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Minimizing the effects of stress during eyestalk ablation of *Litopenaeus vannamei* females with topical anesthetic and a coagulating agent

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Abstract

A topical anesthetic and a coagulating agent were employed in this study to determine if observable signs of eyestalk ablation stress could be alleviated in adult female *Litopenaeus vannamei* broodstock. The experimental design included four separate treatments, with each tested group consisting of 15 female shrimp weighing approximately 40 g. Results show that survival of the shrimp was 100% for all treatments, but reaction to the eyestalk ablation event varied between treatments. Initiation of normal swimming or recovery and the onset of feeding following ablation and treatment also varied among the four groups. The results suggest that the use of a topical anesthetic prior to eyestalk ablation reduces the visible reactions to stress experienced by *L. vannamei* broodstock, allowing for a more humane eyestalk ablation method.

Keywords: Shrimp eyestalk ablation; *Litopenaeus vannamei*; Topical anesthetic; Coagulating agent

1. Introduction

Litopenaeus vannamei is a commercially important species of penaeid shrimp cultivation (Wyban and Sweeney, 1991). Controlled maturation and reproduction in a hatchery

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environment is an important aspect contributing to the success of the culture of this species (Ogle, 1991). To induce ovarian maturation and spawning of *L. vannamei*, unilateral eyestalk ablation is performed on female broodstock (Zaib-Un-Nisa and Ahmed, 2001). This induction method is considered to enhance production of larvae (Racotta et al., 1998; Palacios et al., 1999), but information is scarce on the physiological and metabolic effects of this procedure (Racotta et al., 1998; Rajesh and Laxminaryana, 1993). Most shrimp hatchery guidebooks emphasize the need to perform ablation quickly in order to minimize the effects of stress (Wyban and Sweeney, 1991; Primavera, 1983). It may be that the event of unilateral eyestalk ablation causes stress in these animals, resulting in an increased rate of respiration and a decreased level of metabolic activity when compared with unablated shrimp (Rosas et al., 1993).

It has been suggested that the enucleation method of eyestalk ablation decreases the effects of stress (Wyban and Sweeney, 1991) and also may allow for faster coagulation of the wound (Bray and Lawrence, 1992). Previous studies suggest that the lack of hemolymph clotting in crustaceans may indicate stress in the organism (Jussila et al., 2001). It has also been proposed that applying antibiotics to the ablation wound limits the amount of body fluid lost and minimizes microbial infection (Primavera, 1983). This study presents possible methods for alleviating stress due to the pain encountered by the event of eyestalk ablation. It was hypothesized that the use of a topical anesthetic prior to ablation of the eyestalk would minimize the trauma experienced by individual female shrimp. The use of a coagulating agent with an antibiotic ingredient may also lessen the time required for full hemolymph clotting of the ablation wound, minimizing hemolymph loss, and help to combat any bacterial infection that may occur afterwards. Results are based on observable signs of stress, such as swimming and feeding behavior and reaction to the event of eyestalk ablation.

2. Materials and methods

2.1. Location of study

The experiment was conducted at the UFSC Laboratório de Camarões Marinhos (LCM), located near Florianópolis, Santa Catarina, Brazil.

2.2. Biological material

Adult female (14 months old) *L. vannamei* broodstock, averaging 40 g ($\pm$ 2 g), were held in an indoor concrete broodstock conditioning tank (5 m diameter) for 7 days prior to the commencement of the experiment.

2.3. Environmental conditions

Average water temperature in this facility was maintained at 29 °C, with average salinity being sustained at 34 ppt. To facilitate environmental conditions required for broodstock maturation, the conditioning room was kept on a photoperiod of 12.5-h light

and 11.5-h dark. After shrimp eyestalk ablation and subsequent treatments (if any), the animals were transferred to one of two circular concrete observation tanks. The dimensions of these tanks were 1.5 m in diameter, 0.45-m depth and a seawater capacity of 1870 l.

Subsequent to ablation and treatment, the shrimp were fed a broodstock conditioning diet of 28 g of thawed *Artemia* adults (1100 h), 50 g of thawed squid and mussel (0300 and 1700 h), and 5 g of dry ration (1400 and 2300 h).

2.4. Experimental design

To facilitate the purpose of the experiment, four different treatments were applied to four groups consisting of 15 adult female shrimp each.

In three of the four treatments, shrimp underwent eyestalk ablation using the enucleation method described by Wyban and Sweeney (1991). This involved the slicing of the distal portion of the eye with a regular razor blade and then expelling the contents of the eyestalk through the incision. For Treatment 3, which involved only the use of anesthetic prior to ablation, no incision was made prior to releasing the eyestalk contents. In this case, pinching of the eyestalk to expel the eye material was performed. During the ablation process for all groups, shrimp were held out of the water in the jackknife position.

Treatment 1: Neither the anesthetic (Xylocaine®) was applied prior to eyestalk ablation nor the coagulating agent (Fibrase®) after. Fifteen shrimp constituting the control group were removed from the conditioning tank with a small hand net, enucleated, and transferred to one of the two observation tanks.

Treatment 2: Following eyestalk ablation, 15 shrimp were placed in a 23-l plastic pail of salt water. The enucleated eyestalks of these individuals were then treated with the coagulating agent (Fibrase®-Pentosan polysulfate sodium), which was applied by hand, rubbing the substance over the entire ablated eyestalk, penetrating the wound. The treated shrimp were then placed into the remaining observation tank. Swimming and eating behavior was then observed and compared in each tank for 48 h. After the observation period, the shrimp were removed from the tanks, which were prepared for the next phase of the experiment.

Treatment 3: Anesthetic (Xylocaine®-containing 2.5% Lidocaine) was applied prior to eyestalk ablation. In this case, an incision was not made on the distal portion of the eye prior to releasing the contents of the eyestalk. Ablation was achieved by compressing the eyestalk with the fingers before transferring to one of the two observation tanks.

Treatment 4: Anesthetic was applied before ablation, which was performed using the enucleation method. Fibrase® was immediately applied to the wound following ablation. Shrimp were then transferred to the remaining observation tank where swimming and feeding behavior was observed and compared for 48 h.

2.5. Response parameters observed

The first parameter observed was the presence or absence of a reaction, or recoil response, to the actual ablation of the eyestalk. For 48 h subsequent to eyestalk ablation

and treatment, all groups of shrimp were observed for signs of stress due to handling and/or the event of ablation. The parameters observed included: swimming behavior, both immediately following ablation treatment and throughout the 48-h observational period, and response to the introduction of various types of food. The latter parameter involved the observation of time elapsed between food introduction and onset of feeding by the shrimp.

At the conclusion of the 48-h observational period, the survival of each group of shrimp constituting the four different treatments was determined.

3. Results and discussion

Qualitative and quantitative parameters observed in *L. vannamei* broodstocks during application of Fibrase®, Xylocaine® and Xylocaine®/Fibrase® immediately after eyestalk ablation are summarized in Table 1.

3.1. Response time to food introduction

The time elapsed between the introduction of food to the observation tanks and the onset of feeding varied between treatments. In Treatments 1 and 2, shrimp exhibited a delayed reaction to introduced food, of 30 and 10 min, respectively. Neither of these treatments involved the application of anesthetic prior to eyestalk ablation. Shrimp involved in Treatments 3 and 4, which both received anesthetic applications prior to eyestalk ablation, immediately responded when food was introduced to the tank. This suggests that the use of a topical anesthetic, such as Xylocaine®, prior to ablation of the eyestalk of female *L. vannamei* improves the response time of the shrimp to food

Table 1
Qualitative and quantitative parameters observed in *L. vannamei* broodstocks during application of Fibrase®, Xylocaine® and Xylocaine®/Fibrase® immediately after eyestalk ablation

Parameter	Treatment 1 (Control)	Treatment 2	Treatment 3	Treatment 4
Treatment	No treatment	Fibrase®	Xylocaine®	Xylocaine®/Fibrase®
Elapsed time before feeding onset	30 min	10 min	Immediate	Immediate
Swimming behavior	80% (12/15) exhibited lateral, erratic, or "spiral" swimming behavior	~65% (10/15) exhibited lateral, erratic, or "spiral" swimming behavior	Normal or calm swimming behavior ~ 6% (1/15) exhibited "spiral" swimming	100% (15/15) exhibited normal or calm swimming behavior. No "spiral" swimming observed
Survival	100%	100%	100%	100%
Other observations	Reaction to ablation	Reaction to ablation	No reaction to ablation	No reaction to ablation

introduction after the ablation process. This indicates that the use of anesthetics reduces post-ablation stress, allowing for normal feeding conditions resulting in better nutritional condition of spawning female shrimp.

3.2. Swimming behavior

Swimming behavior also varied between treatments. In Treatment 1, shrimp exhibited very erratic swimming behavior. Eighty percent of the shrimp in this group were swimming very rapidly upon introduction to the tank, frequently crashing into the walls of the tank. Shrimp exhibited lateral swimming and were swimming in a spiral or whirling fashion. Primavera (1983) suggests that eyestalk-ablated females exhibiting a circular pattern of swimming is an indication of trauma. One hour after ablation, some of this type of behavior was still evident, but had ceased within 2-h post-ablation.

Erratic swimming behavior was again observed in Treatment 2 shrimp. Sixty-five percent of shrimp in this group also exhibited a slow recovery response upon transference to the observation tank. Lateral swimming was observed, with shrimp swimming spirally and crashing into the tank walls. After 1 h of observation, no erratic swimming was observed and shrimp appeared to prefer the base of the tank.

Shrimp in Treatment 3 exhibited a calmer response upon entrance to the observation tank. All shrimp displayed preference for the base of the tank with the exception of one animal, swimming in an erratic behavior, displaying the same whirling pattern mentioned in previous treatments. One-hour post ablation, no erratic swimming behavior was observed and shrimp seemed to prefer the base of the tank.

For Treatment 4, no erratic swimming was observed upon introduction to the observation tank following eyestalk ablation. The female shrimp showed preference for the bottom of the tank almost immediately, a situation that is characteristic of female broodstock in unstressed conditions (Schuweitzer, 2001). This behavior continued 1-h post-ablation.

These observations suggest that the use of topical anesthetics decreases the recovery time of this species of shrimp following ablation of the eyestalk, as little abnormal or erratic swimming behavior was observed in both Treatments 3 and 4. It can be suggested that erratic swimming behavior increases the incidence of injury to shrimp and adds to an already stressful situation. The use of Xylocaine® may have lessened the stress of eyestalk ablation, allowing for more normal swimming behavior and therefore less chance of injury.

3.3. Survival after 48 h

In all four treatments, no mortality was observed during the 48-h observational period.

3.4. Other observations

In Treatments 1 and 2, all shrimp reacted to eyestalk ablation by displaying a recoil reaction, or ‘‘flinching’’, when the incision was made. This suggests that eyestalk ablation is traumatic (Primavera, 1983), but due to the widespread use of eyestalk ablation in the shrimp culture industry, signs of stress are not uncommon (Treece and Fox, 1993).

In Treatments 3 and 4, no reaction to the ablation process was observed. Shrimp did not display a recoil response and appeared relatively unaffected by the process, suggesting that the anesthetic reduced the amount of trauma experienced by the shrimp.

4. Conclusion

The results obtained in this experiment indicate the use of the topical anesthetic Xylocaine® reduces the amount of observable reaction to the event of eyestalk ablation of female *L. vannamei*. Xylocaine® was used in both Treatments 3 and 4, in which no recoil response was observed during the ablation process. No incision was performed on shrimp constituting Treatment 3, however, no physical reaction to compression of the eyestalk was observed in any individual making up this group, again suggesting that the anesthetic used was effective.

It was hypothesized that applying a coagulating antibiotic agent (Fibrase®) may have aided in reducing stress in this situation. However, this experiment did not produce conclusive evidence to support this hypothesis. This study suggests that the use of Xylocaine® alleviates stress due to eyestalk ablation of *L. vannamei* female broodstock. Xylocaine® application prior to eyestalk ablation is now included as standard protocol at the Laboratório de Camarões Marinhos, as it is considered by staff to be a more humane (ethic) approach to the ablation process.

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References

Bray, W., Lawrence, A., 1992. Reproduction of *Penaeus* species in captivity. In: Fast, A., Lester, J. (Eds.), Marine Shrimp Culture: Principles and Practices. Elsevier Publishers, Amsterdam, The Netherlands, pp. 93–170.

Jussila, J., McBride, S., Jago, J., Evans, L.H., 2001. Hemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). Aquaculture 199, 185–193.

Ogle, J., 1991. Maturation of *Penaeus vannamei* based upon a survey. Gulf Res. Rep. 8 (3), 296–297.

Palacios, E., Carreõo, D., Redriguez-Jaramillo, M., Racotta, I., 1999. Effect of eyestalk ablation on maturation, larval performance, and biochemistry of white pacific shrimp, *Penaeus vannamei*, broodstock. J. Appl. Aquac. 9 (3), 1–23.

Primavera, H., 1983. Broodstock of Sugpo, *Penaeus monodon* Fabricius. Extension Manual No. 7, 3rd ed. Southeast Asian Fisheries Development Center, Tigbauan, Philippines.

Racotta, I., Palacios, E., Carreño, D., 1998. Effect of ablation on reproductive performance and biochemical

composition of eggs, nauplii, and spawner tissues in *Penaeus vannamei*. Proceedings of the Symposium held in Brest, 19–20 November 1998, vol. 27. Ifremer, Plouzane, France, p. 184. 2000.

Rajesh, B., Laxminaryana, A., 1993. Metabolic effects of eyestalk removal in *Penaeus indicus* H. Milne Edwards. Mariculture Research under the Postgraduate Programme in Mariculture: Part 2, CMFRI, Cochin (India), 1993. CMFRI Spec. Publ., vol. 53, pp. 76–79.

Rosas, C., Fernandez, I., Brito, R., Diaz-Iglesia, E., 1993. The effect of eyestalk ablation on the energy balance of the pink shrimp, *Penaeus notialis*. Comp. Biochem. Physiol. 104A (1), 183–187.

Schuweitzer, R., 2001. UFSC Laboratório do Camarões Marinhos, Florianópolis, SC, Brazil. Personal communication.

Treece, D., Fox, M., 1993. Design, Operation and Training Manual for an Intensive Culture Shrimp Hatchery. Texas A&M University Sea Grant College Program, Texas, USA.

Wyban, A., Sweeney, N., 1991. The Oceanic Institute Shrimp Manual, Intensive Shrimp Production Technology. The Oceanic Institute, Honolulu, HI, USA.

Zaib-Un-Nisa, N., Ahmed, M., 2001. Natural and induced spawning of Pakistani penaeid shrimps in the laboratory. Pak. J. Zool. 33 (2), 125–130.