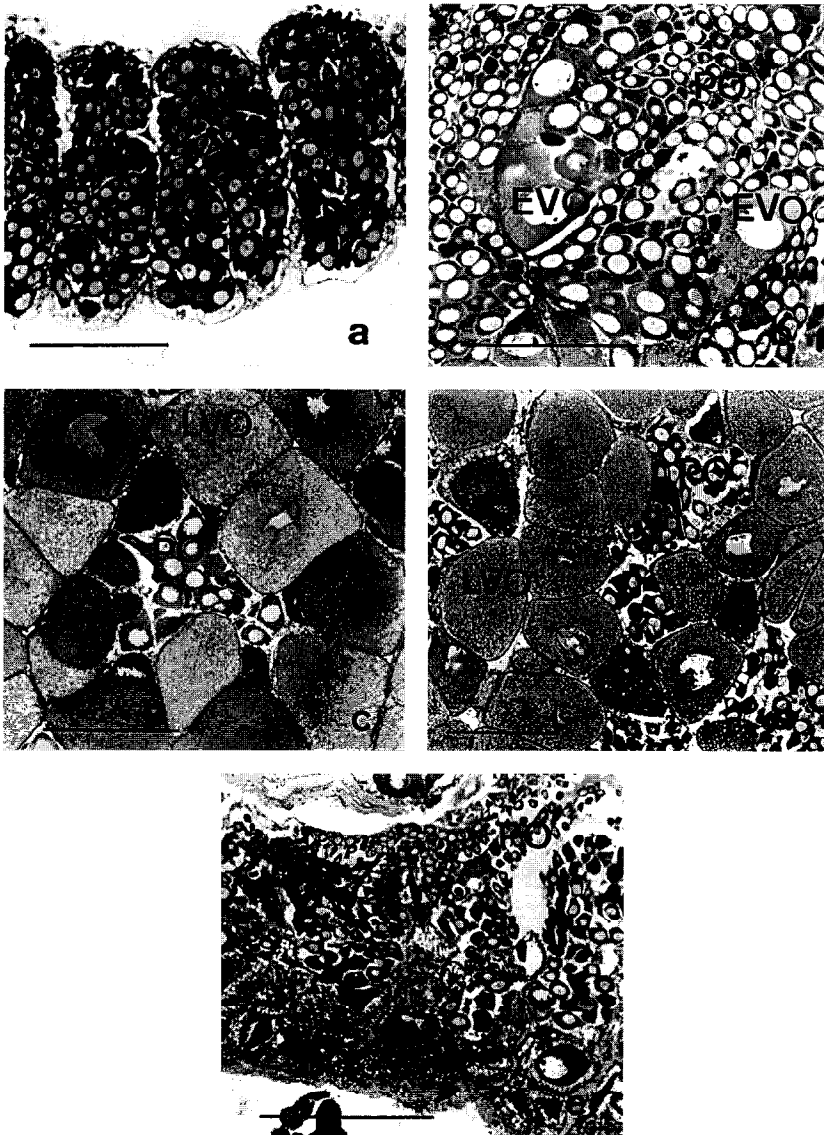
Fig. 3. Sections of pond-reared *Penaeus kerathurus* ovaries stained with toluidine blue. (a) stage I (March); (b) stage II (April); (c) stage III (May); (d) stage III (July); (e) stage V (July). DO: degenerating oocytes; EVO: early vitellogenic oocytes; LVO: late vitellogenic oocytes; MO: mature oocytes; PO: previtellogenic oocytes. Arrow: empty space due to oocyte resorption. Scale bars: 200 μm.

females sampled in July showed stage IV or V ovaries (Fig. 2d,e) with minimum percentages of previtellogenic oocytes and maximum percentages of vitellogenic (about 25%) and mature (about 20%) oocytes (Table 2).

Table 2
Monthly evolution of ovarian stage and frequencies (as percentages) of distinct oocyte categories in wild-caught and pond-reared *P. kerathurus*.

	Wild-caught shrimp			Pond-reared shrimp		
	Stage	% PO	% PVO (% VO + % MO)	Stage	% PO	% PVO (= % VO)
February	I ($n = 13$)	100 ± 0.0	0.0 ± 0.0 [a]	-	-	-
March	I ($n = 5$)	100 ± 0.0	0.0 ± 0.0 [a]	I ($n = 5$)	100 ± 0.0	0.0 ± 0.0 [a]
April	I ($n = 1$), II ($n = 3$), III ($n = 1$)	89.2 ± 9.2	10.8 ± 9.2 [ab] ($10.8 \pm 9.2 + 0.0 \pm 0.0$)	I ($n = 2$), II ($n = 3$)	96.2 ± 1.8	3.8 ± 1.8 [ab]
May	II ($n = 5$)	86.9 ± 3.4	13.1 ± 3.4 [b] ($13.1 \pm 3.4 + 0.0 \pm 0.0$)	III ($n = 4$), V ($n = 1$)	67.9 ± 7.9	32.1 ± 7.9 [bc,*]
June	III ($n = 3$), IV and V ($n = 1$)	74.2 ± 4.6	25.8 ± 4.6 [bc] ($18.8 \pm 6.2 + 6.9 \pm 6.8$)	III ($n = 3$), V ($n = 2$)	73.3 ± 3.4	26.7 ± 3.4 [c]
July	IV ($n = 3$), V ($n = 2$)	54.7 ± 7.1	45.3 ± 7.1 [c] ($25.7 \pm 6.9 + 19.6 \pm 9.8$)	III ($n = 2$), V ($n = 3$)	80.1 ± 8.8	19.9 ± 8.8 [c,*]

PO: previtellogenic oocytes; PVO: postvitellogenic oocytes, including non-mature vitellogenic oocytes (VO) and mature oocytes (MO).
Data are means ± s.e.m. Values within a given column bearing different superscript letters were significantly different ($P < 0.05$). PVO values within a given row showing an asterisk were significantly different ($P < 0.05$) between the two populations in the corresponding month.

3.4. Pond-reared shrimp ovarian histology

As noted for the wild population, in captive animals no vitellogenic activity was observed in March (Fig. 3a), with 100% of previtellogenic oocytes. Similarly, vitellogenesis started in April (Fig. 3b), though the percentage of vitellogenic oocytes was slightly lower than in wild shrimp (Table 2). A marked difference in the ovarian histology relative to wild shrimp was noticed in May (Fig. 3c), when most ovaries were found at stage III and the frequency of vitellogenic oocytes reached the maximum value of 32% (Table 2). It is noteworthy that pond-reared shrimp oocytes evolved normally throughout vitellogenesis to reach the diameter of mature oocytes (approximately 200 μm); however, cortical rods never differentiated in any of the animals examined. Therefore, a true stage IV in terms of histological features was never present in captive shrimp, and stage III ovaries proceeded directly into stage V. Thus, in June and July the specimens examined possessed ovaries at stage III (Fig. 3d) or V (Fig. 3e), with a decreasing percentage of vitellogenic oocytes (26.7 ± 3.4% and 19.9 ± 8.8%, respectively) accompanied by an increased proportion of younger oocytes (Table 2).

Statistical analysis of these data in the two groups separately showed significant seasonal variations in the frequency of vitellogenic oocytes ($P < 0.05$). Nevertheless, comparison between both groups indicated that in May and July the proportion of vitellogenic oocytes in the wild population was significantly different ($P < 0.05$) from that in pond-reared shrimp.

3.5. Discussion

The present results suggest that female *P. kerathurus* grow normally in extensive culture, reaching body weights that are comparable to those recorded in wild specimens. By contrast, the maturation rate, as indicated by the GSI and ovarian histology, appeared to be negatively affected by pond culture conditions. Two earlier studies (Yano, 1987; Menasveta et al., 1993) have shown that, although *P. japonicus* and *P. monodon* are able to mature in ponds, their reproductive potential decreases relative to that of wild specimens. Yano (1987) observed an earlier maturation in pond-reared *P. japonicus* as compared to wild-caught shrimp; however, spawning did not appear to occur in ponds, though further stimulation in the laboratory succeeded in inducing spawning. In *P. monodon*, the egg production of pond-reared shrimp was found to be much lower than that of wild-caught specimens, and this was thought to be due to the great difference in body size between both populations (Menasveta et al., 1993).

Pond-reared *P. kerathurus* did not reach full maturity, since none of the specimens examined for ovarian histology showed mature oocytes. Curiously, in May the ovarian development was clearly more advanced in cultured shrimp than in the animals captured from the wild, an observation that is conclusively supported by the higher values of GSI and percentage of vitellogenic oocytes, as well as by the later developmental stage of the ovary. However, these values decreased progressively from May to July in cultured shrimp, whereas in wild-caught animals they showed an upward trend (see Fig. 1 and Table 2). A significant histological difference between both populations is that in captive shrimp the ovaries pass directly from stage III to V, since late vitellogenic oocytes never

differentiate cortical rods, even though they may be as large as ~ 220 μm in diameter, coinciding with the size of mature oocytes. This is not consistent with the observation of Rodríguez (1981) on female *P. kerathurus* cultured in similar conditions, which appeared to produce mature oocytes, though spawning in the pond was not recorded.

Among the possible ecophysiological factors that can be involved in ovarian maturation, the salinity and photoperiod are not believed to account for the reduced reproductive potential of pond-reared *P. kerathurus*. In fact, salinity in the pond was maintained within a range of values (35–39.5‰) which is very close to that recorded in the open sea (33–38‰), while the photophase to which captive and wild shrimp were subjected was equivalent, since the monthly sampling was carried out almost simultaneously in both populations. However, the intensity of light is considerably higher on the bottom of the pond than at 25 meters depth, where large mature females live; therefore, the hypothesis that light intensity may affect the reproductive capacity of *P. kerathurus* requires further investigation to complete the present observations.

The slight difference in water temperature recorded between the pond and the open sea (maximum values 2–3°C higher and minimum values 2–3°C lower in the pond) may be responsible for the different pattern of ovarian development observed throughout the experimental period. However, Laubier-Bonichon (1978) concluded that in *P. japonicus* the ovarian development is not dependent on the temperature, though this species proved to be very sensitive to photoperiod in terms of ovarian function.

Another factor to be considered is the quality of the diet. According to Luis and Ponte (1993), the presence of polychaetes in the diet may be important to induce ovarian maturation in laboratory-reared *P. kerathurus* because they contribute essential fatty acids (Luis and Ponte, 1993; Luis and Passos, 1995). In our experiment, no external food was supplied to pond-reared animals; furthermore, an examination of the pond showed the associated fauna to consist mainly of bivalves and small crustaceans but not polychaetes. In any case, a biochemical study, mainly focused on lipid and fatty acid composition, is required to assess the hypothesis of the lack of essential nutrients as one of the causes for reduced maturity in pond-reared *P. kerathurus*. The lower frequencies of postvitellogenic oocytes in captive shrimp during the month of maximal reproductive activity (July) in comparison to those observed in wild-caught shrimp clearly indicate a reduced vitellogenic activity under culture conditions. This fact may reflect nutritional deficiencies, so that, in contrast to what is believed to be the natural metabolic tendency in adult shrimp (Tan-Fermin and Pudadera, 1989), the nutrients could be channelled into somatic growth and body mass maintenance rather than into reproductive biosyntheses.

The oocyte size frequency and oocyte diameter are thought to be good indicators of the stage of ovarian maturity in wild *P. monodon* (Tan-Fermin and Pudadera, 1989). In *P. kerathurus*, the percentage of postvitellogenic oocytes was in fact clearly related to the degree of ovarian maturation of wild shrimp, and indicated differences in ovarian development between wild and captive animals. However, the diameter of each oocyte category was not found to be significantly different between the groups.

The inability to form cortical rods cannot be explained satisfactorily in terms of nutritional deficiency, because in a number of cultured specimens, large oocytes containing as much yolk as mature oocytes of wild specimens failed to develop cortical rods. Probably, the lack of cortical rods in pond-reared *P. kerathurus* specimens may be

attributed, in principle, to altered hormonal activity rather than to energetic or metabolic causes. The penaeid cortical rods are invaginations of the oolemma (hence the widely used term "cortical crypts") that contain a jelly precursor. Upon contact with sea water at spawning, the material of cortical rods is released around the egg and forms a jelly layer whose function is still undetermined, though they are believed to play an important role at early development (Lynn et al., 1991). These specializations of the oocyte surface are synthesized shortly before spawning; therefore, the percentage of mature oocytes may be considered as a reliable index of maturity and spawning activity (Anderson et al., 1984).

In conclusion, extensive culture of *P. kerathurus* appears to be viable in terms of biomass production. However, the full closing of the reproductive cycle is still far from resolved. In addition to a lower vitellogenic capacity, which results in a decreased number of yolky oocytes, a major problem observed in captivity is the inability of late vitellogenic oocytes to produce cortical rods. Consequently, ovaries at stage III proceed into stage V ovaries where large oocytes degenerate. Whether spawning occurs in the pond at the end of stage III is not known, but if so the eggs may have little or no viability, since the penaeid cortical rods are thought to be important in early development. Ultrastructural and biochemical studies are being carried out to investigate the possible causes for cortical rod synthesis inhibition and the repercussions of diet composition in ovarian maturation.

Acknowledgements

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