PRELIMINARY EVALUATION OF EYESTALK ABLATION AND THORACIC GANGLION IMPLANTATION ON SPERMATOPHORE QUALITY AND MOLTING OF MALE *Penaeus vannamei*

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RESUMEN

*Machos de **Penaeus vannamei** (peso promedio = 25.05 ± 1.89 g) se seleccionaron de un estanque semiintensivo, de producción comercial, para estudiar el efecto de la implantación de secciones de ganglio toráxico sobre la calidad de los espermatóforos y el efecto de la ablación ocular sobre la duración del ciclo de muda.*

Los implantes de ganglio toráxico no beneficiaron la calidad de espermatóforos en cuanto a conteo de espermas y peso. Sin embargo, la ablación ocular unilateral mejoró significativamente ($P < 0.05$) esos parámetros. La ablación ocular no acortó la duración del ciclo de muda. Se considera que la ablación ocular tiene un efecto diferente sobre la muda, dependiendo del ambiente natural de cada especie.

ABSTRACT

*Males of **Penaeus vannamei** (mean weight = 25.05 ± 1.89 g), selected from a commercial semi-intensive pond, were used to study the effect of thoracic ganglion implantation on spermatophore quality, and the effect of eyestalk ablation on molt cycle duration.*

Thoracic ganglion implantation did not benefit spermatophore sperm count and spermatophore weight, whereas eyestalk ablation significantly ($P<0.05$) improved spermatophore quality. Eyestalk ablation did not shorten molt cycle duration. It is believed that eyestalk ablation has a different effect on molting depending on the natural environment of each species.

INTRODUCTION

Endocrinology studies concerning penaeid shrimp maturation have been particularly focused on ovarian maturation. Therefore, vitellogenesis in malacostran crustacea is an important research subject (Charniaux-Cotton 1985; Yano and Chinzei 1987; Quackenbush and Keeley 1988; Quackenbush 1986).

Knowledge that has been accumulated about crustacean reproductive endocrinology is now being tested and applied to penaeid shrimp in such a way that eyestalk ablation has become the most important commercial technique for ovarian-maturation induction. The bihormonal theory of ovarian regulation (Adiyodi and Adiyodi 1970; Eastman-Reks and Fingerman 1984), which is based on the antagonistic action of ovary inhibiting hormone (OIH) and ovary stimulating hormone (OSH), has been partially studied in penaeid shrimp (Quackenbush 1986). Yano et al. (1988) demonstrated an ovary-stimulating effect by implantation of thoracic ganglion in *Penaeus vannamei*. Nevertheless, the bihormonal approach appears to be only part of a more complex system since the mechanisms of actions of the OIH remain unclear and the existence of an ovarian hormone is not yet demonstrated (Charniaux-Cotton 1985).

Moreover, Quackenbush (1989) found that OIH appears to inhibit yolk synthesis directly rather than by acting to suppress vitellogenesis via the inhibition of a gonad-stimulating factor.

On the other hand, the endocrine mechanisms that control male penaeid shrimp maturation are still unclear. In the Decapoda, the start and intensity of spermatogenic activity are controlled by eyestalks through androgenic glands (Pochon-Masson 1983). This enhancing influence of eyestalk ablation was demonstrated over spermatophore quality in *P. vannamei* (Leung-Trujillo and Lawrence 1985). Such a finding can be promising for commercial shrimp maturation.

Knowledge about male gonad-stimulating hormone in penaeid shrimp is not available. Nevertheless, in the crab *Paratelphusa hydrodromous*, implantation of thoracic ganglion into males results in a precocious maturation of the testis and an eventual hypertrophy of the vas deferens (Gómez 1965). Moreover, thoracic ganglion of adult male *Sesarma* was effective in accelerating ovarian development in young females of *Potamon* (Otsu 1963). Adiyodi and Adiyodi (1970) stated that no studies were available linking sperm production with thoracic neurosecretion. To our knowledge, no more information on this subject exists, particularly dealing with penaeid shrimp.

Haemolymph ecdysteroid levels and molt inhibiting hormone (MIH) control molting in crustaceans (Quackenbush 1986). Molt cycle duration in *P. setiferus* was found to be significantly shorter for unilaterally eyestalk ablated animals (Robertson et al. 1987).

This study was undertaken to determine if thoracic ganglion implantation would enhance spermatophore quality in *P. vannamei* as eyestalk ablation does, and at the same time evaluate the effect of eyestalk ablation and thoracic ganglion implantation on the molt cycle duration.

MATERIALS AND METHODS

Animals and Culture Conditions

Male *P. vannamei* (mean weight = 25.05 ± 1.89 g) were selected during harvesting of a commercial semi-intensive earthen pond at Criadero de Camarones de Chomes S.A., Costa Rica. Animals were then packed in plastic bags with chilled seawater (25 °C) and oxygen. Bags were transported in ice chests to the Aquaculture Laboratory at Universidad Nacional, Heredia, where experimental shrimp were acclimated to the culture water.

Males were stocked (12 shrimp/m²) in recirculation culture units with external biological filters and automatic control of temperature (Earth Shyokai Co., Ltd, Tokyo, Japan). Water was exchanged weekly at approximately 10-15% of the total volume. A fresh-frozen diet consisting of shrimp heads *(P. occidentalis)*, marine bloodworms *(Nereis viridens)*, giant squid *(Dosidicus gigas)*, and bivalves *(Anadara tuberculosa)* was provided at a ratio of 1:1:1:0.25, respectively, at 13% wet body weight per day. Each food item was given individually at different times: 09:00, 12:00, 14:00, and 17:00 h, respectively. Salinity was maintained at 30 ppt, temperature at 29 °C, and pH at 7.7-7.9. An acclimation period of 13 days was adopted before initiation of treatments.

Thoracic Ganglion Isolation and Implantation

A male *P. stylirostris* (body weight = 36.9 g) with well-developed spermatophores was selected from a semi-intensive culture pond and used as donor.

The donor was disinfected superficially with ethyl alcohol and transversely dissected at the level of the cephalotorax-abdomen junction. The cephalotorax was then longitudinally dissected and tissue was removed until exposing the entire thoracic ganglion.

The whole thoracic ganglion was removed and transferred to a petri dish containing sterilized and chilled modified crustacean physiological solution (Ro et al. 1990). The modification consisted in the non inclusion of HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethane sulfonic acid]). The entire ganglion was cut into pieces of 3-4 mm. in length. Thoracic ganglion pieces were then implanted into male *P. vannamei* recipients at a rate of one

ganglion section per shrimp. Implant was inserted into the tail by using a surgical procedure. Shrimp were held between two dry paper towels, exposing the terminal part of the tail, which was disinfected with ethyl alcohol. Surgery was done in the sixth abdominal segment by practicing a 5 mm. incision, cutting through cuticle and muscle in the lateral area located between the hindgut and the abdominal ganglion. Sterile blades # 11 were employed for this purpose.

Out flowing blood was absorbed by applying a sterile piece of absorbent pad (Johnson & Johnson, NY, USA). The graft was then placed into the muscle using disinfected super fine point tweezers. After implanting, all remaining blood was absorbed, and the incision was plugged with a drop of cyanoacrylate (Krazy Glue Inc., NY 10010, USA). After 30 sec. a sterile saline solution (0.85%) was poured over the glue to insure hardening before transferring the recipient to the culture tank. The whole procedure from ganglion isolation to implantation of the group took 40 min.

Experimental Design

Before initiation of treatments, the experimental group was analyzed for molt stages using the technique described by Robertson et al. (1987). Male *P. vannamei* were divided into three groups of 4 animals each, selecting only those shrimp at postmolt to intermolt stage. The first group was unilaterally eyestalk ablated by cauterization; the second group was implanted with a thoracic ganglion piece of matured *P. stylirostris;* the third group was used as control, receiving the surgical procedure without implant. Females were not stocked with males to eliminate this factor as an exogenous spermatophore-quality enhancing variable.

Each experimental animal was individually marked for identification by cutting the uropods in a similar way to that described by Makinouchi and Primavera (1987). The parameters measured were total body weight, to the nearest 0.1 g., compound spermatophore weight, to the nearest 0.01 g., sperm count per compound spermatophore, percentage of abnormal sperm, and molt cycle duration.

Spermatophores were removed by manual ejaculation following a modified procedure of that described by King (1948). The modification consisted in manually ejecting with gentle pressure a portion of the spermatophore, and employing disinfected fine tweezers to completely expel the unit. Males were ejaculated before treatment and every 14 days, until 3 compound spermatophores were obtained.

Sperm count was performed by homogenizing the compound spermatophore in a glass tissue grinder, containing 3.0 ml. of artificial seawater, prepared according to Cavanaugh (1956). The suspension was then mixed several times to insure homogeneity and 3 samples were counted using an hemacytometer. The total 25 fields of the camera were counted.

Percentage of abnormal sperm was obtained by recording, over a transect of a microscope slide, number of normal sperm: spherical body and an elongate spike (Talbot et al. 1989) and number of abnormal sperm: malformed heads and bent or missing spikes. At least 100 cells for one of the categories were recorded. Additionally, molts were recorded evaluating uropod marks.

Data Analysis

Data on sperm count were used to calculate a sperm count improvement variable (SCIV), according to the following equation:

$$SCIV = C_t - C_i$$

where:

C_t= sperm count (millions) in third spermatophore

C_i= sperm count (millions) at initiation

The estimation of this variable was performed in order to reduce any error introduced by the inherently high level of variability in sperm count associated with penaeid shrimp (Leung-Trujillo and Lawrence 1985). Spermatophore weight at third spermatophore regeneration, SCIV, and data on molt cycle duration were statistically analyzed for significant differences using analysis of variance

Table 1

Sperm count and spermatophore weight response of male *Penaeus vannamei* under different conditions

Parameter		*Treatment*		
		Ablation	*Control*	*Implantation*
n[a]		4	4	4
SCIV[b]	-Mean	10.06	1.91	0.72
	-SEM[c]	4.23	2.26	0.74
	-Tukey's W[d]	A	B	B
Spermatophore weight(g)	-Mean	0.03	0.01	0.01
	-SEM	0.005	0.005	0.000
	-Tukey's W	A	B	B

a. Number of shrimp per treatment; mean size=25.05 g. (SEM=1.89).
b. Spermatophore improvement variable (millions).
c. Standard error of the mean.
d. Treatment means with the same letter are not significantly different (P<0.05).

(AOV) for one-way classification and the Tukey's W procedure for comparing treatment means (Ott 1984). Variance heterogeneity was reduced by transforming (squared root of Y + 3/8) the SCIV prior to analysis.

RESULTS

Experimental males performed well under our culture conditions. None of them died during the progress of the experiment, and no case of male reproductive system melanization (MRSM) was recorded as documented in other reports (Chamberlain et al. 1983; Chamberlain 1988; Alfaro 1990).

The response of eyestalk ablation and thoracic ganglion implantation over sperm count

Table 2

Comparison in molt cycle duration between different treatments for male *Penaeus vannamei*

Treatment	*n*[a]	*Molt cycle duration (days)*		*Analysis of variance*[c]
		Mean	*SEM*[b]	
Ablation[d]	7	14.4	1.5	
Control[d]	7	13.3	1.0	null hypothesis
Implantation[e]	7	14.7	2.2	accepted[f]

a. Number of molt cycles analyzed.
b. Standard error of the mean.
c. H0: u1=u2=u3.
d. Treatment performed at early postmolt to intermolt.
e. Implants performed at intermolt.
f. Molt cycle duration is not significantly different between treatments (P<0.05).

improvement (SCIV) and spermatophore weight, is shown in Table 1. Sperm abnormalities were higher at initiation (85.7%) than at third spermatophore regeneration (47.0%), regardless of treatment.

The implantation procedure performed on intermolt males allowed the implants to be in place without the possibility of being expelled during molting. None of the males that received a graft molted during the following 7 days from the time of surgery. Additionally, by examination of the gross morphology of the wounded area, we did not observe any melanization process taking place over grafts, as it would be expected for rejected foreign bodies (Fontaine and Lightner 1975; Lackie 1986).

Molts were easily identified individually by the uropod-cutting technique, and molting frequency was established for each treatment (Table 2).

DISCUSSION

Eyestalk ablation, as documented by Leung-Trujillo and Lawrence (1985), significantly ($P<0.05$) improved the sperm production in our experimental males. These young males were capable to generate spermatophores with an absolute sperm count in the range of 6.75 to 21.53 millions at third spermatophore regeneration. On the other hand, either implantation or control group produced sperm counts in the range of 0.00 to 4.63 millions at third spermatophore regeneration. Additionally, spermatophore weight also increased significantly ($P<0.05$) for the eyestalk-ablated group.

Thoracic ganglion implantation, under the protocol followed by this research, did not benefit spermatophore quality. Its SCIV and spermatophore weight were not significantly different from the control group, but these two groups presented significantly lower values than the eyestalk-ablated group.

Yano et al. (1988) found a positive effect of thoracic ganglion implantation over ovarian maturation of *P. vannamei*, and male maturation improvement by thoracic ganglion implantation was reported for other decapods (Otsu 1963; Gómez 1965). Our finding suggests that thoracic ganglion implantation does not have a direct effect over spermatophore sperm count and spermatophore weight as eyestalk ablation does. Nevertheless, other aspects of male maturation like testes and vas deferens development could have been affected but were not evaluated.

There are many questions to be answered in order to understand the mechanisms that control male maturation in penaeid shrimp. From the research of Yano et al. (1988), we see that at 18 days after treatment, some thoracic-ganglion implanted females matured to stages IV and V. In our study, thoracic-ganglion implanted males did not show any improvement in sperm count and spermatophore weight during the 41-days experimental period. Therefore, whichever the mechanisms are, thoracic ganglion implantation appears to have a different effect over ovarian maturation and spermatophore quality. Nevertheless, other aspects of male maturation like testes and vas deferens development, and number of thoracic ganglion pieces implanted, which could have different effects over recipients, should be investigated.

Young *P. vannamei* did not show significant variations in molting frequency between treatments. The molt cycle lasted from 12 to 18 days, with the mean values presented in Table 2. Contrary to the finding of Robertson et al. (1987) for *P. setiferus*, we did not find a significantly shorter molt cycle for the eyestalk-ablated group. Quackenbush (1986) states that ablation affects molt cycle duration early in the molt cycle. In the present study, ablation was performed at postmolt to intermolt, and animals were followed for at least 2 molt cycles. It could be that eyestalk ablation has a different effect over molting depending on the natural environment of each species, in a similar way that ovarian maturation is triggered by different environmental factors in the wild.

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