

Production of all-male stock by neofemale technology of the Thai strain of freshwater prawn, *Macrobrachium rosenbergii*

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Abstract

The giant freshwater prawn, *Macrobrachium rosenbergii* is commercially cultured in many tropical countries. Males of this species grow much faster than females so that, obviously, culture of an all-male population produces significantly higher yield than an all-female or mixed-sex culture. Although sex of *M. rosenbergii* is determined by genetics, sex reversal from males to females can be triggered by the removal of androgenic glands at an early stage. The empirical information obtained from two families showed that almost all offsprings of the neofemales were male. Therefore we conducted this study to explore the result of such mating with sufficient number of crosses in the Thai strain of *M. rosenbergii*. The histology of the gonads during the threshold period of sex determination was also studied. The results showed that the gonadal differentiation of *M. rosenbergii* did not occur until the stage that both gonopore complexes and appendix masculinae appeared in males. The bilateral removal of androgenic glands of a total of 87 postlarvae at stage I (presence of the gonopore complexes but not the appendix masculinae) resulted in 80.4% survival. Among the survivors 30% developed as female and 27.14% eventually matured. Mating of 12 neofemales with normal males resulted in all-male offspring in eight crosses; 88–99.49% males in two crosses. The remaining two crosses produced offspring with 1:1 sex ratio and hence were attributed to mislabeling of the normal female as neofemales. The 10 control crosses (normal male × normal female) produced varying offspring sex ratios from 1:1 (six crosses) to 1.72♀:1♂ (pooled data from four crosses). Fecundity of the neofemales was not different from that of the normal females. Our results suggest that the application of the neofemale technology to produce all-male stock for aquaculture is promising.

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1. Introduction

Aquaculture of the giant freshwater prawn, *Macrobrachium rosenbergii* has been established for more than 30 years and is expanding worldwide with approximately 23.5% increment in the world annual

production between 1998 and 2003 (FAO, 2004). However, culture technologies of this species are still varying. The differential growth between sexes is of great concern in *M. rosenbergii* culture wherein males grow much faster than females (Holthuis, 1980).

Culture of all-male prawn gave significantly higher yield with shorter culture period than the mixed-sexes and the all-female culture (Sagi et al., 1986; Cohen et al., 1988). Moreover the manual sexing was not justified by the relatively small increase of income (Hulata et al.,

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1988). Therefore, the alternative biotechnological method has been explored for producing an all-male stock of freshwater prawn, among which the neofemale technology is promising (reviewed by Sagi and Aflalo, 2005).

The androgenic gland of crustaceans is the only source of hormone that controls sex differentiation to maleness and the development of male characters (Ruppert et al., 2004). The ablation of the androgenic gland at early stage of development caused sex reversal to females (neofemale) (Nagamine et al., 1980) while the implantation of the androgenic gland in female *M. rosenbergii* resulted in sex reversal to males (neomale) (Malecha et al., 1992).

Sagi and Cohen (1990) based on two crosses (a total of 567 offspring) showed that the mating of the neofemale with the normal male resulted in 99.1% and 100% male offspring. Although their results have shown the potential of the technique for producing all-male offspring of *M. rosenbergii* for aquaculture, more information is needed because it is likely that sex determination of freshwater prawn may not completely rely on the ZW system (Malecha et al., 1992). The authors observed the fluctuation of sex ratio among control crosses (normal males $\times$ normal female) towards femaleness. Moreover the mating between the neomale (ZW) and the normal female produced varying sex ratios that ranged between 14% and 44% male in 10 crosses (Malecha et al., 1992).

Recently, Aflalo et al. (2006) who failed to identify male postlarvae at a sufficiently early stage of development for successful sex reversal by the androgenic gland ablation, overcame the problem by using a two-step procedure. Whereby in phase I, 25–60 days postlarvae were andrectomized with a low success rate of sex reversal. In phase II, the presumed all-male postlarvae of 20–30 days old produced from the neofemales were andrectomized resulted in improved success rate of sex reversal.

Therefore we conducted this study to explore the possibility of using the neofemale technology to produce all-male offspring in Thai strain of freshwater prawn which has been domesticated from the wild stock from Chaophraya River for more than 30 years (Rabanal, 1979).

Moreover despite a study on morphological sex differentiation in male the histology of gonads in very early stages was not available. Our study reports the histological sex differentiation of freshwater prawn in relation to the appearance of the stages defined by Nagamine et al. (1980) based on external sex characteristics. The results obtained enable defining a precise stage for efficient removal of the androgenic gland.

2. Materials and methods

2.1. The stock of *M. rosenbergii*

The stock of the freshwater prawn used in our study was a stock used for commercial aquaculture. It has been domesticated since 1970 from a natural population collected from central Thailand without the benefit of genetic knowledge.

2.2. Histology of males at stages I and II

According to Nagamine et al. (1980) the male freshwater prawn postlarva was first identified based on the presence of only the gonopore complexes (Stage I, 6–10 mm carapace length—CL). It reached stage II when both the gonopore complexes and appendix masculinae were present (10–28 mm CL). They found that the removal of the androgenic glands at stage I resulted in successful sex reversal. Nevertheless, histology of these stages was not provided.

Therefore we studied the histology of freshwater prawn postlarvae to elaborate the histology of the gonad in relation to the presence of male external characters. Thirty postlarvae from a single family were collected at each of the three sequential stages: (1) 1 week prior to the first development of the gonopore complexes, (2) when the gonopore complexes first observed, 15 each of specimens with and without the gonopore complexes were collected, (3) when the appendix masculinae were observed, 15 each of the specimen with and without gonopore complexes and the appendix masculinae were collected. The samples were preserved in Davidson's fixative for 24 h and subsequently transferred to 70% alcohol. The histological study was performed following the method described by Humason (1979).

2.3. Removal of androgenic glands

The sex determination study showed that the external male sex character was first observed at 45 days of the postlarval stage at which it developed the gonopore complexes, an opening of a vas deferens, at the base of the fifth walking leg which is visible under a stereo microscope.

To remove the androgenic gland both fifth walking legs were cut and the androgenic gland which was visible under a stereomicroscope was pulled out using forceps. The walking legs regenerated soon after the operation. Then the andrectomized prawns were individually reared in plastic boxes of about 1000 cm³ floating

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