

The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective

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Abstract

Males of the freshwater prawn *Macrobrachium rosenbergii* (De Man) grow faster and reach a larger size at harvest than females of the species. It is thus obvious that culture of monosex all-male populations would be economically advantageous. Sexual differentiation in crustaceans is regulated by the androgenic gland (AG), which plays a pivotal role in the regulation of male differentiation and in the inhibition of female differentiation. In *M. rosenbergii*, AG removal from immature males resulted in sex reversal, with complete female differentiation. Similarly, AG implantations into immature females lead to the development of the male reproductive system. Sex-reversed *M. rosenbergii* animals were capable of mating with normal specimens to produce offspring. Early attempts in Israel and more recently, attempts in other countries to establish all-male populations through manual segregation showed that for the production of monosex prawn populations to be economically feasible, intervention via the AG is probably required. However, a suitable biotechnology is still to be developed, and an androgenic hormone has yet to be identified in decapods. Three lines of aquacultural and biotechnological research and development are proposed for the future: (1) Establishment of monosex cultures through manual segregation, together with the application of selective harvesting and claw ablation, as well as examination of different monosex culture strategies under a variety of economic conditions. (2) Microsurgical intervention in the AG, leading to the development of functional neo-females, which would subsequently be mated with normal males to produce all-male pro-

geny. (3) Elucidation of AG bioactive products to enable biochemical or molecular manipulation of sex differentiation.

Keywords: monosex culture, sex reversal, androgenic gland, *Macrobrachium rosenbergii*, decapoda, crustacea

Introduction

By the late 1970s, monosex culture strategy had become common practice in fish-based aquaculture (Mires 1977; Tayman & Shelton 1978), and more recently attempts have been made to apply this aquaculture technology to crustacean culture (Curtis & Jones 1995; Sagi, Milstein, Eran, Joseph, Khalaila, Abdu, Harpaz & Karplus 1997a). It was soon realized that differences between males and females of the same cultured species in terms of growth rate, alimentary needs and behavioural patterns dictate the need to establish management systems specifically tailored to one sex or the other. One of the inherent advantages of non-breeding monosex culture populations is that energy is diverted from reproduction to growth. Another is the obvious ecological merit derived from culturing monosex populations should the introduced species escape into the environment. In general, the escape of an introduced aquatic species into natural water bodies carries two inherent dangers: (1) the dissemination of new pathogens and (2) the establishment of an exotic population that impacts negatively on native species and thus reduces natural biodiversity. An example of one such species is the freshwater prawn, *Macrobrachium rosenbergii*

(De Man), which has been introduced, as an aquaculture species into a number of countries.

Advantages of all-male *M. rosenbergii* culture

Growth in culture systems may be affected by a wide variety of factors, including gender, sexual maturity and age (Hartnoll 1982; Botsford 1985; Aiken & Waddy 1992). A number of crustacean species exhibit bimodal growth patterns in which males exhibit superior growth to females or vice versa (Hartnoll 1982). The first attempt at monosex culture of *M. rosenbergii* was carried out in a small-scale, intensive, cage-culture system (Sagi, Ra'anan, Cohen & Wax 1986). An all-male population yielded 473 g m^{-2} within 150 days, whereas all-female and mixed populations produced 248 and 260 g m^{-2} , respectively, in the same grow-out period. In addition to giving higher yields, the prawns of the all-male population reached market size at a faster rate, a factor that prolonged the fresh product marketing period and enabled the now-vacant pond to be used for further production (Sagi *et al.* 1986). Subsequent to this small-scale cage experiment, similar results were obtained when monosex prawn culture was tested under intensive monoculture conditions in earthen ponds (Cohen, Sagi, Ra'anan & Zohar 1988): the all-male stocking gave higher marketable yields, increased average weights, higher calculated income per unit area and shorter times to harvest. When the procedure was tested in polyculture ponds, all-male stocking yielded an increase in 18% in net income, but the authors suggested that the improvement in income was not sufficient to justify the implementation of manual sexing under the economic conditions prevailing in Israel at that time (Hulata *et al.* 1988). It thus became obvious that an efficient biotechnology for producing monosex prawn populations is required if the monosex culture strategy is to be economically viable.

Selective harvesting of *M. rosenbergii*

Fujimura and Okamoto (1972) were the first workers to recommend the periodic selective removal (with a large mesh net) of the larger, faster growing animals as a means of managing the variable growth pattern of *M. rosenbergii* prawns. The periodic selective harvesting of large animals reduced competition for resources and promoted increased growth rates among

the remaining prawns. The importance of selective harvesting is more marked in all-male monosex cultures, since the intervention influences the male social hierarchy, as follows: the largest males in the population, the blue-claw males, are the dominant animals. These dominant prawns, which have terminally moulted, are challenged by the fast-growing orange-claw males. At the bottom of the hierarchy are the small males, which are the slowest growing animals (Ra'anan & Sagi 1985). Selective harvesting of the largest (above the market size threshold) blue-claw and orange-claw animals at 2–3-week intervals may be a suitable biotechnology in countries with warm climates: the removal of the larger prawns enables the smaller males to undergo 'compensatory' growth and occupy the size niche vacated by the large males. It has been shown that this practice increased yields in all-male populations cultured either in cages (Sagi *et al.* 1986) or in earthen ponds (Cohen *et al.* 1988).

Claw ablation of *M. rosenbergii* males

The 'leapfrog' growth pattern of *M. rosenbergii*, suggested by Ra'anan and Cohen (1985), results in a gradual descent of the social rank of blue-claw males. When a new and larger blue-claw male appears in a population, the ranks of all the other blue-claw males in that population will fall. It has been suggested that the deposed males might grow again following claw autotomy leading to a series of moults resulting in increased body mass and claw size, which may in turn improve the social status of the male. Schmalbach, Harpaz, Kahan, Galun and Frankenberg (1984) proposed that voluntary claw autotomy in blue-claw males may take place when the ratio of 1:2.8 of body length to claw length is reached, resulting in a subsequent enhanced cheliped growth. The level of 20 hydroxyecdysone in the haemolymph of *M. rosenbergii* about to perform voluntary claw autotomy was found to be three times higher than that in regularly moulting prawns (Harpaz, Kahan, Moriniere & Porcheron 1987). The above autotomy studies were carried out only on isolated individuals under laboratory conditions. Long-term studies are thus needed to elucidate the dynamic changes in male prawn dominance hierarchies and changes in social status that follow claw autotomy and the subsequent rapid increase in body weight and claw size. It has recently been proposed that if a blue-claw animal has not crossed the harvest size boundary at its final orange-claw to

blue-claw metamorphic moult, claw ablation could be used to improve yields by promoting further moulting and growth until the individual re-establishes a blue-claw rank with a larger size.

The androgenic gland (AG) in crustacea

It has been suggested that in crustaceans, the AG is the sole source of the hormone(s) responsible for sex differentiation, i.e., the commitment of an embryo to either the female or the male pathway (Charniaux-Cotton 1954). In male crustaceans – unlike male vertebrates – the endocrine and gametogenic functions are clearly separated into two distinct organs: the AG and the testis respectively (Ginsburger-Vogel & Charniaux-Cotton 1982; Charniaux-Cotton & Payen 1988). Thus, sex differentiation can be manipulated through the removal of the AG, without damaging the gonads. In *M. rosenbergii*, the AG is composed of strands of cells surrounded by a thin layer of connective tissue, forming a pyramidal cluster loosely associated with the posterior portion of the ejaculatory duct (Veith & Malecha 1983).

To date, the only androgenic substances that have been purified from the AG of decapod crustaceans are lipidic in nature; for example, Veith and Malecha (1983) found that the AG of *M. rosenbergii* stained positive for lipids. Similarly, Berreur-Bonnenfant, Meusy, Ferezou, Devys, Quesneau-Thierry and Barbier (1973) extracted a lipoidal substance from the AG of the crab *Carcinus maenas*. They showed that injection of that substance into a sexually active female *Orchestia* amphipod inhibited vitellogenesis. The active substance was characterized by Ferezou, Barbier and Berreur-Bonnenfant (1978) as farnesylacetone, a compound that is not species specific. Farnesylacetone acts by influencing protein and RNA synthesis in its target organs (Berreur-Bonnenfant & Lawrence 1984). In the light of the findings described above, it is surprising that the ultrastructure of the AG in different crustaceans resembles that of a vertebrate protein-producing cell rather than that of a steroid-producing cell (King 1964).

Recent histological evidence in *M. rosenbergii* supports the idea of a proteinaceous androgenic hormone (Awari & Kiran 1999). Different cell-type populations, which vary significantly among orange-claw, orange-claw to blue-claw and blue-claw morphotypes, exhibit different concentrations of AG total protein content (Sun, Weatherby, Dunlap, Arakaki, Zacarias & Malecha 2000). Sodium dodecyl

sulfate-polyacrylamide gel electrophoresis of AG extracts from the different morphotypes reveals four polypeptides, which increase quantitatively from the sexually immature orange-claw morphotype to the sexually mature blue-claw morphotype (Sun *et al.* 2000). These findings, taken together with certain histological studies in decapods (King 1964; Sagi 1988), have given rise to the belief that a proteinaceous androgenic hormone will eventually be purified from decapod crustaceans.

Further support for the above-mentioned belief comes from findings regarding the role of the AG in sex differentiation in isopods (Legrand 1955; Katakura 1961; Juchault & Legrand 1964). The masculinization effect of the AG on both primary and secondary female characteristics has been thoroughly investigated (Legrand, Juchault, Mocquard & Noulin 1968; Katakura & Hasegawa 1983; Hasegawa, Hirose & Katakura 1993) and purification, identification and full DNA sequencing of the isopod androgenic gland hormone (AGH) have already been performed (Martin, Sorokine, Moniatte, Bulet, Hetru & Van Dorsselaer 1999; Okuno, Hasegawa, Ohira, Katakura & Nagasawa 1999). Complete amino acid sequencing showed a structure that seems to be a pro-AGH in the form of a protein containing three peptide chains, designated A chain, B chain and C peptide. It has been suggested that this complex is the AGH precursor that is analogous with the pro-insulin superfamily peptides (Martin, Sorokine, Moniatte & Van Dorsselaer 1998; Martin *et al.* 1999; Okuno *et al.* 1999).

Role of the AG in sex determination

Charniaux-Cotton (1954) was the first worker to suggest a regulatory role for the AG, showing that bilateral AG ablation in *Orchestia gammarella* blocked the differentiation of secondary male characteristics and decreased spermatogenesis. Tuir (1977) described the effects of the AG on both primary and secondary male characteristics in a number of gonochoristic and hermaphroditic decapod crustaceans. It was recently shown that injection of AG extracts into the crayfish *Procambarus clarkii* affected the appearance of external male characteristics (Taketomi, Murata & Miyawaki 1990). It was also shown that injection of AG extracts into juvenile *Cherax destructor* crayfish that were assumed to be females led to the development of male genital openings (Fowler & Leonard 1999).

A study on maturing *M. rosenbergii* males that had been andrectomized at the youngest developmental stage indicated that these prawns exhibited a high degree of feminization, including initiation of oogenesis and the development of oviducts and female gonopores (Nagamine, Knight, Maggenti & Paxman 1980a). Reimplantation of the AG into the andrectomized prawns reversed the effect of the andrectomy. Androgenic gland implantation into female prawns led to masculinization, as manifested by the development of the appendices masculina, the male gonopore complex, mature masculine chelipeds and the initiation of spermatogenesis in the ovaries (Nagamine *et al.* 1980a; Nagamine, Knight, Maggenti & Paxman 1980b). Males andrectomized in later developmental stages were either partially feminized or not feminized at all (Nagamine *et al.* 1980a). A wide range of abnormalities in gonadal development has been observed in andrectomized males, depending on the age at which the andrectomy was performed. Development of reduced testes was observed in males andrectomized at a relatively old age. In younger andrectomized males, gonads that were partly testicular and partly ovarian ('ovotestes') or abnormally lobulated ovaries developed (Sagi, Snir & Khalaila 1997b). Surgical removal of the AG from juvenile *M. rosenbergii* resulted in complete sex reversal, leading to the development of functional females capable of mating and producing progeny (Sagi *et al.* 1997b). Functional sex reversal of *M. rosenbergii* females by implanting AG into the youngest and smallest identified female prawns has also been reported (Malecha, Nevin, Ha, Barck, Lamadrid-Rose, Masuno & Hedgecock 1992). In both cases, progeny was obtained when fertile sex-reversed animals were crossed with normal prawns, and the sex ratio of the offspring supported the homogametic male theory of Katakura (1989). It has also been demonstrated that AG ablation affects growth rates and morphotypic transformation in *M. rosenbergii* populations (Sagi, Cohen & Milner 1990); for example, orange-claw and blue-claw morphotypes did not develop in males and rectomized at critical stages, the effects being evident in both body length and claw morphology (Kuris, Ra'anan, Sagi & Cohen 1987; Sagi 1988).

In summary, in different crustaceans, the AG exerts well-defined male-specific morphological, physiological and behavioural effects. Many crustaceans exhibit a sexual dimorphic growth pattern, which might be a manifestation of the effects exerted by the AG, as found in *M. rosenbergii* (Sagi *et al.* 1997b). Research and development on a biotechnology for

the control of sex determination or sex reversal in bimodal growth species via intervention in AG activity could play a key role in producing monosex cultures. For such species, the applied merit of study of the AG might equal or even outweigh its basic scientific significance.

Prospects for future research

The mechanism of sex determination has not been studied extensively in crustaceans (Katakura 1989), and only limited cytogenetic data are available for decapods (Legrand, Legrand-Hamelin & Juchault 1987). An understanding of the control pathways of sex determination will facilitate the development of biotechnologies for the culture of monosex populations of crustacean species. Three lines of future aquacultural and biotechnological research and development are proposed:

1. *Monosex culture through manual segregation together with the application of selective harvesting and claw ablation:* Reports on attempts in recent years in several countries to culture *M. rosenbergii* in all-male monosex populations suggest that higher yields, shorter culture times, lower production costs and higher selling prices can be obtained with monosex cultures. All-male populations are obtained by manual segregation of juveniles, an extremely labour-intensive procedure that requires skilful workers. This disadvantage is compounded by the fact that approximately half of the nursed population, i.e., all the females, are discarded. Manual segregation could also be performed at later stages of nursery or during early stages of grow-out. The latter could be easily performed by less skilled workers but has a disadvantage of investing in growing the female fraction for a longer period of time. Despite the disadvantages, manual segregation to produce all-male cultures combined with selective harvesting of the largest males and claw ablation of moult-arrested blue-claw males (Fig. 1, approach no. 1) could produce large prawns that command high prices. It is thus worthwhile to examine specifically different culture strategies on a large scale under a variety of economic conditions.

2. *Microsurgical removal of the AG and sex reversal:* For some decapod crustaceans, studies of sex-determination mechanisms based on karyotype information have suggested the male to be the heterogametic sex, with variant formulae probably being derived from an XY–XX scheme (Ginsburger-Vogel &

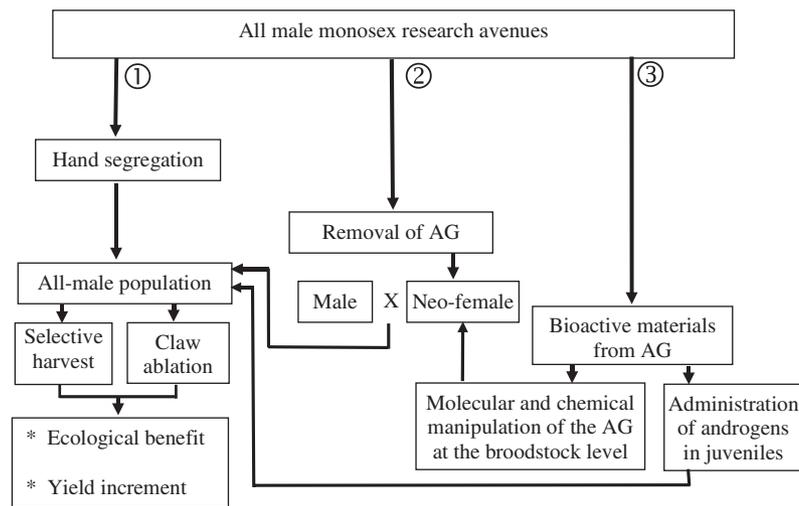


Figure 1 Three research avenues to produce all-male *Macrobrachium rosenbergii* populations. Approach no. 1 – hand segregation combined with selective harvesting and claw ablation; approach no. 2 – removal of the androgenic gland (AG) from juveniles (by microsurgical intervention) to create neo-females, which would be mated with males to produce all-male progeny; approach no. 3 – elucidation of the bioactive materials in the AG to facilitate biochemical or molecular manipulation of sex differentiation. Such materials could be administered to juveniles for sex reversal to produce monosex population. Alternatively silencing the production of these bioactive materials could lead to sex reversal at the broodstock level. Thereafter, the biotechnology would continue with the second approach. The different lengths of arrows 1–3 represent time relativity for the implementation of each approach.

Charniaux-Cotton 1982). In contrast, most controlled breeding studies in crayfish, lobsters, spiny lobsters, shrimps and prawns have suggested a WZ–ZZ scheme, with the male being the homogametic sex (Sagi & Cohen 1990; Malecha *et al.* 1992; Austin & Meewan 1999; Benzie, Kenway & Ballment 2001; Parnes, Khalaila, Hulata & Sagi 2003). In *M. rosenbergii*, AG ablation (by microsurgical intervention) of juveniles at an early developmental stage and age leads to the development of functional neo-females (Sagi & Cohen 1990). Mating neo-females with normal males (Fig. 1, approach no. 2) could result in all-male progeny, a premise supported by the homogametic male theory (Katakura 1989; Sagi & Cohen 1990; Malecha *et al.* 1992).

3. *Elucidation of AG bioactive products*: Unlike the AGH of isopods, the exact nature of the AGH of decapod Crustacea has not yet been identified. Moreover, the morphological, physiological and behavioural effects of the gland observed to date have raised the question of whether a 'one factor show' for all the above-described effects is applicable in higher crustaceans. At present, there is no definitive answer to this question, since the effects of purified AGH on internal sexual characteristics have not yet been determined.

Elucidation of such factors will enable future biochemical or molecular manipulation of sex differentiation in crustaceans. It is possible that administration of such active substances at an early developmental stage could cause masculine sex differentiation and lead to the creation of an all-male population (Fig. 1, approach no. 3). Another possibility that will be opened by the elucidation of active androgens could be chemical ablation or the silencing of genes related to specific sex differentiation stages, which could lead to sex reversal. In such a case, the resulting neo-females could be used as broodstock to create all-male progeny (combining Approach nos. 2 and 3, Fig. 1).

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