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Short Communication

Sex Reversal of the Giant Freshwater Prawn *Macrobrachium dacqueti* through Androgenic Gland Ablation

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Abstract: The male *Macrobrachium* species (giant freshwater prawn) typically achieve better growth and a larger harvest size than females. Hence, it is clear that the monosex culture of all-male prawn populations would be inexpensively advantageous. This study aimed to determine whether androgenic gland (AG) ablation induces sex reversal of giant freshwater prawn *Macrobrachium dacqueti*. The AG of the prawn was ablated through bilateral microsurgery (AG ablation) and let them recover for two months. The weight and length were also measured every 15 days. Results revealed that the removal of AG from the males of immature *M. dacqueti* resulted in sex reversal, with 70% female differentiation. Successful neo-female prawns exhibited the development of an ovary with orange coloration as it matures. Andrectomized *M. dacqueti* did not develop the appendix masculina in the second pleopod, an indicator of a suspected neo-female prawn. A significant increase in weight and length was observed within two months compared to the control. Based on the result of the study, sex reversal of *M. dacqueti* is possible through AG ablation. Therefore, sex-reversed (neo-females) *M. dacqueti* can be used to breed with normal males to produce all-male progenies since both parents possess male hormones, thereby rendering a huge advantage for prawn culture.

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1. Introduction

The farming of giant freshwater prawns (*Macrobrachium* spp.) is important and is practiced worldwide, which contributed significantly to aquaculture production in many Southeast Asian countries such as Malaysia, Thailand, and Indonesia (Romana-Eguia et al., 2006). About 472 000 metric tonnes, or 5% of the total world crustacean aquaculture production, were contributed by *Macrobrachium* species in 2018 (FAO, 2020). This decapod has become a world food source owing to its great protein content, palatability, and bigger size (Tan and Wang, 2022). *Macrobrachium dacqueti* was previously known as *M. rosenbergii dacqueti* and was recently separated into different species by taxonomists (De

Bruyn et al., 2004; Wowor and Ng, 2007; Iketani et al., 2011). *M. dacqueti* is one of the most commercially significant crustaceans and is famously farmed all around the world (Wowor and Ng, 2007; Iketani et al., 2011). In the Philippines, *M. dacqueti* is among the important freshwater prawn with high economic value (Eguia et al., 2009). In recent years, this species of freshwater prawn has been not only extensively farmed but also fished and researched species from Indochina, South Asia, and Southeast Asia (Shy et al., 2013).

In the course of increasing production of giant freshwater prawns due to the increasing demand, the monosex culture (i.e., all-male populations) is more desirable and advantageous due to its fast-growing characteristics that can reach greater biomass than females during the harvest period (Aflalo et al., 2006; Aflalo et al., 2014; Levy et al., 2018). Recently, the use of monosex farming of crustaceans has been considered as a promising approach to increase production yield, especially when there are huge differences in growth rates and behavioral patterns between females and males (Tan et al., 2020).

Androgenic gland (AG) ablation as a sex reversal potential for monosex mariculture has been well explored in different species of decapods (Alfaro-Montoya et al., 2016; Tropea et al., 2011). AG ablation plays an important role in male differentiation among decapods (Tropea et al., 2011; Dunn et al., 2020; Tan et al., 2022). The AG is responsible for prawn sexual differentiation and is important for the male primary and secondary sexual characteristics (Tan et al., 2020). For instance, when the males of *M. rosenbergii* ablated at a very early development stage, sex-reversal was accomplished, making the males transformed into completely functional females (neo-females) (Sagi and Aflalo, 2005; Aflalo et al., 2006).

AG's role in the sex differentiation of Malacostraca has been largely studied, primarily employing two alternative techniques; AG implantation in females and andrectomy (AG ablation) in males. The alteration of AG is viable since, in male crustaceans, the endocrine and gametogenic functions are segregated into different organs, the AG and the testis, respectively. Hence, sex differentiation can be determined by removing the AG without impairing the gonads (Tropea et al., 2011), and by aberrating the male AG activity, de-masculation of crustaceans can be achieved (Ford, 2008). It is believed that the insulin-like AG produced by the male's AG in decapods governs the male sex differentiation, behavior as well as growth (Sroyraya et al., 2010; Huang et al., 2014).

The microsurgical ablation of the AG, also known as "andrectomy," is accomplished by the removal of the fifth pair of walking legs together with the AG and pulling off a significant portion of the sperm duct to ensure that the AG has been fully removed. Bilateral androgenic gland ablation can be performed as early as the formation of the post-larval stage of the prawn (Aflalo et al., 2006; Tan et al., 2020). To perform AG microsurgical removal, skillful personnel is needed, and under farm conditions, this can be realistically applied without any sophisticated instruments. Using this procedure, it is likely that the production of neo-females is high, and all-male prawn post-larvae mass production can be attained commercially when neo-female breed with normal males (Tan et al., 2020). Previous studies only focused on the sex reversal of *M. rosenbergii* and other crustaceans. Hence, this study aimed to determine whether sex reversal for *M. dacqueti* would be possible through AG ablation. Additionally, the weight and length of successfully sex-reversed individuals were also monitored.

2. Material and Methods

2.1. Study site

The study was conducted at the Fish Health Laboratory, Iloilo State College of Fisheries (ISCOF)-Main Tiwi Campus, Barotac Nuevo, Iloilo, Philippines.

2.2. Acquisition and acclimatization of experimental animal

Giant freshwater prawn *M. dacqueti* specimens were acquired from the Bureau of Fisheries and Aquatic Resources - National Integrated Fisheries Technology Development Center (BFAR-NIFTDC), Dagupan City, Pangasinan, Philippines. These were transported to the study site and acclimatized for one week.

2.3. Andrectomy process (AG ablation)

M. dacqueti prawns (30-day-old post larvae, PL_{30}) were separated into two groups: control and experimental. The control prawns (mixed male and female, n=100) were kept in normal culture, and the experimental group (all male, n=100) underwent the andrectomy process (AG ablation). Each of the experimental prawns was ablated through bilateral microsurgery of their androgenic glands. This was done by the removal of the fifth pair of walking legs together with the AG and pulling off a significant portion of the sperm duct to ensure that the AG had been entirely removed. The andrectomized prawns were placed in the recovery aquarium. A total of 100 andrectomized prawns were used in the experiment, and 70 individuals survived after two months of culture.

2.4. Culture and maintenance

The andrectomized and control prawns were stocked in 20 aquariums (10 for control and 10 for andrectomized prawns) with a stocking density of 10 prawns per aquarium. The prawns were fed at a 5% feeding rate, same water management and supply of aeration were applied. The prawns were maintained at 27°C temperature, 0 ppt salinity, 6.5-6.8 pH, and satiated dissolved oxygen (6-9 ppm). The duration of the culture was two months after the andrectomy.

2.5. Sampling

Sampling was done every 15 days for two months. Once no mortality was observed after AG ablation (1-7 days), monitoring of weight and length was commenced. All individuals were sampled for measurements of length and weight using a vernier caliper and an electronic top-loading balance scale, respectively. A total of 70 andrectomized prawns and 90 control prawns survived after the culture period.

2.4. Data analysis

The data on the weight and length of two sets of groups were subjected to a t-test using SPSS software version 20. The significant difference was set at 0.05.

3. Results and Discussion

Successful neo-female prawns developed an ovary. The ovary is located distal behind the eyes and is dorsally visible on the prawn's cephalothorax. As a prawn's ovary matures, its orange color is noticeable. In this study, out of the 100 ablated prawns, all 70% (70 individuals) of *M. dacqueti* that survived possessed light orange-colored gonads (Figure 1).

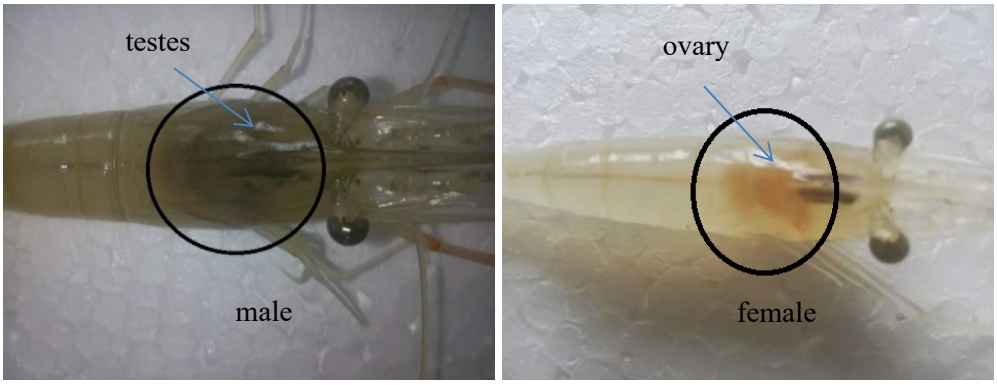

Figure 1. Gonadal development of *Macrobrachium dacqueti* before (left) and after AG ablation (right).

Mortalities (30% or 30 individuals) were caused by both the procedure and cannibalism. According to Aflalo et al. (2006), there is a possible ovarian development if a light orange-colored gonad is visible through the prawn's transparent cuticle, as observed in their study. This suggests that the AG-ablated *M. dacqueti* samples in this study successfully underwent sex reversal from male to neo-female

individuals. AG-ablated (andrectomized) *M. rosenbergii* (PL_{15} and PL_{30}) developed ovaries after 105 days of succeeding metamorphosis (Aflalo et al., 2014).

The appendix masculina (AM) is an accessory organ of a male situated medially on the second pair of pleopods between the appendix interna and endopodite. The absence or presence of an AM is the simplest way to determine females and males in most prawns (Hobbs et al., 1977). Studies use the existence of AM after ablation as an indicator of failed sex reversal. The absence of the AM, therefore, indicates a suspected neo-female prawn. As observed after andrectomy (AG ablation), the ablated prawns did not develop AM on their second pleopod (Figure 2 A). In the study of Aflalo et al. (2006), about 32% of the andrectomized *M. rosenbergii* males did not develop appendix masculina after 30 days, which indicated that sex reversal was completed.

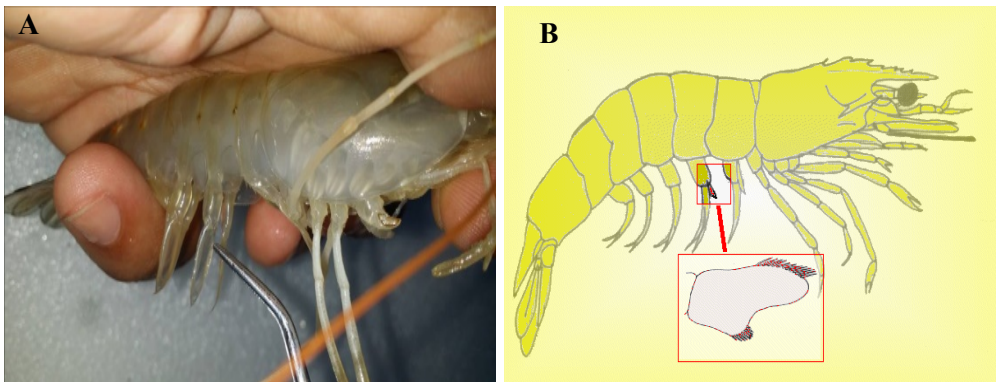

Figure 2. Appendix masculina (AM) of *M. dacqueti*. Absence of AM after AG ablation (left). Normal AM in unoperated prawn (right) (https://aquaculture.ugent.be).

In decapods, the first successful sex reversal was produced with *M. rosenbergii*. In order to obtain neo-males, the AG tissues were implanted into immature *M. rosenbergii* females. By crossing these neo-males, all-female offspring were produced (Nagamine et al., 1980; Tan et al., 2020). When the males of *M. rosenbergii* AG ablated at a very early development stage, sex reversal was accomplished, making the males transformed into entirely functional females (neo-females) (Sagi and Aflalo, 2005; Aflalo et al., 2006). In this study, sex reversal was successful at a higher rate (70% or 70 individuals), evident from the presence of an ovary and the absence of an appendix masculina. The mechanism behind successful sex reversal from males to neo-females of *M. rosenbergii* is attributed to different signaling pathways (e. g., Hippo, Rap1, PI3K, thyroid hormone, oxytocin, and apoptosis), which play essential roles in the sex reversal process by regulating gonad development, cell proliferation, and maintaining homeostasis (Tan et al., 2022). However, in other decapods, the use of AG ablation did not successfully accomplish the sex reversal in *Litopenaeus vannamei* (Alfaro-Montoya et al., 2016).

The AG is normally found associated with the terminal male gamete duct's portion. As stated by Diwan (2005), the bilateral andrectomy of AG in some shrimp has an effect on sex reversal. Specifically, shrimp males who have undergone andrectomy have lost secondary sexual characteristics and demonstrated a lack of sperm in the lumen of their testicular acini. AG in decapod is not necessary for spermatogenesis completion, and their non-existence leads only to the reduction of spermatogenesis intensity (Diwan, 2005). AG controls the male sex differentiation, growth, and behavior in crustaceans, where insulin-like AG plays a crucial role (Sroyraya et al., 2010; Huang et al., 2014; Sun and Li, 2021). The absence of AG in *Penaeus indicus* showed to impede spermatogonial differentiation (Mohamed and Diwan, 1991). In terms of the androgenic hormone's chemical nature, some studies indicated that the AG has the ability to produce various compounds, such as proteins and the hexahydroxy farnesylacetone, terpenes, and farnesylacetone, and the precise role of these compounds is still unknown (Laufer and Landau, 1991). Charmantier et al. (1997) stressed that the AG hormone regulates spermatogenic activity in the testis and is accountable for maintaining and developing secondary sexual characteristics in male crustaceans.

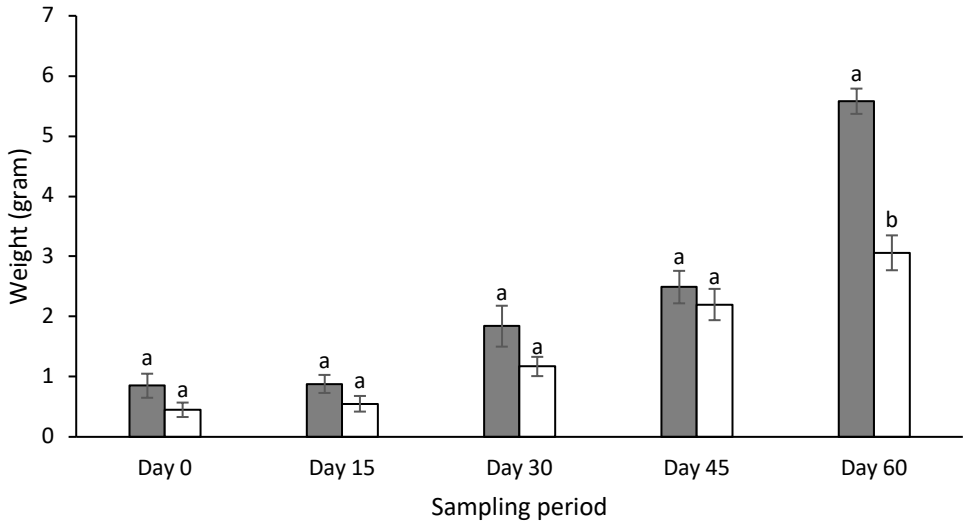

Figure 3. Weight of sex-reversed (dark gray bar) and control (light bar) *M. dacqueti* after 60 days.

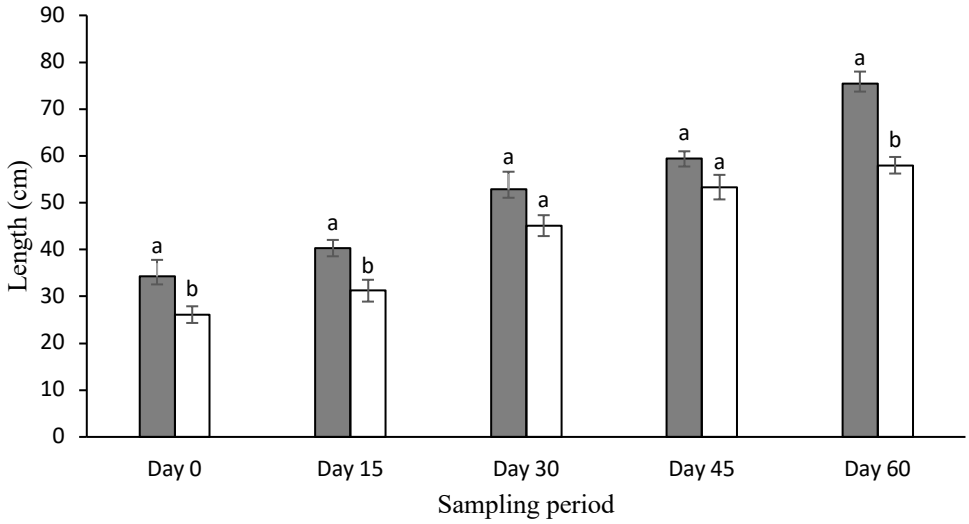

Figure 4. Length of sex-reversed (dark gray bar) and control (light bar) *M. dacqueti* after 60 days.

The weight and length of sex-reversed *M. dacqueti* after 60 days in this study were significantly higher (p<0.05) than the control prawns (Figures 3 and 4). The result of the study shows that the AG-ablated prawns grow faster than the control prawns because they still possess male genes. Earlier reports under the same genus, like in *M. rosenbergii*, indicated AG-ablated males resulted in lower growth significantly compared to unoperated male groups (Sagi et al., 1990). In the present study, since the control prawns were mixed (male and female), the female prawns may have affected the result of the growth of the control. In other decapods, the AG ablation of the *Cherax quadricarinatus* males had no influence on the somatic growth parameters (growth increment and specific growth rate) (Tropea et al., 2011). In *Litopenaeus vannamei*, although sex reversal was unsuccessful, andrectomized males were significantly higher compared to control both in terms of body weight and length four months after surgery of PL_{70} (Alfaro-Montoya et al., 2016).

Conclusion

In conclusion, our study demonstrated that sex reversal of *Macrobrachium dacqueti* is feasible through AG ablation. This practical approach can be used in a monosex culture by producing an all-male population through mating neo-females with normal male individuals, thereby enhancing the

production yield in prawn culture. However, the relationship between AG ablation and the growth of the prawn should be given attention in future studies.

Compliance with Ethical Standards

Authors' Contributions

R.C.V.: Design the experiment.
P.P.T., M.A.V., and R.C.V.: Conducted the experiment and collected the data.
R.C.V., J.O.A., and A.B.T.: Wrote the initial and final draft of the manuscript.
R.C. V. and A.B.T.: Analyzed the data.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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