REVIEW ARTICLE

MONOSEX PRAWN CULTURE: FROM A BIOTECHNOLOGICAL STANDPOINT

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ABSTRACT:

In the aquaculture of Macrobrachium rosenbergii, the cultivation of monosex (all-male) prawns is advantageous due to the faster growth rates of males compared to females. Traditionally, manual selection of males during farming has been practiced but is labour-intensive and often fails to achieve a male-only population. The androgenic gland (AG) is pivotal for sexual differentiation and the development of male characteristics. Microsurgical removal of the AG (andrectomy) during early development can transform male prawns into fully functional neo-females. The androgenic gland-specific insulin-like hormone (Mr-IAG) is crucial for male spermatogenesis and sexual development in M. rosenbergii. RNA interference (RNAi) offers a precise method to silence gene expression post-transcriptionally, effectively inducing complete and functional sex reversal by silencing Mr-IAG. Both microsurgery and RNAi target the AG to achieve efficient sex reversal. Neo-females resulting from these methods can mate with normal males to produce all-male prawn offspring, thereby enhancing production efficiency and profitability in commercial prawn farming.

KEYWORDS: *Freshwater, Prawn, Genetic Modification, All-Male Population, Selective Breeding, Monosex-Culture, Sex Reversal*

INTRODUCTION:

Prawn aquaculture is a crucial source of protein and a source of income for millions of people, contributing significantly to global food security. However, there are frequently issues with conventional methods' effectiveness, use of resources, and influence on the environment. It was quickly discovered that the necessity to create management methods specifically suited to one sex or the other originates from the differences between males and females of the same cultured species in terms of development rate, nutritional requirements, and behavioural characteristics. Monosex culture groups that do not reproduce naturally have the advantage of

focusing their energy on growth rather than reproduction (Sagi *et al.,* 2005). The freshwater prawn, *Macrobrachium rosenbergii*, is one such species that has been introduced as an aquaculture species in several nations.

Selective breeding programs have also been initiated for several commercially important crustacean species (Jerry, Purvis, Piper, & Dennis, 2005; Jones, McPhee, & Ruscoe, 2000) and various marine penaeid species (Argue, Arce, Lotz, & Moss, 2002; De Donato, Manrique, Ramirez, Mayer, & Howell, 2005; Preston, Crocos, Keys, Coman, & Koenig, 2004). Information on phenotypic variation and both non-additive and additive genetic variation is crucial for the successful development of any breeding project (Gjerde, 1986). Over generations, selective breeding can yield significant long-term economic benefits.

Alternatively, monosex culture is a promising approach to increasing production yield. In crustaceans, monosex culture is used when there are significant differences in behavioral patterns and growth rates between males and females (Curtis & Jones, 1995; Sagi, Ra'anan, Cohen, & Wax, 1986). All-male cultures of *Macrobrachium rosenbergii* have been conducted through manual segregation, resulting in a substantial increase in harvest yield and income by over 60% (Mohanakumaran, Salin, Raju, & Sebastian, 2006).

Aflalo *et al.* (2006) developed a novel technique to produce all-male populations through male sex reversal, employing a two-phase biotechnological approach. This involves removing the androgenic gland (AG) of male prawns during sexual differentiation to create neo-females, which are genetically male but phenotypically female. These neo-females are then mated with regular males to produce monosex (all-male) offspring. Studies indicate that AG removal is crucial for manipulating sex reversal in *M. rosenbergii*, although the roles of the AG and testis in male reproductive system development are not yet fully understood.

PROCEDURE:

Generally, Androgenesis is done through the genetic inactivation of the egg and its activation with healthy sperm from the matching species. To reestablish diploidy, shock therapy must be given. Most likely, dispermy or another mechanism is involved in the process.

The neo-males were crossed with regular females to produce all female offspring. A little hole was made on the right ventral side of the abdomen, close to the cephalothorax, during the female sex reversal phase. Through this opening, the AG tissues were injected into the musculature of the abdomen. To determine the emergence of a male gonopore complex, the moulting of the injected female prawns was studied. Researchers concluded that the male gonopore complex's presence is a more reliable predictor than the appendix masculinum (Nagamine *et al.,* 1980a, b). Neo-males had a female gonopore complex, albeit with aberrant setae, in addition to a male gonopore complex, which made them simpler to recognise as they grew in size. However, following the transplanting of AG tissues and the injection of AG cells, neo-males finally grew into males with normal phenotypes (Malecha *et al.,* 1992). Neo-males ultimately underwent differentiation to become small males, orange-claw males, and blue-claw

males, which are the three adult morphotypes (Kuris *et al.,* 1987). To produce an all-female offspring, neo-males with blue-claws were mated with normal females.

Male *M. rosenbergii* post-larvae (PL) that had had their AG removed had a successful transition into neo-females (andrectomized prawn) that had functional ovaries (Aflalo *et al.,* 2006; Sagi & Cohen, 1990) or ovotestes (Nagamine *et al.,* 1980a, b). When the neo-females mated with normal males, they were able to produce all-male offspring; however, the success rates of neo-female production via andrectomy technology were low and inconsistent, which posed a barrier to the mass production of neo-females for the production of all-male prawn PL at a commercial scale. The success of creating neo-females depended on the stage of the PL during andrectomy. Previous research indicated that the PL stage, when the appendix masculina was absent, was the best time to increase the success rates of feminization via an antrectomy (Aflalo *et al.,* 2014). Aflalo *et al.* (2006) shown that feminization could be highly successful in the early phases (PL20–30).

1. RNA interference (RNAi)-mediated gene silencing associated with sexual differentiation:

RNA interference (RNAi) is a precise method of post-transcriptional gene silencing or interference in cells, primarily involving small interfering RNA (siRNA). Endogenously synthesized, siRNA can silence gene activities, and in vitro synthesis can produce three types: siRNA, short hairpin-loop RNA (shRNA), and long double-strand RNA (dsRNA). siRNA can be externally synthesized through in vitro transcription and introduced into cells via microinjection or transfection. Within cells, shRNA and dsRNA are cleaved into siRNA fragments by the enzyme Dicer. These fragments then guide the RNA-induced silencing complex (RISC) to target RNA sequences, effectively silencing specific genes (Agrawal *et al.,* 2003; Qi and Hannon, 2005; Watson, Fusaro, Wang, & Waterhouse, 2005). RNAi can temporarily silence gene expression without genetically modifying the organism. Microinjection has proven to be the most efficient and cost-effective method for inducing RNAi in crustaceans to silence gene expression. In addition to rapid and large-scale RNAi production, innovative methods are needed. For example, Kamath and Julie (2003) demonstrated the effectiveness of feeding the platyhelminth Caenorhabditis elegans with bacteria containing dsRNA as a mass-scale administration method (Kamath & Julie, 2003).In crustaceans, RNAi can interfere with genes involved in embryonic or organ development, cellular metabolism, and reproduction mechanisms (Kato, Kobayashi, Watanabe, & Iguchi, 2011). Kato *et al.* (2011) showed that microinjecting dsRNA to silence the gene Doublesex1 (Dsx1) in male embryos led to the development of female secondary characteristics. The androgenic gland, crucial for male sexual differentiation in malacostracan crustaceans, produces an insulin-like gene known as C. quadricarinatus insulin-like androgenic gland-specific factor (Cq-IAG) in the red-claw crayfish *Cherax quadricarinatus*. Silencing Cq-IAG using RNAi confirmed its role in male sexual differentiation (Rosen *et al.,* 2010). In *C. quadricarinatus*, intersex individuals exhibit both male

reproductive systems and secondary sex characteristics, which can permanently inhibit ovarian development. Silencing Cq-IAG in these individuals caused male feminization, reduced sperm production, low-quality sperm, testicular degeneration, and yolk protein accumulation in developing oocytes. Similarly, Ventura *et al.* (2009) showed the significance of Mr-IAG in the spermatogenesis and development of male primary and secondary sexual characteristics in juvenile M. rosenbergii males through RNAi experiments. Given the maturity of RNAi technology in inducing sexual dimorphism, it can be regularly applied to produce neo-females and enhance the mass production of all-male prawns.

2. Using RNA interference technology, sexual dimorphism and monosex culture of prawns:

The rapid development of gene silencing technology in decapod crustaceans has opened up significant opportunities for its application. The first commercial use of dsRNA-based technology was in the prawn aquaculture industry (Ventura *et al.,* 2009). In aquaculture, reproductive and sexual maturity can influence crustacean growth rates (Aiken, 1992; Hartnoll, 2001). Monosex culture, particularly of all-male prawns, has proven beneficial for commercial farming. Manual segregation of male prawns for culture was initially performed on a small scale, resulting in higher growth rates (Sagi *et al.,* 1986). In India, cultivating all-male *Macrobrachium rosenbergii increased* income by 60% (Mohanakumaran *et al.,* 2006). However, manually separating males and females was labour-intensive and time-consuming, making it inefficient for large-scale implementation. The discovery of RNAi allowed for specific dsRNA to silence the insulin-like androgenic gland hormone (Mr-IAG) in *M. rosenbergii* (Ventura *et al.,* 2009). This process involved a two-step method to synthesize dsRNA. First, forward and reverse oligos were designed based on the Mr-IAG mRNA sequence, which were then annealed using polymerase chain reaction (PCR) with Mr-IAG as the template DNA. The PCR products were purified and transcribed into dsRNA. A 2 µg/g dose of dsRNA was microinjected into the sinus of the fifth set of walking legs at an early developmental stage. The dsRNA effectively silenced Mr-IAG, causing sex reversal from males to neo-females, which were identified using molecular sex markers (Ventura *et al.,* 2011a, b). After three months, these neo-females mated with normal males to produce all-male offspring (Ventura & Sagi, 2012). This success marked the first commercial method of sex reversal in crustaceans using RNAi silencing technology. Prawns produced with this technology are considered safer for consumption as they are free from hormones, chemicals, and genetic modifications. Additionally, dsRNA-based silencing technology is temporary and affects only the current generation, not being transmitted to the next. Thus, the all-male prawns can be consumed safely and do not face the regulatory hurdles associated with genetically modified organisms (Stein and Rodriguez-Cerezo, 2010).

3. Sex reversal by AG ablation microsurgery:

Sexual maturity, reproductive activity, and gender are key factors influencing animal growth in aquaculture systems (Aiken, 1992). Earlier management strategies involved

separating males and females to redirect energy from reproduction to growth. Monosex culture has been a longstanding practice in fish aquaculture (Beardmore *et al.,* 2001; Gomelsky, 2003). In crustacean farming, monosex culture, particularly of prawns and crabs, is implemented because males typically grow faster than females (Curtis & Jones, 1995; Lawrence, 2004). In crustaceans, sexual differentiation and the development of male primary and secondary characteristics are controlled by the androgenic gland (AG) (Sagi *et al.,* 1990). For *Macrobrachium rosenbergii*, sexual differentiation can be manipulated by removing the AG without damaging the gonads, which is crucial for producing all-male prawns. When the AG of male prawns is removed at an early developmental stage, oogenesis and the development of female reproductive organs occur, a process known as feminization. These males, now neo-females, develop functioning female organs (Nagamine *et al.,* 1980a, b). Neo-females can mate with normal males to produce all-male offspring (Katakura, 1989; Sagi, Snir, & Khalaila, 1997). The microsurgical removal of the AG involves excising the AG located at the base of the fifth pair of walking legs, along with part of the sperm ducts, to ensure complete removal. This full removal is necessary for successful sex reversal to neo-females (Aflalo *et al.,* 2006). Three months post-andrectomy, DNA from prawns with female phenotypes is extracted and checked against molecular sex markers to confirm neo-females (Ventura *et al.,* 2011a, b). These neo-females are then separated, and their gonadal development is monitored every two weeks. When ovarian development is visibly advanced, indicated by the orange-colored ovary seen through the transparent cuticle, the neo-females are ready to mate with normal males. Mated neo-females begin to carry eggs, which mature over time, changing color from orange to gray (Aflalo *et al.,* 2006). This process results in the production of all-male progeny. While the microsurgical removal of the AG requires skilled personnel, it can be done on farms without sophisticated equipment. Typical success rates range from 3–5%. Using this technology, a large number of neo-females can be produced, enabling the commercial mass production of all-male prawn post-larvae.

ADVANTAGES:

The growth of prawn culture systems is profoundly influenced by factors such as gender and sexual maturity. In many crustacean species, including *Macrobrachium rosenbergii*, males often exhibit faster and larger growth compared to females, a phenomenon known as bimodal growth. Monosex culture trials with *M. rosenbergii* have consistently shown that all-male populations can achieve higher yields and reach marketable size more quickly than mixed or all-female populations. This approach not only boosts production rates but also extends the period during which fresh prawns can be marketed, thereby optimizing pond utilization and increasing economic returns.

Early experiments in monosex culture, such as those conducted with *M. rosenbergii*, demonstrated significant advantages. For instance, studies reported that all-male populations achieved substantially higher yields compared to mixed or all-female stocks within similar

grow-out periods (Sagi *et al.,* 2005). These findings were consistent across different culture systems, from small-scale intensive cage cultures to larger earthen ponds under monoculture conditions. While initial trials indicated economic benefits in terms of increased average weights and shorter time to harvest, the implementation of monosex culture in polyculture ponds showed a notable increase in net income, albeit with varying economic feasibility depending on local conditions and production costs.

DISADVANTAGES:

While monosex prawn culture has some benefits, such as faster growth rates and possibly higher yields, it also has some serious drawbacks. The loss of genetic variety within communities is a serious worry since it can make people more vulnerable to illnesses and environmental stresses. This vulnerability derives from the reliance on specific genetic lines or sex-reversed individuals.

Furthermore, because residual hormone levels exist in prawns meant for human consumption, the process frequently entails the use of hormonal treatments for sex reversal, which can be expensive and raise issues about food safety and the environment. Additionally, there's a chance that sex reversal methods won't provide the intended monosex populations, leading to unexpected or mixed sex ratios that reduce productivity.

Customer preferences and market demand present another difficulty. Market preferences for particular prawn sizes or genders may not necessarily correspond with monosex cultures, which could have an impact on market acceptance and profitability. The increased investments and operating costs necessary for the implementation and upkeep of monosex culture systems further impact the economic sustainability of such endeavours.

Concerns regarding animal welfare and the moral implications of these procedures in aquaculture also surface when prawn genetics and reproductive systems are manipulated. Sustaining these concerns necessitates continued biotechnology breakthroughs, prudent management techniques, and a deep comprehension of market dynamics and regulatory frameworks.

CONCLUSIONS:

Removing a key gland (AG) in young prawns disrupts their development, turning them into "neo-females" that can reproduce with normal males, but only produce male offspring. Two methods achieve this: microsurgery (practical for farms) and RNAi technology (more complex, requiring labs). Both methods can produce enough neo-females for large-scale production of all-male prawn offspring. All-male prawn culture offers significant advantages: faster growth, higher yields (due to no need for sex separation), and lower costs, resulting in substantially higher profits for prawn farmers.

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