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# Environmental sex determination, external sex differentiation and structure of the androgenic gland in the Pacific white shrimp *Litopenaeus vannamei* (Boone)

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## Abstract

Environmental effects on sex determination in *Litopenaeus vannamei* were studied by rearing day 1 postlarvae at three temperatures, under three photoperiods, at high density and by starving. None of the environmental conditions affected sex determination or differential development of gender in this species. From day 50, the development of the endopodite of the first pair of pleopods revealed the first external differentiation, showing a triangular structure with three setae in females, whereas a tubular structure remained in males. Juvenile shrimp sex differentiation took place from days 50–90, independent of size, only if postlarvae reached a development threshold of 150 mg of body weight and 20 mm of body length previously. Histology and scanning electron microscopy of the vas deferens revealed that the androgenic gland (AG) is a single 2-mm cord attached in the subterminal ejaculatory region, just before the distal vas deferens narrows. The AG is composed of large oval cells containing vacuolated cytoplasm, and each cell has a prominent rounded nucleus, similar to all descriptions of the AG in Malacostracans, so we assume that it should have the same function in sex differentiation.

**Keywords:** *Litopenaeus*, sex determination, sex differentiation, vasa deferentia, androgenic gland

## Introduction

Penaeid species are gonochoric and present external fertilization (Malecha & Hedgecock 1989), which allows chromosome manipulations such as induction of triploidy (Dumas, Ramos & Campos 1999; Li, Xiang, Zhang, Zhou, Zhang & Wu 2003), production of meiotic gynogenetics (Dai, Zhenmin, Zhang & Liu 1993; Cai, Ling, Ke & Chen 1995) and inter-specific hybridization (Benzie, Kenway & Ballment 2001). Penaeid species also exhibit sexual dimorphism, and as observed in wild populations, females are larger than males. Some studies on shrimp aquaculture emphasize sexual size dimorphism and its possible advantages in improving production. In the Pacific white shrimp *Litopenaeus vannamei* (Boone), sexual size dimorphism begins at about 10 g (Chow & Sandifer 1991) and becomes significant around 17 g (Pérez-Rostro, Ramírez & Ibarra 1999; Pérez-Rostro & Ibarra 2003a), a size in shrimp aquaculture usually reached by harvest time. Genetic–environmental effects seem to affect the size and weight of sexual dimorphism (Pérez-Rostro & Ibarra 2003b). In a study of the brown shrimp *Farfantepenaeus californiensis* (Holmes), one of the most important species for the northeastern Pacific fishery, Campos-Ramos, Magallón-Barajas, Portillo-Clark, Porchas-Cornejo and Naranjo-Páramo (1994) showed that after 8 months of culture beginning in July and harvesting in early March, females attained 18 g versus the 14 g of males. Possibly, the larger size of females is influenced by faster female growth during the winter temperatures (16–20 °C) in northwestern México. Investigations of sexual dimorphism as a possible advantage in shrimp aquaculture include Asian species such as *Penaeus japonicus* (Bate; Nakamura, Matsuzaki & Yonekura 1992; Anonymous 1999; Li, Byrne, Miggiano, Whan, Moore, Keys,

Crocos, Preston & Lehnert 2003), *Penaeus monodon* (Fabricius; Hansford & Hewitt 1994; Lumare, Scordella & Zonno 1998), the Indian species *Penaeus indicus* (H. Milne-Edwards; Mohan & Siddeek 1995) and *Fenneropenaeus chinensis* (Kishinouye; Yin, Song, Ma & Yu 1986; Li & Xiang 1997; Li, Xiang *et al.* 2003). Monosex female culture could be a strategy to produce shrimp with larger tails for the market. Research on sex determination and the achievement of female monosex culture in future shrimp aquaculture will require determining the gender at early juvenile stages. Juvenile shrimp secondary sexual characters are acquired through progressive moulting, until development of external sex characters and gonad differentiation to female or male. However, it is not known whether sex differentiation in marine shrimp is size dependent or age dependent. The easiest way to sex shrimp is by just observing the presence or absence of the endopodite modification, the male petasma, in the first pair of pleopods that develops from the early juvenile (postlarval) stage. However, to do this by eye with confidence, shrimp must be cultured for at least 4 months (Pérez-Rostro & Ibarra 2003a). There is no information on when (size or age), or how to sex young postlarvae of *L. vannamei*.

Although it is known that sex in gonochoric Crustacea is primarily genetic (Charniaux-Cotton 1960), little is known about sex determination systems for penaeid species (Benzie 1998). Previous studies on *L. vannamei* (Campos-Ramos 1997), as well as many other karyological studies of penaeid species, have indicated no differentiated mitotic sex chromosomes. There are few studies on sex determination in penaeids; induced gynogenesis in *F. chinensis* yielding only female meiogenetic shrimp suggests that this species could present an XX/XY system (Cai *et al.* 1995). In contrast, Benzie *et al.* (2001) suggested that the female of penaeids could be the heterogametic sex (WZ/ZZ system) after the gender of hybrid progeny from a cross of female *P. monodon* with male *P. esculentus* (Haswell) was skewed to the male. Li, Byrne *et al.* (2003) obtained a genetic mapping of *P. japonicus* using AFLP markers, and found a sex marker mapped on the maternal genome, implying that the female may be the heterogametic sex in this species. Interestingly, Li, Xiang *et al.* (2003) found that induction of triploidy in *F. chinensis* skewed the sex ratio to the female, but the authors did not propose any sex determination mechanism to explain it. Steroid hormones such as oestradiol, which was administered to *P. penicillatus* (Alcock; Zhongqing 1990) and *L. vannamei* (González-Gómez 2001) larvae and postlarvae, do not seem to be involved in sex determination and differentiation of these species.

Sex differentiation is the result of a chain of gene expression events triggered by sex determination genes located in sex chromosomes or autosomes. However, depending on how strong or labile the sex-genetic mechanism is in a particular species, it may be overruled by environmental influences. Environmental sex determination (ESD) occurs in some crustacean species in which abiotic factors such as temperature (some copepod species), density, starvation (cladocera) and photoperiod (amphipods), among others (simbionts or parasites), influence the phenotype sex (see reviews of Legrand, Legrand-Hamelin & Juchault 1987; Korpelainen 1990). There is no study on environmental factors influencing sex determination in Penaeidae. Nevertheless, Pérez Farfante and Robertson (1992) found two hermaphrodites of *L. vannamei* among the fourth generation of a broodstock cultured at a shrimp farm in Venezuela, suggesting a link to shrimp culture conditions in captivity.

Whole sex determination and differentiation is more interesting particularly in Malacostraca (i.e. isopods, amphipods and decapods) because the organ responsible for maleness is the androgenic gland (AG). This gland was discovered in the amphipod *Orchestia gammarellus* (Pallas; Charniaux-Cotton 1953, 1954, 1960), and it was shown that an immature female implanted with an AG reverts to male (neomale), while an immature male with the AG removed (andrectomy) reverts to female (neofemale). According to Suzuki (1999), the AG in the isopod *Armadillidium vulgare* (Latreille) secretes a hormone that is a sex-reversing factor but not a sex-determining factor. As freshwater prawn aquaculture developed in tropical countries, it was observed that males clearly grow faster than females, so the findings of Charniaux-Cotton on *O. gammarellus* were applied to freshwater decapods. The hormone secreted by the AG appears to control male gender differentiation in freshwater prawns (Nagamine, Knight, Maggenti & Paxman 1980a; Sagi, Snir & Khalaila 1997), and to inhibit female vitellogenesis (Nagamine, Knight, Maggenti & Paxman 1980b; Fowler & Leonard 1999; Manor, Aflalo, Segall, Azulay, Ventura & Sagi 2004). Andrectomy and implantation of the AG has given partial or total gonad sex reversal results in commercially freshwater prawns such as *Macrobrachium rosenbergii* (de Mann; Nagamine *et al.* 1980a, b; Sagi, Cohen & Milner 1990), *Procambarus clarkii* (Girard; Taketomi & Nishikawa 1996), *Cherax destructor* (Clark; Fowler & Leonard 1999) and *Cherax*

*quadricarinatus* (von Martens; Khalaila, Katz, Abdu, Yehezkel & Sagi 2001; Barki, Karplus, Khalaila, Manor & Sagi 2003). Andrectomy of freshwater *M. rosenbergii* has allowed crossing sex-reverted individuals with normal individuals to determine the sex determination mechanism, which, in this species, constitutes a WZ/ZZ system (Sagi & Cohen 1990). In contrast, sex reversal technology on marine penaeid shrimp has not received proper scientific attention as yet.

We focused this investigation of *L. vannamei* on the following objectives: (1) to analyse ESD, and discover whether any of the most common environmental variables in shrimp culture influence gender; (2) to record the process of external sexual differentiation in concordance with further gonad differentiation; (3) to determine whether sex differentiation in marine shrimp is size dependent or age dependent; (4) to determine when and how it is possible to sex young postlarvae and (5) to describe the AG by histological and scanning electron microscope (SEM) techniques.

## Materials and methods

Day 1 postlarva shrimp (pl1) and harvest size shrimp (body weight: 15–18 g) were obtained at Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR) facilities. Shrimp were reared using filtered and aerated seawater ranging from 34 to 38 g $L^{-1}$ of salinity, maintained at constant temperature by submergible 300-W heaters, and 50% of water volume was changed three times a week with seawater at the same temperature as the bath.

## Feeding regime

Postlarva shrimp food consisted of brine shrimp nauplii and commercial microencapsulate (250–500 μm) up to pl20, and then live and frozen adult brine shrimp were gradually substituted for nauplii over a few days, and brine shrimp flakes and commercial crumbly dry pellets were substituted for microencapsulate. The shrimp were fed to apparent satiation.

## Effect of temperature on gender

We evaluated the influence of temperature on the gender of *L. vannamei* by rearing triplicate postlarvae during 90 days at two temperatures: 27 ± 1 °C (control) and 32 ± 1 °C, in rectangular 300-L baths at a density of 250 postlarvae in each experimental unit. Postlarval shrimp were acclimatized to the high temperature for 2 days. We also evaluated the effect of low temperature on gender. In this case, we acclimatized 1200 postlarvae within 2 days to 18 ± 0.5 °C, and reared them for 60 days in a 1000-L oval bath inside a sealed, temperature-controlled room with automatic air-conditioning. Surviving shrimp were split into three replicates from day 61, and the temperature was increased to 27 ± 0.5 °C within 1 week to observe sex differentiation.

## Effect of photoperiod on gender

The effects on gender of continuous light, shadow and darkness were evaluated after rearing triplicate postlarvae for 2 months at 27 ± 0.5 °C. Three 300-L indoor rectangular baths were each fitted above with two 40 W fluorescent lamps to provide continuous artificial light to shrimp; three baths covered with a double mosquito net provided continuous shadow, and three baths inside a dark room provided continuous darkness. Density was maintained around 70 postlarvae in each experimental unit.

## Effect of high density on gender

The effect of high density on gender was evaluated by rearing 3000 postlarvae for 2 months in one 500-L concrete outdoor tank at 27 ± 1 °C, with no replicates.

## Effect of starving on gender

About 2000 postlarvae were starved for 7 days in one 1000-L outdoor oval bath at 27 ± 1 °C, and the effect was evaluated by rearing the surviving shrimp for 2 months, with no replicates.

## Sexing shrimp

The gender of shrimp after photoperiod, high-density and starving treatments was determined using the method of temperature external sex differentiation (see results).

## Statistical analyses

We used a $\chi^2$ test to evaluate the sex ratio in each environmental assay. To justify pooled data, an overall

 

$\chi^2$ test (heterogeneity$\chi^2$) of the three replicates in a given treatment was performed (Zar 1996).

## Evaluation of shrimp growth at three temperatures

Individual shrimp weights and lengths from each replicate ($n = 30$) of the three temperatures tested were recorded every 15 days using a digital balance and a ruler.

## External sex differentiation at three temperatures

Every day from day 10 on, we killed three randomly chosen postlarvae from each replicate at each temperature treatment to analyse the following sex structures: (a) the development of the endopodite by cutting off the first pair of pleopods, (b) the development of the appendix masculine by cutting off the second pair of pleopods, (c) the development of the male genital pores located medially in the coxopod of the fifth pereiopods and (d) the pair of oblique sharp ridges on the anterior sternite XIV of the female thelycum. These structures are described by Pérez Farfante (1988), Treece and Yates (1988) and Dall, Hill, Rothlisberg and Sharples (1990).

## Examination of gonads

To confirm that observation of early morphological characters correctly identified gender, we randomly selected six females and six males from each temperature that were morphologically sexed at day 60. Shrimp were separated by gender, and reared for another 4 months at 27 ± 1 °C. Shrimp were fixed in Davidson (Howard & Smith 1983; Bell & Lightner 1988) for 24 h, and then in 70% ethanol until processed with the haematoxylin and eosin histology technique (Bell & Lightner 1988).

## Endopodite morphology

Additionally, we compared the sex endopodite morphology of the first pair of pleopods in females and males among early sexed pl55, and 100 pre-adult shrimp between 15 and 18 g body weight.

## Description of male AG through histology and SEM

Both the middle and distal vasa deferentia, containing the attached AG from six pre-adult male shrimp (15–18 g), were removed using dissecting tweezers. Gentle finger pressure over the coxa of the fifth periopod was applied until the spermatophore and the terminal ampoule came out of the male gonophore. Then, the terminal ampoule was held with dissecting tweezers, and was gently and slowly pulled out, along with the distal and middle vasa deferentia. Half of the vasa deferentia were fixed in 4% formalin, processed using the haematoxylin and eosin histology technique. The other half of the vasa deferentia were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer pH 7.0 for 24 h at 4 °C, followed by two 30-min washes in sodium cacodylate buffer, and dehydration in ethanol. Digitized images and measurements of the AG were obtained with a Hitachi S-3000N SEM (Hitachi High Technologies, Dallas, TX, USA), after samples were critical point dried and sputter palladium coated.

## Recording images

A compound light microscope (Olympus, Tokyo, Japan) and a dissecting stereoscope microscope (Olympus), with a digital camera attached, and the software IMAGE PRO PLUS 4.0 (Media Cybernetics) were used to monitor progressive temperature development of the external gender morphology, and for histological examination of gonads and AG.

## Results

None of the gender data of juvenile *L. vannamei* reared under different environmental conditions (Table 1) were significantly different from 1:1 female: male ($P > 0.05$), even though low-temperature, high-density and starving treatments had low survival rates at 60 days of 25%, 3% and 3% respectively.

At the high and control temperatures, development of the endopodite at the first pair of pleopods started with the appearance of one or two apical setae between days 25 and 28, followed by the emergence of a tiny undifferentiated protuberance. Between days 30 and 34, a large, tubular undifferentiated structure appeared with the two apical setae previously observed (not shown). By day 40, the large structure in some individuals became wider on one

**Table 1** Pooled gender data and $\chi^2$ analyses of *L. vannamei* (Boone) postlarval subjected to three different temperatures, three photoperiods, high density, and starving

| | *n* | ♀ | ♂ | $X^2$ |
|---|---|---|---|---|
| Temperature (°C) | | | | |
| 32 | 244 | 120 | 124 | 0.06 |
| Control 27 | 267 | 125 | 142 | 1.08 |
| 18 | 230 | 113 | 117 | 0.07 |
| Photoperiod | | | | |
| Light | 166 | 90 | 76 | 1.18 |
| Shadow | 173 | 90 | 83 | 0.28 |
| Darkness | 179 | 84 | 95 | 0.67 |
| High density | 85 | 49 | 36 | 1.98 |
| Starving | 59 | 27 | 32 | 0.42 |

Number of individuals (*n*); females (♀) and males (♂).
*L. vannamei*, *Litopenaeus vannamei*.

side in the proximal and middle areas, giving the structure a triangular shape, and then from days 50 to 53, three or four setae appeared sequentially along the ridge of the widest part of the structure (Fig. 1a). By day 58, individuals having the three setae developed a pair of oblique sharp ridges on the anterior part of sternite XIV that characterizes the female thelycum of this species (Fig. 1b). In contrast, individuals having a tubular appendage (Fig. 1e) developed the male gonophores located medially at the coxopod of the fifth pereiopods (Fig. 1f), and the appendix masculine at the second pair of pleopods (Fig. 1g). By day 70, the distal male petasma developed a round, dense structure originating at the distal part of the lobe (Fig. 1e). Both endopodites of the first pair of pleopods were close to the same length. Gonad histology revealed that individuals with an early developed triangular structure with setae had an ovary (Fig. 1d). In contrast, specimens with a tubular appendage had testes (Fig. 1h). The early differentiated female endopodite had a morphology similar to that of pre-adult females (Fig. 1c), whereas the early differentiated male endopodite continued developing into the male petasma during the juvenile stage. In both 90-day-old and pre-adult specimens, the distal male protopodite of the first pair of pleopods had a rectangular articulation notched into the distal region that characterizes the male gender (Fig. 1i).

Shrimp growth in controls (8.6 mg day$^{-1}$) was notably different from the high-temperature samples (17.0 mg day$^{-1}$). However, differentiation of external sexual characteristics took place in close to the same 50–60-day period, after juvenile shrimp reached a threshold body weight of 150–200 mg, and a length of 20–25 mm (Fig. 2). Low temperature (18 °C) inhibited postlarval growth, delaying sexual differentiation. At higher temperatures, however, sex differentiation structures appeared in the same order as described above.

Vas deferens histology revealed that the *L. vannamei* AG is attached at the distal part of the middle vas deferens, at the subterminal ejaculatory region, just before the distal vas deferens narrows. The AG is a single, compact cellular mass in parallel to the vas deferens, and connected to the muscle layer through an epithelial lining (Fig. 3a). Its length is about 2.5 mm, and it is tubular, having around 100-μm diameter in the middle. The clearly distinguishable cellular mass is composed of large oval cells, in which a vacuolated cytoplasm appears, and each cell has a prominent, rounded nucleus (Fig. 3b). Scanning electron microscopy of the AG shows a main cord-like structure attached in the distal part of the middle vas deferens (Fig. 3c), from each end of which thinner cords branch out (Fig. 3d), extending towards the wider middle and the narrower distal vas deferens respectively.

## Discussion

None of the different environmental conditions starting from pl1 appeared to affect gender determination or affected differentially the development of female and male of *L. vannamei*. It appears that penaeids possess a stable genetic sex determination system. We suggest that the two *L. vannamei* hermaphrodites reported by Pérez Farfante and Robertson (1992) are linked to a bottleneck effect or endogamy among siblings and not to environmental conditions during shrimp culture.

According to Nakamura *et al.* (1992), the male petasma in *P. japonicus* appears as a large, triangular lobe, whereas in females, the endopodite is a small, slender lobe (no photographs shown). Our observations of *L. vannamei* indicate that the length of the endopodite is comparable in both genders for the three months after the endopodite emerges from the protopodite, and that the early triangular structure with setae identifies a female. According to Charniaux-Cotton and Payen (1985), the depression on the inner face of the male protopodite occurs in *P. japonicus* before appendix masculine and petasma differentiation. This structure also occurs in male *L. vannamei*. However, we were not able to differentiate gender precisely using this structure until pl90, when all external sexual structures were already developed.

 

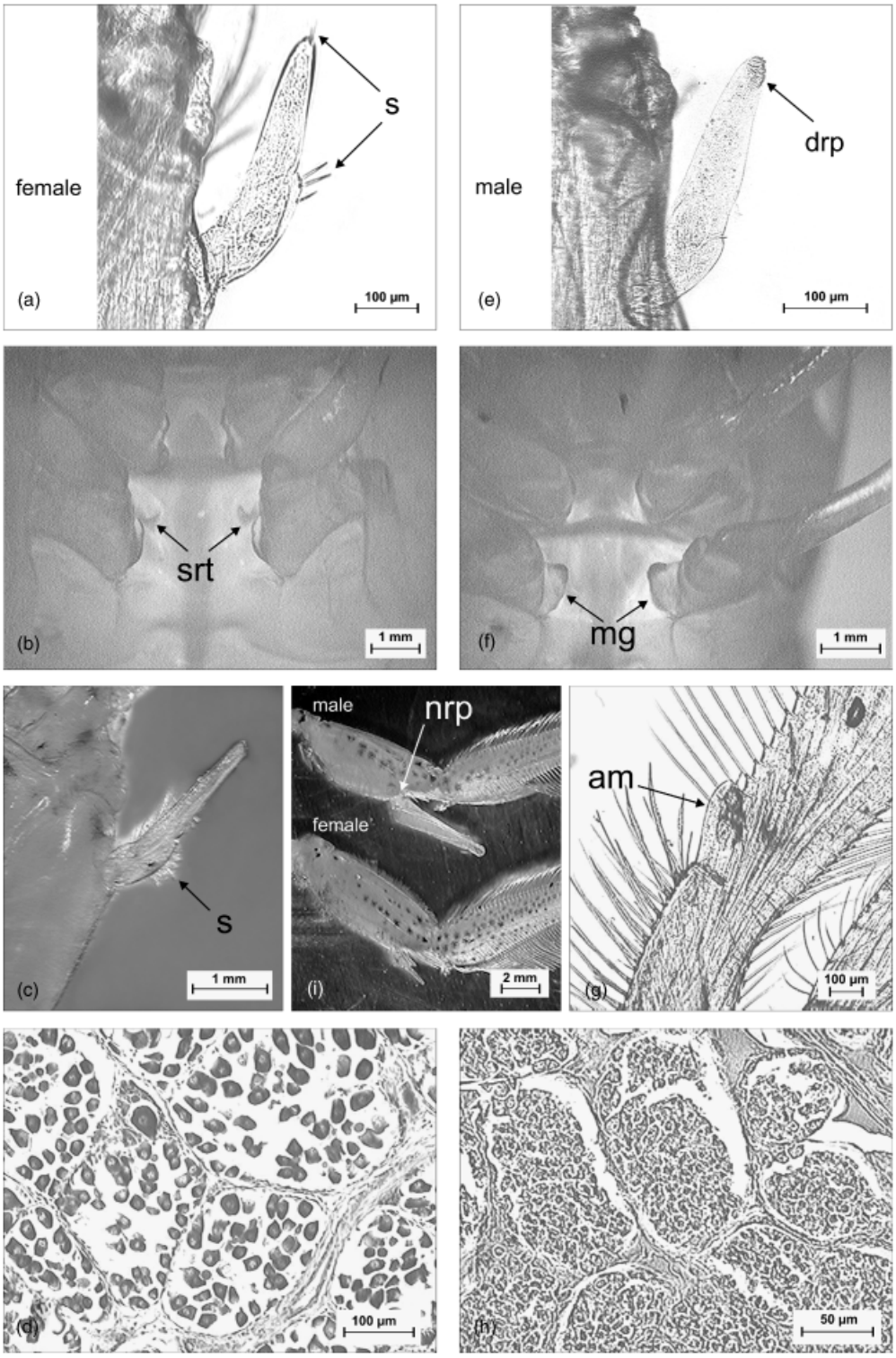


**Figure 1** Gender differentiation of *Litopenaeus vannamei* (Boone) into female (a–d) and male (e–f) early female endopodite × 160 (a) showing setae (s); thelycum × 15 (b) showing sharp ridges in thelycum (srt); pre-adult female endopodite × 80 (c) ovary × 400 (d) early male petasma × 160 (e) showing an apical dense region of petasma (drp); male sternite × 12 (f) showing gonophores (mg); second male pleopod × 80 (g) showing appendix masculine (am); testes × 400 (h) Endopodite of pre-adult shrimp × 18 (i) female pleopod showing endopodite with setae, and male pleopod showing the petasma. An arrow indicates the notched region of the protopodite (nrp).

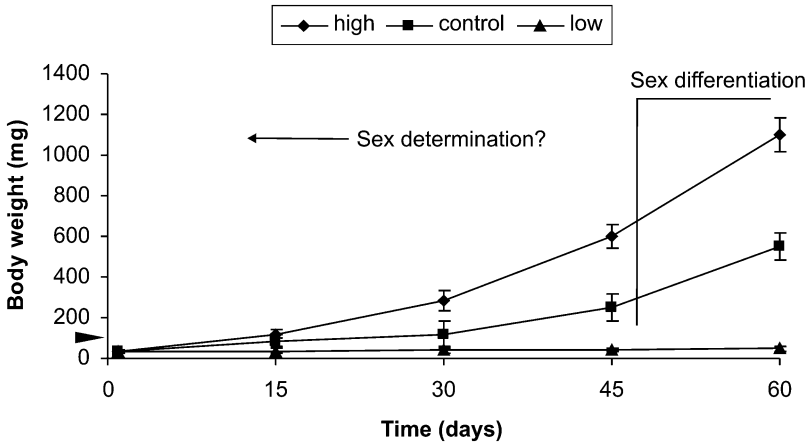


**Figure 2** Mean body weight ± SD of *Litopenaeus vannamei* (Boone) postlarvae at three temperatures: 27 ± 1 °C (control), 32 ± 1 °C (high) and 18 ± 0.5 °C (low). The arrowhead indicates a threshold of body weight.

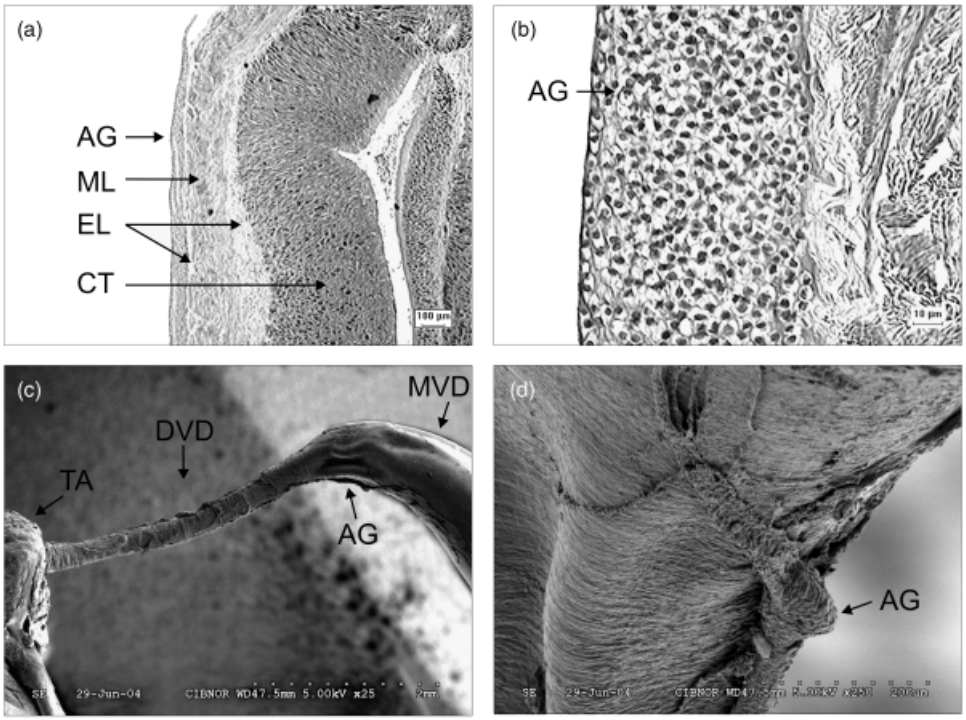


**Figure 3** Androgenic gland (AG) of *Litopenaeus vannamei* (Boone). Histology of AG attached at the distal part of the middle vas deferens (a × 64 and b × 800). Scanning electron microscope of the putative AG showing a single cord attached to the distal part of the ejaculatory bulb × 25 (c) in which each end of the gland branches out into thinner cords × 250 (d) GA, Androgenic glandGA; ML, muscular layer; EL, epithelial lining; CT, connective tissue; TA, terminal ampoule; DVD, distal vas deferens; MVD, middle vas deferens.

Juvenile shrimp sexual characteristics are revealed through successive stages of moult. We expected that the gender of shrimp growing faster at the high temperature would be differentiated faster than that of the smaller control group shrimp of the same age. However, gender differentiation took place at about the same age independent of size, both between shrimp at the two temperatures, and among shrimp at each temperature. Possibly, there is an age window in sex differentiation during development

 

under rearing conditions, after postlarvae reach a threshold size. This window might be altered during early postlarval development under prolonged, unfavourable environmental conditions, such as low temperature and high density.

According to Yin *et al.* (1986) and Li and Xiang (2002) on *F. chinensis*, and Charniaux-Cotton and Payen (1985) and Nakamura *et al.* (1992) on *P. japonicus*, external differentiation between female and male takes place during the second month after postlarval transformation, which concurs with our observations.

In synthesis, our observations on *L. vannamei* indicate that the progress of male external sex differentiation structures begins with the endopodite around pl50, which agrees with Nakamura *et al.* (1992), followed by both the male gonophores and the appendix masculine in the second pair of pleopods by day 58, and finally the depression on the inner face of the male protopodite by day 90.

The male reproductive system in penaeids consists of a pair of testis, each with six lateral lobes located in the cardiac region, dorsally to the midgut gland. The vas deferens extends dorsally from the edges of the two posterior testis lobes, and continues laterally in a dorsal–ventral direction until exiting, through a genital pore in a ventral opening located medially in the coxopod of the fifth pereiopod (Dall *et al.* 1990; Treece & Yates 1988). Each vas deferens presents a short, narrow proximal portion that widens until forming the middle ejaculatory vas deferens, which folds once about half way along its length, forming a double fixture (Treece & Yates 1988), and is also called the horseshoe-shaped vas deferens (Charniaux-Cotton & Payen 1985; Dall *et al.* 1990). The distal part of the middle vas deferens narrows sharply, and continues as a relatively long narrow tube, forming the distal vas deferens and ending in the terminal ampoule (Dall *et al.* 1990; Treece & Yates 1988). According to Charniaux-Cotton & Payen (1985), the AG attaches at the subterminal ejaculatory region of the vas deferens in decapods. Payen (unpublished, cited in Charniaux-Cotton & Payen 1985) obtained the first image of the AG tissue from the marine penaeid *P. kerathurus* (Forskäl). Observations on the location of the AG in *P. japonicus* (Nakamura *et al.* 1992), *F. chinensis* (Li & Li 1993), *L. vannamei* and *L. stylirostris* (Stimpson; Alfaro 1994) and those of the present investigation show that the AG is a cord-like cellular mass attached to the distal surface of the ejaculatory bulb wall (the distal part of the middle vas deferens). Additionally, it appears that in *L. vannamei*, thinner cords branch out from both ends of the main cord of the AG, and extend towards the wider middle vas deferens, and towards the narrower tube that forms the distal vas deferens respectively. In adult *L. stylirostris* specimens of 30 g body weight (Alfaro 1994), and pre-adult and adult specimens of *F. chinensis* (Li & Xiang 1997), the AG covered both sections of the vas deferens, which suggests that the AG continues its development from juvenile to adult by extending its size along the vas deferens.

Our description of the AG in *L. vannamei* extends the previous investigation of Alfaro (1994) by providing further histological description of the gland in this species. The histology of the AG resembles practically all those in the literature for other Malacostracans, such as amphipods, isopods and decapods. The AG tissue is unique, and ineludibly easily identifiable. The difference between *L. vannamei* and freshwater prawns is that the AG in the former attaches to the vas deferens as a single main cord, while it is adjacent leaving free segments or cords in the latter (Nagamine *et al.* 1980a; Fowler & Leonard 1999). In *M. rosenbergii* males, it is clear that male secondary sexual characteristics do not develop in the absence of the vasa deferentia-AG (Nagamine *et al.* 1980a). Unlike marine shrimp, freshwater prawns have the advantage of different male developmental phenotypes related to claw colour. These phenotypes allow the quantification of developing stages of males, giving an external indication of how andrectomized males or implanted females are responding completely or partially to sex reversal. Successful sex reversal of the male Malaysian prawn to female is achieved only if andrectomized before gonophore complexes are evident in individuals having 1 g of body weight when the gonads are not fully differentiated (Nagamine *et al.* 1980a; Sagi & Cohen 1990).

According to Chim, Payen & Laubier (1982, unpublished, cited in: Charniaux-Cotton & Payen 1985), the undifferentiated phase of the genital apparatus in *P. japonicus* continues through the mysis larval stages and extends at least to postlarva day 6 before the male sperm ducts form. Nakamura *et al.* (1992) observed that by day 20, the internal vasa deferentia were already formed. We assume that the vas deferentia in *L. vannamei* appear before endopodite differentiation. The strongest evidence for penaeids shows that during male *P. japonicus* organogenesis, the AG appears by day 55, just before gonad differentiation into testes by day 60 (Charniaux-Cotton & Payen 1985; Nakamura *et al.* 1992). We assume that the AG and testes in *L. vannamei* are differentiating by the

time the gonophores and appendix masculine are developing externally.

The AG tissue that is unique among malacostracans tells us that its function in sex differentiation must be the same in all species. However, the evidence for *P. japonicus* up to now shows that the AG is not involved in this process because the male gender has been already differentiated earlier by organogenesis of the internal sperm ducts (Charniaux-Cotton & Payen 1985; Nakamura *et al.* 1992). It appears that the function of the AG is involved in the onset of spermatogenesis in *P. japonicus* (Charniaux-Cotton & Payen 1985), spermatogonial differentiation in *P. indicus* (Mohamed & Diwan 1991) and further development of petasma in *F. chinensis* (Li & Xiang 1997), which makes it clear that more research is needed. Nevertheless, our certainty that there are no environmental conditions affecting gender, and that the AG is present in the Pacific white shrimp gives us confidence to proceed with experimental essays. However, these procedures will require novel micromanipulation of marine shrimp specimens. Additionally, we can state that it is practical to determine the gender of 60-day-old *L. vannamei* juveniles for further monosex culture experiences.

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