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Squeezed from both sides for aqua feeds in Asia

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Monosex culture of prawns

Androgenic gene silencing and a fast growing all maleline

Work conducted by Dr Amir Sagi, professor in the Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel is now available to *Macrobrachium rosenbergii* commercial farms in Asia. Below, Sagi discusses developments in the industry and the role of this novel technology.

Sexual bimodal growth

In many crustacean species, a sexual bimodal growth pattern is exhibited where females grow larger than males of the species or vice versa. In several cultured species such as the Australian red-claw crayfish *Cherax quadricarinatus* and the giant freshwater prawn *M. rosenbergii*, males grow faster and reach higher weights than females. Thus, an all-male monosex population culture of the species is desirable.

M. rosenbergii is native to the tropical Indo-Pacific region of the world and is an economically important crop in China, India, Vietnam and many other Asian countries. It has high demand as a food item and as an export product. The reduction of wild stocks has resulted in a gradual increase in the volume of traditional prawn culture production up to 2009. China, India and Vietnam together produced more than 200,000 tonnes annually valued at USD 2.4 billion (FAO 2009). Bangladesh, Thailand and other countries produced smaller amounts. However a decrease in culture during recent years calls for the introduction of advanced technologies to increase yields.

Monosex culture

Differences between males and females of the same cultured species, in growth rate, alimentary needs and behavioural patterns, dictate the need to establish management procedures specifically adjusted to one sex or the other. Moreover, since a monosex culture population is inherently non-breeding, energy is diverted to growth. Reproduction can be carried out in such systems under separate, controlled conditions. The monosex culture strategy has become a common practice in fish culture and attempts have been made to apply it to crustacean culture.

A small scale experiment conducted as early as 1986 in Israel in hapa nets by hand segregating *M. rosenbergii* monosex populations resulted with significantly higher yields when all-male populations were cultured. More recently (India, 2006), an economic analysis of all-male population culture showed income increase by ~60% over mixed and all-female populations, taking into account the expenses under Indian conditions, caused by labour-intensive hand segregation and related losses (Figure 1).

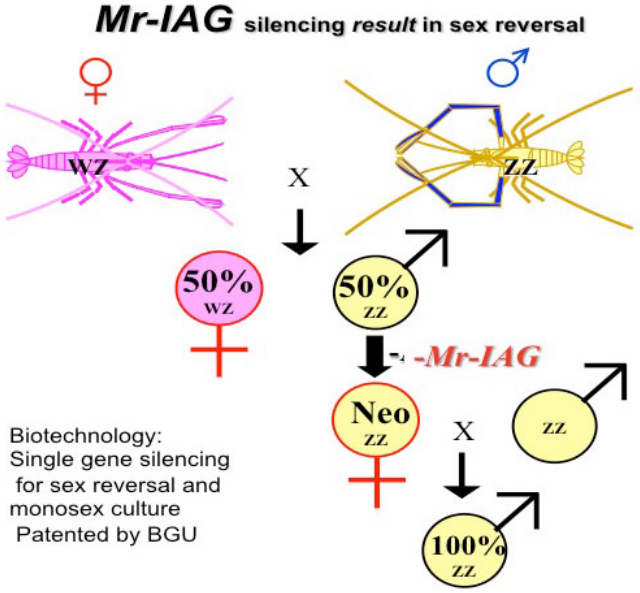
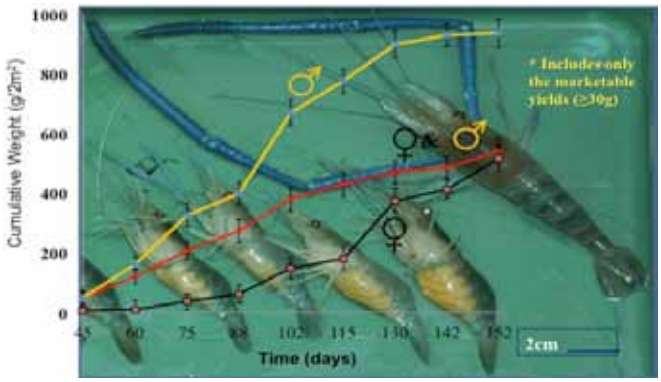


Figure 1. *M.rosenbergii* dimorphic growth in favour of all male culture and selective harvest* (modified from Sagi et al, 1986).



Sex reversal

In *M. rosenbergii* a fully functional sex reversal could be achieved by microsurgical manipulation of the androgenic gland (AG) in early post-larval males. Although AG manipulation dates back half a century, a biotechnological approach for the generation of all-male populations was only recently devised, and it involves the microsurgical removal of the AG from juvenile males. AG removal from immature males results in sex reversal. Since *M. rosenbergii* males are the homogametic sex, bearing two homologous sex chromosomes (ZZ) as in several other studied crayfish and shrimp species, sex-reversed males produce 100% male progeny. Indeed, we found that sex-reversed animals (neo-female) are capable of mating with normal males to produce an all-male offspring population. This population may result in an increase in yield since females at the end of a culture season may weigh 26-45 g, while males may reach up to 100-120 g. This biotechnology has been implemented in Vietnam, Thailand and India.

The novel technology: Gene silencing and the BGU line

The gene silencing technology was developed at the National Institute of Biotechnology at Ben Gurion University of the Negev (BGU). It is based on a recent finding of a new gene encoding an insulin-like androgenic gland hormone from *M. rosenbergii* (termed Mr-IAG and fully patented).

The patent is licensed to Tiran Shipping (1997) Ltd with exclusive, worldwide rights to utilise the IP and to commercialise the licensed products. Silencing of this gene could cause complete sex reversal of a male into a functional neo-female. The technology includes the application of gene silencing in males of the fast growing BGU line of *M. rosenbergii*. Via the use of specific molecular sex markers, the identified males are transformed through a temporal single gene silencing into neo-females.

The neo-females are grown and bred to produce all-male populations (see chart). The technology does not use hormones or chemicals and it is not producing genetically modified prawns. Thus this is a completely non-GMO technology. Because it does not involve genetic modification of the organism, thereby bypassing the regulatory pipeline required of genetically-modified crops, this is a boon for monosex biotechnology. As the intervention is temporal, it is not transmissible to next generations. Indeed, this approach may be of tremendous merit in the aquaculture industry. Moreover, it could also form part of a sustainable solution for the management of invasive and/or pest crustacean species, where the production of non-reproducing male or female populations is sought.

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