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Reproductive readiness of the shrimp *Litopenaeus vannamei* grown in a brackish water system

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Abstract

The reproductive readiness of the marine shrimp *Litopenaeus vannamei* (formerly *Penaeus vannamei*) cultured in brackish water was characterized by applying morphological, physiological, and molecular tools. The shrimp were cultured on two commercial shrimp farms in brackish water that was pumped from artesian wells that tap into a geothermal aquifer. The shrimp populations exhibited a bimodal growth curve with the females being significantly bigger than the males at the end of the growout period. Some male shrimp started to develop spermatophores about 6 months after the first post-larval (PL₀) stage, and some with developed, normal-looking, white spermatophores were observed 8 months after PL₀. The sperm count in these males was $10.1 \times 10^6 \pm 5.8 \times 10^6$ cells per compound spermatophore, and $81.6 \pm 19.8\%$ of the cells were spiked. Melanization of the males, which eventually affected about a third of the male population, first became evident before the appearance of white spermatophores. Female ovaries were transparent and appeared to be arrested in a previtellogenic stage. However, beyond a weight/age threshold of 20 g/8 months, some of the ovaries had become opaque and the vitellogenin gene was found to be expressed in the ovary—but not in the hepatopancreas—of 7 out of 10 females. A unique case of a 46.8 g female with a fully developed ovary that was found in the brackish-water-held broodstock is reported.

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Keywords: Pacific white shrimp; *Litopenaeus vannamei*; Crustacea; Decapoda; Penaeidae; Geothermal brackish water; Reproduction; Spermatophores; Melanization; Vitellogenin; Bimodal growth

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1. Introduction

The development of inland shrimp production in water from saline aquifers is currently viewed as an economically and environmentally sustainable alternative to traditional coastal aquaculture. Recent protests concerning the destruction of mangrove habitat as a result of coastal shrimp aquaculture have drawn attention to environmentally more controllable inland shrimp production (Anon, 2003). Reduced-salinity culture systems for marine shrimp, which are operated under low or zero water exchange, can be set up on farms far removed from potentially contaminated coastal waters (Decamp et al., 2003). They limit the risk of introducing devastating shrimp pathogens, ease the problem of waste disposal or treatment, and facilitate the use of effluents for the production of agricultural crops. Inland culture of the Pacific white shrimp *Litopenaeus vannamei* (formerly *Penaeus vannamei*), in low-salinity well waters is currently being conducted successfully on a small scale in the USA and Israel, with adequate growth rates and acceptable shrimp production being obtained (Smith and Lawrence, 1990; Samochoa et al., 1998; Appelbaum et al., 2002; McGraw et al., 2002; Saoud et al., 2003). Procedures for the acclimation of *L. vannamei* post-larvae (PL) from the salinity of seawater hatcheries to that of brackish water growout ponds are well documented (McGraw et al., 2002). However, there is no data on the reproductive performance of shrimp grown from the first PL stage to adult size in low-salinity water.

Although penaeid reproduction in captivity has been under investigation for more than half of a century (Wickins and Lee, 2002), the obstacles standing in its way are currently more challenging than ever, and the questions raised have not found easy solutions (Browdy, 1998). Penaeid male reproductive performance is currently regarded as one of the major drawbacks in the fragile shrimp aquaculture industry. To date, there are no reliable bioassays for the evaluation of male broodstock performance, but some generalizations can be drawn from the current body of knowledge, at least for *L. vannamei*: (1) White spermatophores showing high sperm counts and a high percentage of spiked sperm are considered most suitable for fertilization, while melanized spermatophores are not. (2) Penaeid males do mature sexually in low-salinity waters (Ogle, 1992). (3) High water temperatures have a negative effect on sperm quality and lead to a deterioration in sperm quality and the spermatogenic apparatus that could be lethal to the shrimp (Perez-Velazquez et al., 2001). (4) Males with melanized spermatophores are considered sexually inadequate and should be removed from an active broodstock (Wyban and Sweeney, 1991).

A large volume of data on penaeid female reproductive parameters and performance in a marine-like environments has accumulated over the years, with the most recent publications being devoted to the cloning and analysis of a number of complete penaeid vitellogenin cDNAs and genes (Tsutsui et al., 2000 Garcia-Orozco et al., 2002; Tsang et al., 2003; Avarre et al., 2003; GenBank accession numbers for *Penaeus semisulcatus*, *Marsupenaeus japonicus* and *Metapenaeus ensis* are AY051318, AB033719 and AF548363, respectively). The vitellogenic process in shrimps has been studied from morphological, anatomical physiological and biochemical points of view, and the basic environmental and nutritional requirements for gonad maturation have been determined. The relevant data for *L. vannamei* may be summarized as follows: (1) It is still not clear whether female maturation is size- or age-dependent, although it has been established

that the females most suitable for reproduction are those heavier than 35 g and younger than two years of age (Ogle, 1992). (2) Seawater of 20 ppt salinity is considered the lower limit for gonad maturation and spawning. However, larvae hatched at that salinity have subsequently to be transferred to water of at least 28 ppt salinity, which thus becomes the de facto lower limit commercial for shrimp production (Ogle, 1992). (3) The sites of vitellogenin (Vg) synthesis are the hepatopancreas and the ovary (Tsutsui et al., 2000; Tseng et al., 2001, 2002; Avarre et al., 2003). (4) Generally, females do not reproduce spontaneously in captivity, even when they are grown in natural seawater. Endocrine manipulation combined with a special diet and specific environmental parameters are required to induce the onset of vitellogenesis and maturation of the ovary. This reproductive data does not cover the reproductive performance of *L. vannamei* grown inland in low-salinity water and it is also not known whether this environment affects the ability of the shrimp to mature sexually if transferred to seawater. To bridge this knowledge gap, the current study provides the first thorough investigation of the reproductive performance of marine shrimp grown in brackish water. Such knowledge is crucial to the success of commercial maturation facilities for the growout of larvae in brackish water.

The above notwithstanding, a unique case of a fully developed ovary in a 46.8 g, *L. vannamei* female of a broodstock held in brackish water is reported for the first time in this study.

2. Materials and methods

2.1. Brackish water systems

The shrimp farms that participated in this study are situated in the Ramat Negev area, west of Beer-Sheva, Israel (34°45'N, 31°00'E). The water for the acclimation tanks and growout ponds is pumped from a number of artesian wells that tap into a huge (estimated 200 billion cubic meters) fossil (tens of thousands of years) geothermal aquifer, known as the Nubian sandstone aquifer (Issar, 1985). Physical and chemical analyses of wells in the region indicate substantial variation among them in parameters such as temperature, pH, salinity and ion composition (Weinberger et al., 1991). The water pumped into the ponds is a mix of a number of wells. This brackish water (2–3 ppt salinity; osmolarity 103.0 ± 20.1 mosM) has a unique composition that differs considerably from that of seawater, as shown in Table 1. The water is cooled (from about 40 to 28 °C) and degassed before use. All water systems on the farms are located in green-roofed greenhouses, enabling a constant temperature of 28 °C to be maintained.

2.2. Animals

The farms that participated in this study obtained *L. vannamei* as PL₈, with a mean weight of about 2 mg, from a pathogen-free source in the USA (Shrimp Improvement Systems, FL). The PL were acclimatized to the local brackish water in small tanks and then transferred to growout ponds containing water of the same composition. The shrimps were fed with

Table 1

Ranges of ion concentrations in geothermal brackish water from the Nubian aquifer of the Negev highlands, Israel compared to natural seawater

Ions	Range of geothermal Negev brackish water used in this study	Natural seawater [average values adapted from Walton (1981)]	Units
Salinity	2–3	35	g/l
Na	568–1180	10,760	mg/l
Ca	160–267	412	mg/l
Mg	79–111	1297	mg/l
K	16–53	399	mg/l
Fe	104–1290	3.1	µg/l
Mn	7–20	1.2	µg/l
Zn	8–94	6.9	µg/l
B	0.88–2.48	4.6	mg/l
Ba	31–50	20	µg/l
Cr	3–7	0.3	µg/l
Cl	971–2060	19,353	mg/l
SO ₄	260–600	2712	mg/l

commercial crustacean pellets (Rangen Buhlder, ID, USA). During a long-run observation, 25 males and 25 females were sampled and examined externally every 2 weeks from each of three concrete growout ponds (440 m²) at one of the farms from October 2000 to March 2001. The animals were then weighed individually and carapace length was measured. Some of the sampled shrimps were transferred on ice to the laboratory for further analysis. Other animals, destined for RNA isolation, were transported live to the laboratory.

2.3. Spermatophore development and analysis

On the basis of the external examination, each male animal was assigned to one of the following spermatophore development categories:

- 1) Translucent—males with a developed ampoules that can be seen through the brown-beige cuticle as a small whitish area
- 2) White line—males with ampoules containing a developing spermatophore that is visible as a thin white line inside the ampoule
- 3) White—males with white spermatophores that are larger than those evident in white-line males
- 4) Partially/completely brown—males with spermatophores whose external surface are partly or entirely brownish-black, indicating melanization.

Categories 3 and 4 were adapted from those proposed by Alfaro and Lozano (1993). Males from each of the above-described categories (at least 25 per category) were transferred on ice to the laboratory for further analysis. After 8–12 h on ice (Bray and Lawrence, 1998), each animal was dissected ventrally between the bases of the walking legs. The pair of ampoules was pulled out, cut away from the sperm duct, and the two halves of the compound spermatophore, if present, were then squeezed out of the ampoule.

Sperm count and viability/maturity were evaluated as described elsewhere (Leung-Trujillo and Lawrence, 1987; Heitzmann et al., 1993; Wang et al., 1995).

2.4. Female reproductive parameters

Due to difficulties in dissecting complete pre-vitellogenic ovaries, only six lobes {the anterior pair (head) and the four most anterior of the lateral lobes (thoracic)} from each ovary were dissected out and weighed. The gonadosomatic index (GSI) was calculated for these six lobes (Ceballos-Vazquez et al., 2003). Oocyte diameters were also measured. A fully developed ovary from a 46.8-g female of the broodstock held in the brackish water was also dissected, and the GSI and oocyte diameter were recorded. In addition, high-density-lipoproteins (HDL) were separated from the latter ovary, as described elsewhere (Sagi et al., 1999).

2.5. RNA isolation and reverse transcription

Total RNA was isolated from the muscle, hepatopancreas and testis or ovary of males and females sampled from the same population three times during a two-month period using TRIZOL (Life Technologies, MD, USA). First-strand cDNA was prepared using Expand Reverse Transcriptase (Roche Diagnostics, Mannheim, Germany), oligo d(T)₁₈ primers, and the reaction conditions recommended by the manufacturer.

2.6. Detection of vitellogenin gene expression

Primers for the polymerase chain reaction (PCR) of the *L. vannamei* vitellogenin cDNA were as follows:

sense: 5'-TGGACCTGAATTCAATGTGGATGTG-3' and
antisense: 5'-CTTGGGCAGAACCCAGGTTGTTGAAG-3' or
sense: 5'-CAGAAGAACATGTTGTGGCATTCAAGGTAG-3' and
antisense: 5'-TGTACTGAACAGCGCCATGATGG-3'.

These sequences were chosen based on the high degree of homology between vitellogenin-specific sequences of two complete *Vg* cDNAs from the phylogenetically related shrimps, *M. japonicus* and *P. semisulcatus* (Tsutsui et al., 2000; Avarre et al., 2003). These sequences were later confirmed when aligned to our recently deposited *L. vannamei* *Vg* cDNA (GenBank entry AY321153, Raviv et al., 2003). PCR products were generated using Vent DNA Polymerase (New England Biolabs, Beverly, MA, USA) or AmpliTaq DNA Polymerase (Perkin Elmer, Roche Molecular System, Branchburg, NJ, USA). Thirty PCR cycles consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and elongation at 72 °C for 2 min, were followed by a 10-min extension at 72 °C. An RNA sample from one of the tissues was a negative control for genomic or other contamination. Ovarian cDNA from a female that had been induced to mature in seawater was a positive control. For all RNA samples, PCR using primers to the house-keeping gene *Eft2* (Tan et al., 2000) was a positive control.

2.7. Data analysis

One-way ANOVA and post-hoc LSD tests were performed where necessary, using *Statistica* version 6 package by StatSoft.

3. Results

3.1. Shrimp growth

Females are slightly heavier than the males, with the difference being significant for the first time at about 6.5 months (Fig. 1A). Towards the end of the growout period, the

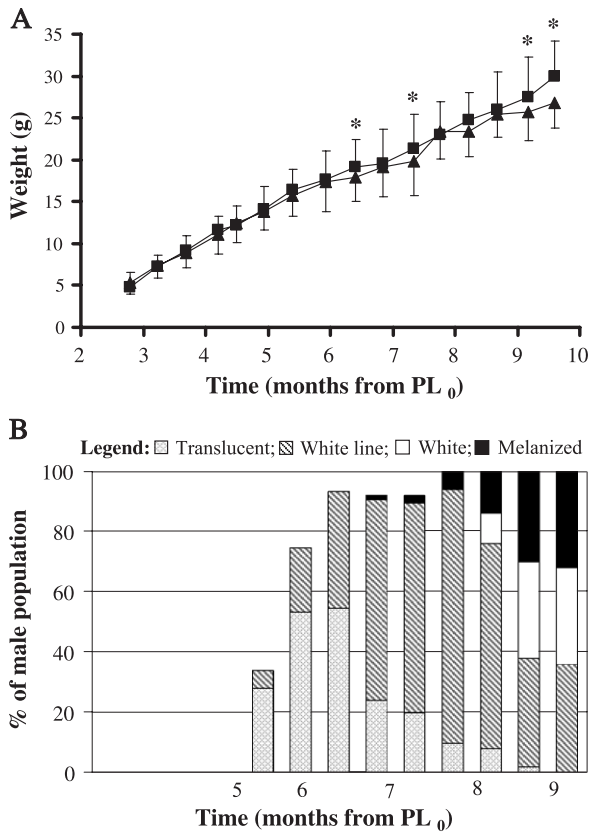


Fig. 1. (A) Growth curves of male (▲ with minus error bars) and female (■ with plus error bars) *L. vannamei* in a commercial geothermal brackish water shrimp farm. Means were calculated from the combined data of three populations (100–120 animals/m²) sampled, with each point representing 25–75 animals. Asterisks indicate a significant difference between males and females ($p < 0.05$). (B) Histogram showing the distribution of males with spermatophores subdivided into different stages in the above shrimp farm population. Different colored sections in the bars represent the percentage of males in the sample that were found with spermatophores in a certain stage.

difference in weight between the males and the females became more marked (mean weight 29.9 ± 4.3 vs. 26.8 ± 3.1 g for females and males, respectively).

3.2. Male morphological–anatomical reproductive parameters

Males with a developed ampoule but no spermatophore (classified as translucent) started to appear about 5.5 months from PL₀ (Fig. 1B). The abundance of such males peaked 6.5 months from PL₀, and declined thereafter as other categories appeared. Individuals with ampoules containing a developing spermatophore with a small number of sperm cells (classified as white line) started to appear at 5.5 months from PL₀. By nearly 8 months from PL₀, they constituted the majority of the population (84%) and thereafter became less abundant as more developed categories appeared. Males with white spermatophores appeared about 8 months from PL₀, and their abundance peaked just before 9 months from PL₀ (32%). Melanization of the spermatophores began to appear in very small percentage of the male population (2%) about 7 months from PL₀. By the end of the observation period (9 months), melanized animals made up 32% of the population, with 8% of the population being fully melanized.

The ampoules of white-line males contained small developing spermatophores with a small amount of sperm ($1.83 \times 10^6 \pm 2.9510^6$ cells per compound spermatophore, Fig. 2). The sperm counts for the white-spermatophore males, which were significantly larger than the white-line males, were significantly ($p < 0.05$) higher than those of the white-line males ($10.1 \times 10^6 \pm 5.8 \times 10^6$ cells per compound spermatophore). These spermatophores contained the highest percentage of spiked cells ($81.6 \pm 19.8\%$). Partially/fully melanized-spermatophore males (with a wide range of body weights) had slightly lower sperm counts than those of the white-spermatophore males but not significantly different. The percentage of spiked cells in the spermatophores of the partially melanized-spermatophore males

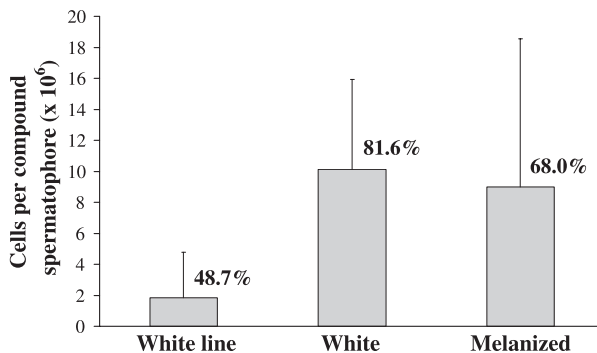


Fig. 2. Reproductive parameters of *L. vannamei* males sampled from a brackish-water-grown population. Columns represent sperm counts \pm S.D. (cells/compound spermatophore) in the different categories (see text for category explanation). The percentages appearing above the columns indicate the % of spiked cells out of the total number of viable cells counted. Only the data from the white-line males category differed significantly ($p < 0.001$) from the other categories (for both sperm counts and percentages of spiked sperm). The translucent males category is not shown in this figure as there were no spermatophores in the ampoules of those males.

was lower ($68.0 \pm 36.9\%$) than that in the spermatophores of white males but again, not significantly different.

3.3. Female reproductive parameters and *Vg* gene expression

The growth curve for *L. vannamei* females grown in a brackish water cultured population shown in Fig. 3 covers a 2-month period during which the female animals increased in weight from 15.3 ± 2.8 to 26.8 ± 4.7 g. During this time, the oocyte diameters increased from 17.3 ± 6.7 to 46.5 ± 10.7 μm , and the color of the ovaries changed from transparent in the smaller females to opaque in the larger ones.

Vg gene expression was examined in 5 females and 5 males selected at random from the above population at three time points during the study. At about 5.5 and 7 months after

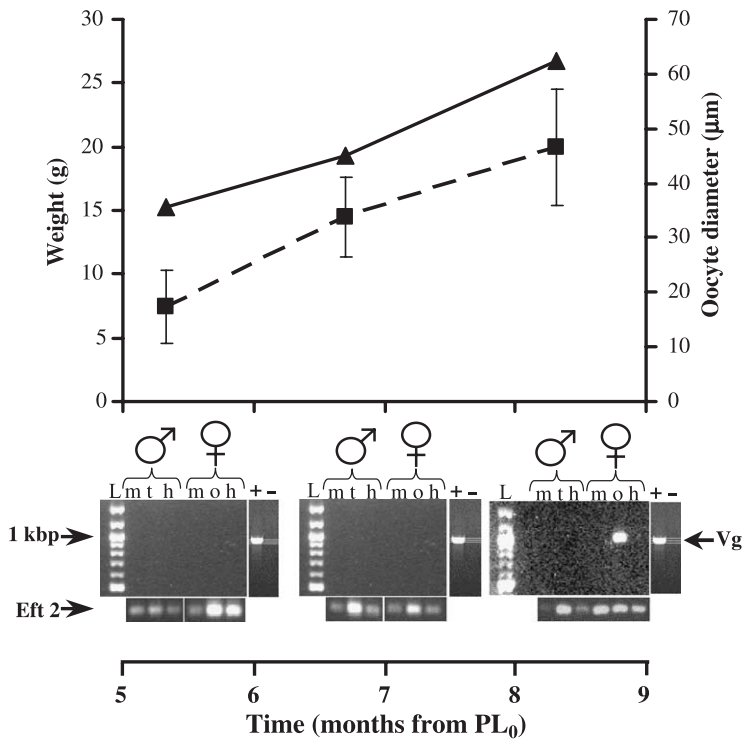


Fig. 3. Growth curve and oocyte diameters of *L. vannamei* females grown in geothermal brackish water and vitellogenin (*Vg*) gene expression. —▲— denotes average weights (error bars were omitted here for clarity) and —■— average oocyte diameters \pm S.D. Weights and oocyte diameters differed significantly among the three time points ($p < 0.001$ and $p < 0.01$ for weights and oocyte diameters, respectively). The pictures below the graph illustrate representative *Vg* gene expression results as determined by RT-PCR and agarose gel electrophoresis as described in the methods. Each of the three pictures corresponds to one of three time points in the graph (from left to right). L—DNA ladder marker, m = muscle, h = hepatopancreas, t = testis, o = ovary, + = ovarian cDNA from a female that had been induced to mature in seawater (positive control), — = RNA from female hepatopancreas (negative control), Eft2 = shows the results using primers to the house keeping gene (Elongation factor 2).

PL₀, no expression of the *Vg* gene could be detected in the animals. However, as the females grew larger than the threshold of 20 g (about 8.5 months from PL₀), some of them expressed the *Vg* gene—but only in their ovaries (see Fig. 3). The examination at this time point was repeated in two different brackish water cultured populations (5 females examined per population), and together 7 out of the 10 females examined expressed the *Vg* gene in the ovary but not in the hepatopancreas. The range of body weights of the females that expressed the *Vg* gene was between 20.7 and 28.1 g and their average GSI was 31.6 ± 13.2 mg. The males did not express the *Vg* gene at any time examined.

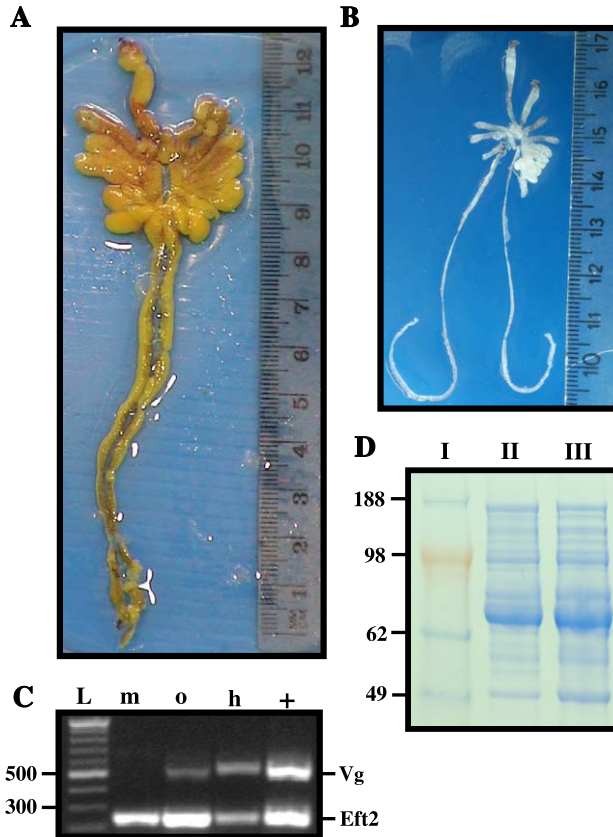


Fig. 4. (A) Vitellogenic ovary of a *L. vannamei* female found in a broodstock held in geothermal brackish water (note that the right anterior lobe is missing). (B) Pre-vitellogenic ovary from a similar-sized *L. vannamei* female. (C) PCR results. Tissues were subjected to RT-PCR as described in the methods and resolved by agarose gel electrophoresis. Lanes are as follows: L: DNA ladder marker, m, o and h: muscle, ovary and hepatopancreas from the vitellogenic brackish water female, respectively. += Ovarian cDNA from a female that had been induced to mature in seawater (positive control), Vg=expected size of band using vitellogenin specific primers, Eft2=expected size of band using primers specific for the house-keeping gene Elongation factor 2. (D) SDS-PAGE (10%) HDL profile of the vitellogenic ovary obtained by ultracentrifugation on a NaBr gradient; lanes are as follows: I: molecular weight marker, II: HDL profile of the vitellogenic brackish water female, III: positive control from an ovary of a female that was induced to mature in seawater.

3.4. Reproductive parameters of the female with a vitellogenic ovary

A female shrimp weighing 46.8 g and having a fully vitellogenic ovary was found during a routine examination of a brackish-water cultured broodstock. A comparison between that ovary and a previtellogenic ovary dissected from a similar-sized female is given in Fig. 4. The only abnormality detected in the vitellogenic ovary was the absence of the right anterior lobe. The average oocyte diameter of the vitellogenic ovary measured without squeezing was calculated as $201.6 \pm 29.0 \mu\text{m}$ and the GSI of the complete ovary was 4.21%. *Vg* gene expression was detected in the ovary and the hepatopancreas of the female with the fully vitellogenic ovary (Fig. 4C). SDS-PAGE (10%) separation of the HDL's extracted from the ovary (Fig. 4D) gave a polypeptide profile that was very similar to that of a vitellogenic ovary that had been induced to mature in seawater.

4. Discussion

The utilization of inland brackish well water for the culture of marine shrimps has a relatively recent history. It is thus not surprising that data on the growth and survival shrimps in such water are scarce (Smith and Lawrence, 1990; Nelson and Flickinger, 1992; Appelbaum et al., 2002; McGraw et al., 2002; Saoud et al., 2003) and that information on the reproductive performance of these marine decapods in brackish water is at best anecdotal (Ogle, 1992). It is, however, well known that crustaceans tend to exhibit bimodal growth patterns, in which one sex grows to larger sizes than the other (Hartnoll, 1982). In decapods, there is little difference between the sexes in the growth pattern prior to maturity with respect to parameters such as the intermolt period and the size. However, after puberty, the females usually molt less frequently, grow more slowly, and finally reach a smaller size than males (Hartnoll, 1982), as has recently been shown in the fresh water crayfish *Cherax quadricarinatus* (Manor et al., 2002). Occasionally, the pattern is reversed, with the females growing faster and reaching a larger size at maturity, as is the case for penaeid shrimps (Choe, 1971; Hartnoll, 1982; Bray and Lawrence, 1992; Primavera et al., 1998; Hoang et al., 2003). The growth curves for *L. vannamei* from juveniles to subadults found in this study thus take the expected form, with the females becoming significantly larger than males towards the end of the growout period.

In contrast to the paucity of data on the culture of marine shrimps in brackish water, the literature abounds with information on sperm, spermatophore development and associated diseases in large sexually mature penaeid shrimp males grown in marine-like environments (Dougherty and Dougherty, 1989; Talbot et al., 1989; Chow et al., 1991; Alfaro and Lozano, 1993; Clark and Griffin, 1993; Heitzmann et al., 1993; Bray and Lawrence, 1998). To the best of our knowledge, only one study has addressed the issue of sperm quality in brackish-water-grown shrimp, but only after transfer of the animals to seawater (Perez-Velazquez et al., 2001), and there are no studies describing the development of the spermatophore from penaeid juveniles to subadults, regardless of water type. In the present study, it is clear (from Figs. 1B and 2) that in brackish water all males developed spermatophores and that melanization started long before white-spermatophore males appeared in the population. In contrast to previous reports that melanization is usually

associated with large males carrying well-developed spermatophores, our data thus suggest that this phenomenon may well start before sexual maturity.

In our study, melanization affected about a third of the male population, while males carrying white, healthy looking spermatophores comprised a similar-sized fraction. Our study also showed that subadult males carrying white, healthy looking spermatophores exhibited sperm counts in the millions, with the majority of the cells being spiked and apparently ripe for fertilization. These data are in agreement with other studies that evaluated similar-sized males of the same species grown in a marine-like environment (Alfaro and Lozano, 1993; Alfaro, 1996).

Studies on penaeid female maturation and vitellogenesis have, to date, dealt only with large, sexually mature females, regardless of the species examined or the animal source (wild vs. pond-reared). The most recent data have appeared in a small number of studies that have approached these issues from a molecular point of view, i.e., sequencing and structure elucidation of the *Vg* gene and determination of *Vg* gene expression site(s) (Tsutsui et al., 2000; Tseng et al., 2001, 2002; Avarre et al., 2003; Tsang et al., 2003). The *Vg* gene encodes the major yolk protein precursor in penaeid shrimps, and the determination of the timing of its expression and the tissues in which it is expressed is fundamental to our understanding of the vitellogenic process and the maturation of the ovary in these animals. The above-cited studies, all based on a very small number of large wild-type females (with natural or artificial induction of ovarian maturation), concluded that in penaeid shrimps, including *L. vannamei*, the sites of *Vg* gene expression are the hepatopancreas and the ovary. Taking all this into account, it is interesting to note that the expression of the *Vg* gene in subadult females, in brackish water, occurs only in the ovary and only after a certain threshold of weight/age has been passed. In our pond population, *Vg* gene expression was detected in ovaries that were undoubtedly pre-vitellogenic at about the same time that the spermatophores of some of the males contained morphologically mature sperm. Further studies are needed to quantify these expression events along a time axis. It still remains to be shown that after being transferred to a marine-like environment, broodstock reared under these conditions will mate successfully and produce viable spawns.

To the best of our knowledge, this study includes the first report of a case of a fully developed ovary in a female grown in brackish water. The ovary looked normal, with the exception of a missing anterior lobe, and had an HDL profile falling in the range (45–205 kDa) previously reported for this species (Quackenbush, 1989; Rankin et al., 1989; Tom et al., 1992). The expression of the *Vg* gene in both the ovary and the hepatopancreas suggests that the vitellogenic process had not been completed. We cannot explain the occurrence of vitellogenesis in this female grown in brackish water conditions, but from this unique case it can be inferred that vitellogenesis under brackish water conditions might be possible.

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References

- Alfaro, J., 1996. Effect of 17-alpha-methyltestosterone and 17-alpha-hydroxyprogesterone on the quality of white shrimp *Penaeus vannamei* spermatophores. *J. World Aquac. Soc.* 27, 487–492.
- Alfaro, J., Lozano, X., 1993. Development and deterioration of spermatophores in pond-reared *Penaeus vannamei*. *J. World Aquac. Soc.* 24, 522–529.
- Anon, X., 2003. Green protests against World Bank funding for shrimp industry. *Mar. Pollut. Bull.* 46, 5.
- Appelbaum, S., Garada, J., Mishra, J.K., 2002. Growth and survival of the white leg shrimp (*Litopenaeus vannamei*) reared intensively in the brackish water of the Israeli Negev desert. *Isr. J. Aquac.-Bamidgch* 54, 41–46.
- Avarre, J.-C., Michelis, R., Tietz, A., Lubzens, E., 2003. The relationship between vitellogenin and vitellin in a marine shrimp (*Penaeus semisulcatus*) and molecular characterization of vitellogenin cDNAs. *Biol. Reprod.* 69, 355–364.
- Bray, W.A., Lawrence, A.L., 1992. Reproduction of *Penaeus* species in captivity. In: Fast, A.W., Lester, L.J. (Eds.), *Marine Shrimp Culture: Principles and Practices*. Elsevier, Amsterdam, pp. 93–170.
- Bray, W.A., Lawrence, A.L., 1998. Male viability determinations in *Penaeus vannamei*: evaluation of short-term storage of spermatophores up to 36 h and comparison of Ca-free saline and seawater as sperm homogenate media. *Aquaculture* 160, 63–67.
- Browdy, C.L., 1998. Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. *Aquaculture* 164, 3–21.
- Ceballos-Vazquez, B.P., Racotta, I.S., Elorduy-Garay, J.F., 2003. Qualitative and quantitative analysis of the ovarian maturation process of *Penaeus vannamei* after a production cycle. *Invertebr. Reprod. Dev.* 43, 9–18.
- Choe, S., 1971. Body increases during molt and molting cycle of the oriental brown shrimp *Penaeus japonicus*. *Mar. Biol.* 9, 31–37.
- Chow, S., Dougherty, M.M., Dougherty, W.J., Sandifer, P.A., 1991. Spermatophore formation in the white shrimps *Penaeus setiferus* and *P. vannamei*. *J. Crustac. Biol.* 11, 201–216.
- Clark, W.H., Griffin, F.J., 1993. Acquisition and manipulation of penaeoidean gametes. In: McVey, J.P. (Ed.), *CRC Handbook of Mariculture: Crustacean Aquaculture*, vol. 1. CRC Press, Boca Raton, pp. 133–151.
- Decamp, O., Cody, J., Conquest, L., Delanoy, G., Tacon, A.G.J., 2003. Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone), within experimental zero-water exchange culture systems. *Aquac. Res.* 34, 345–355.
- Dougherty, W.J., Dougherty, M.M., 1989. Electron microscopical and histochemical observations on melanized sperm and spermatophores of pond-cultured shrimp, *Penaeus vannamei*. *J. Invertebr. Pathol.* 54, 331–343.
- García-Orozco, K.D., Vargas-Albores, F., Sotelo-Mundo, R.R., Yepiz-Plascencia, G., 2002. Molecular characterization of vitellin from the ovaries of the white shrimp *Penaeus (Litopenaeus) vannamei*. *Comp. Biochem. Physiol.* 133 (Part B), 361–369.
- Hartnoll, R.G., 1982. Growth. In: Bliss, D.E., Abele, L.G. (Eds.), *The Biology of Crustacea: Embryology, Morphology, and Genetics*, vol. 2. Academic Press, New York, pp. 111–196.
- Heitzmann, J.C., Diter, A., and AQUACOP, 1993. Spermatophore formation in the white shrimp, *Penaeus vannamei* Boone 1931: dependence on the intermolt cycle. *Aquaculture* 116, 91–98.
- Hoang, T., Barchiesi, M., Lee, S.Y., Keenan, C.P., Marsden, G.E., 2003. Influences of light intensity and photoperiod on moulting and growth of *Penaeus merguensis* cultured under laboratory conditions. *Aquaculture* 216, 343–354.
- Issar, A., 1985. Fossil water under the Sinai–Negev peninsula. *Sci. Am.* 253, 82–88.
- Leung-Trujillo, J.R., Lawrence, A.L., 1987. Observations on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. *Aquaculture* 65, 363–370.
- Manor, R., Segev, R., Leibovitz, M.P., Aflalo, E.D., Sagi, A., 2002. Intensification of redclaw crayfish *Cherax quadricarinatus* culture: II. Growout in a separate cell system. *Aquac. Eng.* 26, 263–276.

- McGraw, W.J., Davis, D.A., Teichert-Coddington, D., Rouse, D.B., 2002. Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *J. World Aquac. Soc.* 33, 78–84.
- Nelson, S.M., Flickinger, S.A., 1992. Effects of geothermal saline spring water on white shrimp, eastern oyster, and fresh-water prawn. *Prog. Fish-Cult.* 54, 28–34.
- Ogle, J.T., 1992. A review of the current (1992) state of our knowledge concerning reproduction in open thelycum penaeid shrimp with emphasis on *Penaeus vannamei*. *Invertebr. Reprod. Dev.* 22, 267–274.
- Perez-Velazquez, M., Bray, W.A., Lawrence, A.L., Gatlin III, D.M., Gonzalez-Felix, M.L., 2001. Effect of temperature on sperm quality of captive *Litopenaeus vannamei* broodstock. *Aquaculture* 198, 209–218.
- Primavera, J.H., Parado-Esteva, F.D., Lebata, J.L., 1998. Morphometric relationship of length and weight of giant tiger prawn *Penaeus monodon* according to life stage, sex and source. *Aquaculture* 164, 67–75.
- Quackenbush, L.S., 1989. Vitellogenesis in the shrimp *Penaeus vannamei*: in vitro studies of the isolated hepatopancreas and ovary. *Comp. Biochem. Physiol.* 94 (Part B), 253–261.
- Rankin, S.M., Bradfield, J.Y., Keeley, L.L., 1989. Ovarian protein-synthesis in the South-American white shrimp, *Penaeus vannamei*, during the reproductive cycle. *Invertebr. Reprod. Dev.* 15, 27–33.
- Raviv, S., Parnes, S., Segall, C., Davis, C., Sagi, A., 2003. *Litopenaeus vannamei* Vitellogenin VTG mRNA, Partial cds. GenBank accession number AY321153 Genbank.
- Sagi, A., Khalaila, I., Abdu, U., Shoukrun, R., Weil, S., 1999. A newly established ELISA showing the effect of the androgenic gland on secondary vitellogenic specific protein in the hemolymph of the crayfish *Cherax quadricarinatus*. *Gen. Comp. Endocrinol.* 115, 37–45.
- Samocho, T.M., Lawrence, A.L., Pooser, D., 1998. Growth and survival of juvenile *Penaeus vannamei* in low salinity water in a semi-closed recirculating system. *Isr. J. Aquac.-Bamidgeh* 50, 55–59.
- Saoud, I.P., Davis, D.A., Rouse, D.B., 2003. Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture* 217, 373–383.
- Smith, L.L., Lawrence, A.L., 1990. Feasibility of penaeid shrimp culture in inland saline groundwater-fed ponds. *Tex. J. Sci.* 42, 3–12.
- Talbot, P.D.H., Leung-Trujillo, J., Lee, T.W., Li, W.-Y., Ro, H., Lawrence, A.L., 1989. Characterization of male reproductive tract degenerative syndrome in captive penaeid shrimp (*Penaeus setiferus*). *Aquaculture* 78, 365–377.
- Tan, S.H., Degnan, B.M., Lehnert, S.A., 2000. The *Penaeus monodon* chitinase 1 gene is differentially expressed in the hepatopancreas during the molt cycle. *Mar. Biotechnol.* 2, 126–135.
- Tom, M., Fingerman, M., Hayes, T.K., Jhonson, V., Kerner, B., Lubzens, E., 1992. A comparative study of the ovarian proteins from two penaeid shrimps, *Penaeus semisulcatus* De Haan and *Penaeus vannamei* (Boone). *Comp. Biochem. Physiol.* 102 (Part B.), 483–490.
- Tsang, W.-S., Quackenbush, L.S., Chow, B.K.C., Tiu, S.H.K., Hec, J.-G., Chan, S.-M., 2003. Organization of the shrimp vitellogenin gene: evidence of multiple genes and tissue specific expression by the ovary and hepatopancreas. *Gene* 303, 99–109.
- Tseng, D.Y., Chen, Y.N., Kou, G.H., Lo, C.F., Kuo, C.M., 2001. Hepatopancreas is the extraovarian site of vitellogenin synthesis in black tiger shrimp, *Penaeus monodon*. *Comp. Biochem. Physiol.* 129A (Part A), 909–917.
- Tseng, D.Y., Chen, Y.N., Liu, K.F., Kou, G.H., Lo, C.F., Kuo, C.M., 2002. Hepatopancreas and ovary are sites of vitellogenin synthesis as determined from partial cDNA encoding of vitellogenin in the marine shrimp, *Penaeus vannamei*. *Invertebr. Reprod. Dev.* 42, 137–143.
- Tsutsui, N., Kawazoe, I., Ohira, T., Jasmani, S., Yang, W.J., Wilder, M.N., Aida, K., 2000. Molecular characterization of a cDNA encoding vitellogenin and its expression in the hepatopancreas and ovary during vitellogenesis in the kuruma prawn, *Penaeus japonicus*. *Zool. Sci.* 17, 651–660.
- Walton, S., 1981. CRC Handbook of Marine Science, vol. 1. CRC Press, Boca Raton.
- Wang, Q., Misamore, M., Jiang, C.Q., Browdy, C.L., 1995. Egg water induced reaction and biostain assay of sperm from marine shrimp *Penaeus vannamei*: dietary effects on sperm quality. *J. World Aquac. Soc.* 26, 261–271.
- Weinberger, G., Rosenthal, E., Kronfeld, J., Flexer, A., Berkovitch, B., 1991. The Underground Geology and

- Salinization Processes in A Multiple Aquifer System in the Central and Northern Negev and Beer-Sheva Vally. The Ministry for Agriculture, Water Commission, Jerusalem, Israel. 41 pp. (in Hebrew).
- Wickins, J.F., Lee, D.O.C., 2002. Crustacean Farming. Blackwell, Oxford. 200 pp.
- Wyban, J.A., Sweeney, J.N., 1991. Intensive Shrimp Production Technology. Argent Chemical Laboratories, Redmond, WA. 174 pp.