Towards Monosex Culture of Prawns (Scampi)

– *As a follow - up to the discovery of an androgenic gland specific insulin-like factor*

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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 two of the most economically important penaeid shrimps, *Litopenaeus vannamei* and *Penaeus monodon*, females grow larger than males (Hartnoll, 1982). On the contrary, in several farmed species such as the Australian red-claw crayfish *Cherax quadricarinatus*, males grow faster and reach higher weights than females (Manor *et al.*, 2002). This is also the case for Scampi, the giant freshwater prawn *Macrobrachium rosenbergii* (Sagi *et al.*, 1986), a species estimated to be farmed at over 200,000 tonnes annually (FAO, 2006). Since males of the latter species reach market size faster than the females, an all-male monosex farming of the species is desirable. In figure 1, there is a male (to the right), which is clearly bigger than the egg berried females from the same population (to the left of the male).

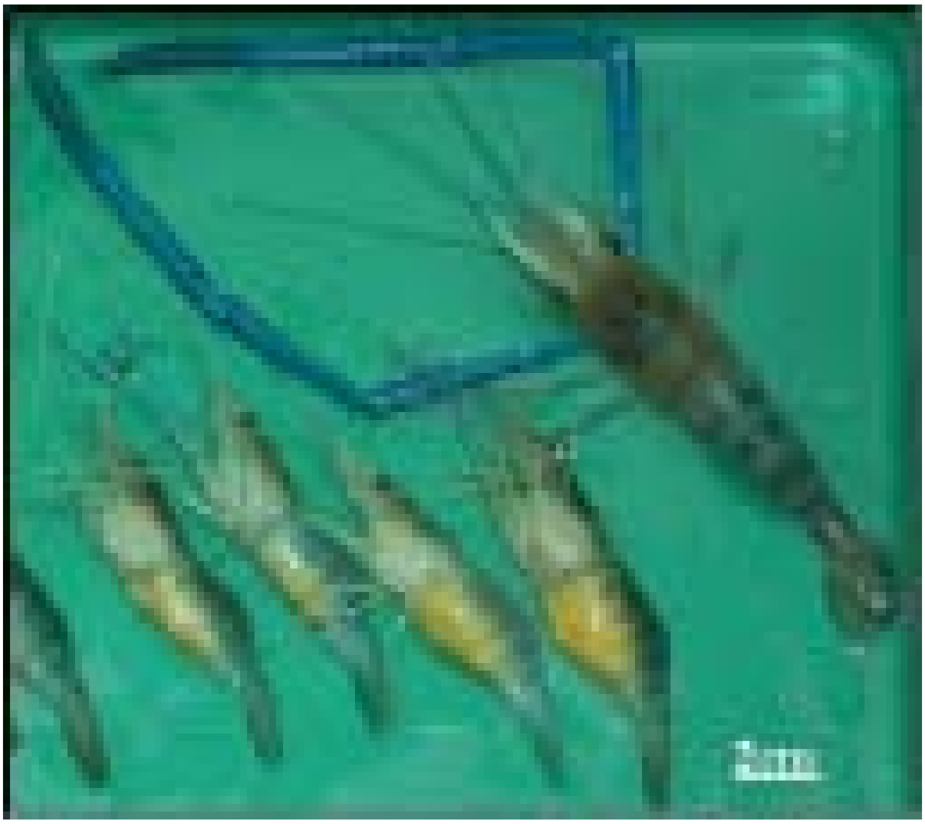

Fig 1: A male (to the right) and several egg berried females from the same population, showing the bimodal growth in favour of the scampi males.

Monosex Farming

Farming of monosex populations is a common procedure in animal husbandry. Differences between males and females of the same farmed 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 farmed population is inherently non-breeding, energy is diverted to growth and unwanted breeding is prevented. The monosex farming strategy has become a common practice in fish farming and attempts have been made to apply it to crustacean farming too. The first experiment was conducted by hand segregation of *M. rosenbergii* monosex populations resulting in significantly higher yields when all-male populations were farmed (Sagi *et al.*, 1986). Two decades later, an economic analysis of hand segregated all-male population farming 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 (Nair *et al.*, 2006).

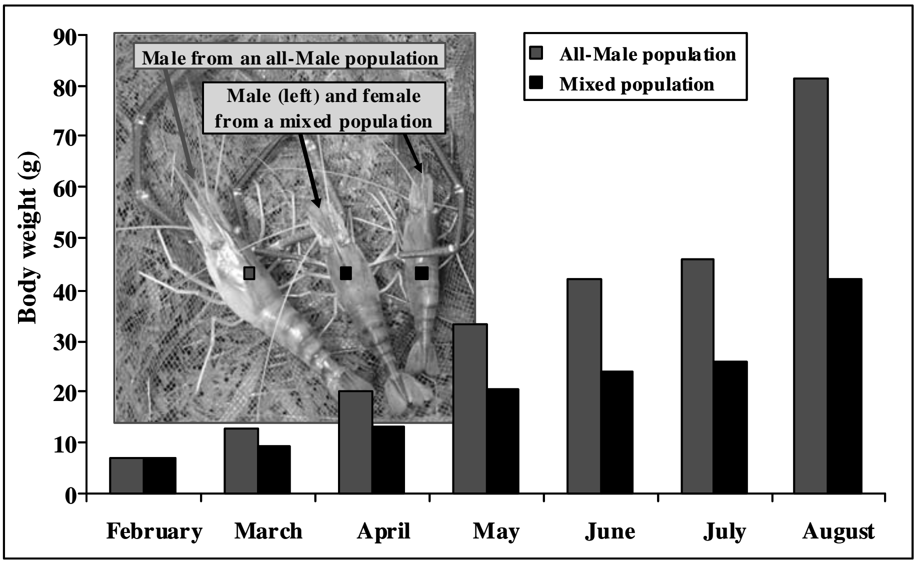

Fig 2: Field experiment in Vietnam (Aflalo *et al.*, 2006), where average weights were recorded monthly, from an all-male population and a mixed population. At the time of harvest, mean weight favoured greatly towards the all-male population. To emphasise this trend, in the background are three individuals from these populations, where the male from the all-male population is visibly much larger compared to male and female individuals from a mixed population.

Field experiment in Vietnam: A field prawn farming experiment was conducted in Vietnam (Aflalo *et al*., 2006) to ascertain the comparative growth levels of a) All male prawn population in one cage concerned,and b) Mixed prawn population (male and female) in another cage. The related data were recorded separately on a monthly basis. As may be seen from the bar graphs in Fig 2, the mean weights of the harvested prawns favoured greatly towards the all-male population. As may also be seen from inset picture in the same Fig 2 printed, above the bar graphs (of February to April), the growth of all-male prawns taken from the related cage is also conspicuously higher (the last one of the three to the left) than the remaining two in the picture (taken from the mixed population in the cage), one being the male and the remaining one to the extreme right, being a female.

The Androgenic Gland

A male specific endocrine gland which was shown to mediate male sexual differentiation in several crustacean species has drawn the attention of researchers interested in devising non-laborious techniques for monosex population farming in crustacean species exhibiting sexual bimodal growth patterns. This gland, known as the androgenic gland (AG), is separated from the gametogenic organ, the testes, enabling a targeted intervention in sex differentiation without physically tampering the gonads (Sagi *et al*., 1997).

Sex Reversal: 2-Phase Procedure

In *M. rosenbergii* a fully functional sex reversal was achieved by microsurgically dissecting out the AGs from early post-larval males. The neo-females (phenotypic females with male genotype) were crossed with males which gave rise to a 100% male progeny (Sagi and Cohen, 1990). However, the elaborated technique for AG ablation requires a highly skilled staff and the success rates, which are based on the identification of males at a very young stage, are low. To overcome the latter drawbacks, a two phase scheme for the production of all-male population was devised where the progeny of successfully reversed males (100% males) served for microsurgical removal of the AG at an early stage without the need to identify males (Figure 3, Aflalo *et al.*, 2006). This two phase procedure increased the success rates and, although it involved a complicated microsurgical operation and time consuming progeny testing, proved

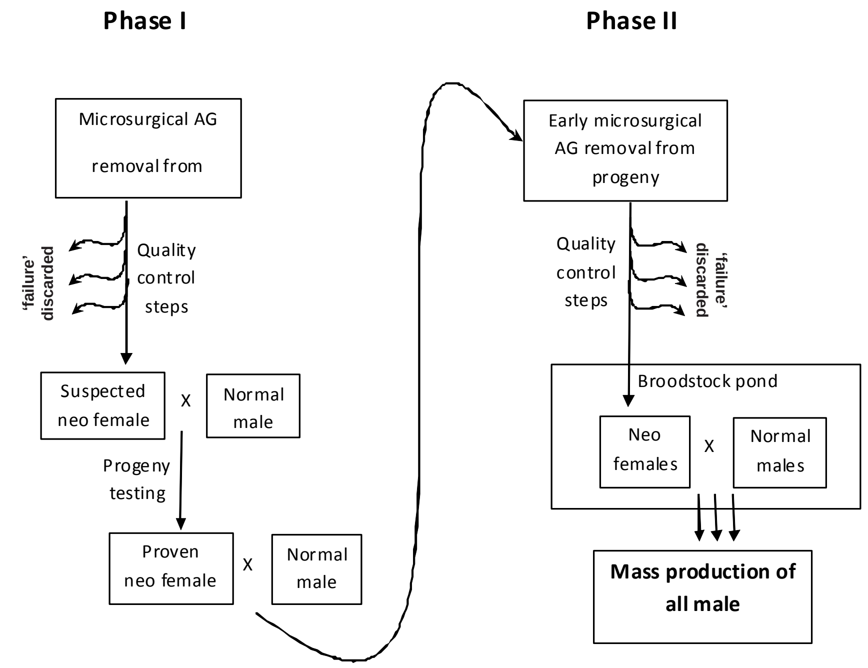

Fig 3: Schematic representation of a novel two-phase procedure for mass production of all-male *M. rosenbergii* populations. (Aflalo *et al.*, 2006).

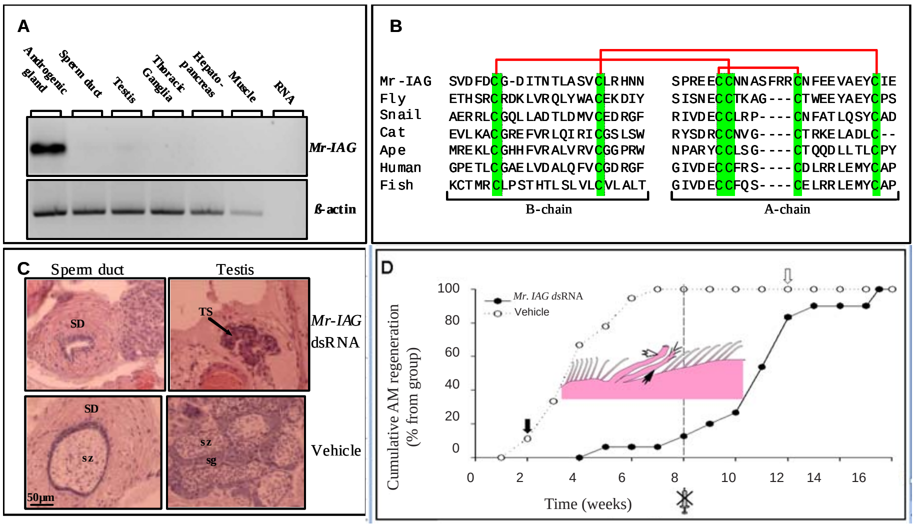

Fig 4: Identification and characterisation of *M. rosenbergii* insulin-like AG (Mr IAG) gene. (A) RT-PCR (reverse transcriptase-polymerase chain reaction) showing expression of *Mr IAG* only in the AG. *â-actin* is used as positive control. RNA is used as negative control. (B) Multiple sequence alignment of the putative mature Mr-IAG peptide with other insulin-like peptides (Based on SMART results and done by ClustalW). Cysteine residues are marked by a green colour. Conserved predicted disulfide bridges are linked (red line). (C) Temporal silencing of *Mr-IAG* using dsRNA injections inhibits testicular spermatogenesis. In the silenced individual there is an empty sperm duct (SD) as oppose to the spermatozoa-filled sperm duct in the control individual. In the silenced individual there are inactive testis lobules (TS) as oppose to the active testis lobules in the control individual, containing both spermatogonia (sg) and spermatozoa (sz). (D) Temporal silencing of *Mr-IAG* using dsRNA injections inhibits regeneration of male secondary characteristic – the *appendix masculina* (AM). At the start of the injections all individuals had one AM removed. By the end of the repeated injections period (8th week), all of the vehicle injected individuals regenerated their AM as oppose to most of the silenced individuals, which did not. This inhibition in AM regeneration was reversible as all silenced individuals regenerated their AM by the 15th week. The end of the repeated-injection period is marked as - (8th week). In pink – an illustrated second pleopod with appendix interna (hollow arrowhead) and appendix masculina (black arrowhead; Source: Ventura *et al*., 2009).

that all male population production to be more feasible (Aflalo *et al.*, 2006) and it is practised at present in the Mekong delta in Vietnam. The increase in yield at harvest is clearly demonstrated in figure 2. However, a more elegant and easy to use technique is still needed to replace the complicated and time consuming microsurgical AG ablation and to enable the scale-up of monosex farming of the species throughout the world.

Androgenic Gland Insulin-like factor

Based on recent findings in a crayfish (Manor *et al.*, 2007), researchers tried to find specific AG genes responsible for male sexual differentiation in *M. rosenbergii*. A subtractive cDNA library of *M. rosenbergii*'s AG has been established from which, among other genes, an AG-specific gene, expressed exclusively in males was found. This gene was termed *Mr-IAG* (insulin-like AG factor from *M. rosenbergii*, Accession #FJ409645, Figure 4A) since its deduced protein sequence contains Cysteine residues and putative cleaved peptide patterns whose linear organisation is similar to those of members of the insulin/insulin-like growth factor/relaxin family (Figure 4B). The function of this gene was elucidated via gene-silencing experiments which indicated that the gene affects spermatogenesis, the development of external male specific sex characters (such as *appendix masculina*) and also growth patterns (Figure 4C, D; Ventura *et al*., 2009).

The gene was shown to be involved in controlling growth and male sexual differentiation, However further research is required to harness this finding to the management of size variation in *M. rosenbergii*. A complete functional sex reversal from males to neo-females might be achievable by manipulating genes such as *Mr IAG* and other AG specific genes and proteins at earlier stages, thereby bearing the potential for the establishment of all-male populations in crustaceans. The advantage of procedures involving temporal gene silencing lies in the fact that a genetically modified organism is not produced, which is restricted in several markets throughout the world.

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The hatchlings arising out of the fertilised eggs released by neo-females would all grow into male seed, to be stocked in ponds.

All male monosex populations could be beneficial not only directly for yield increase in aquafarming, but also for restocking endangered and over-fished species, population control of invasive crustacean species as well as for sustainable aquafarming where it might reduce leakage of genetically selected lines into the natural ecosystem.

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Synthetic artemia shows promise

A highly nutritional feed which can replace artemia completely as a major feed source in shrimp hatcheries has been developed by Ziegler. According to Tim Ziegler, Vice President of Sales at Ziegler, the product comes at a critical time when economic conditions are forcing the industry to look for more cost-effective solutions.

Neil Gervais, Hatchery Product Manager for Ziegler and the main driving force in the development of the new product, reports that feedback from testing labs had shown that overall survival rates have increased up to 30% with the new feed. Time to reach the PL-10 stage was also shortened by 2-3 days. Tanks using 100% of the new feed consistently outperformed control tanks which used natural artemia from cysts, in terms of survival, growth and staging, he said.

Preliminary economic modeling suggests that hatcheries will be able to reduce direct feeding costs by US$50 – 100 and total production costs by US$100 – 200 per million PLs produced. Furthermore, production managers will now have access to a more predictable and bio-secure supply of larval feed. ☯☯☯

Half of all fish eaten comes from farms

According to a new report by an international team of researchers, 50% of all the fish eaten globally comes from aquaculture. The report also concludes that, while the industry is becoming more efficient on marine resources by consuming large amounts of feed made from wild fish harvested from the sea. The team's findings are published online in the Proceeding of the National Academy of Sciences.

The study noted that global production of farmed fish nearly tripled in quantity between 1995 and 2007, partly because of increasing consumer demand for long-chain omega-3 fatty acids. To maximize growth and enhance of fishmeal and fish oil made from less valuable wild-caught species, including anchoveta and sardine. As a consequence, aquaculture's consumption of fishmeal and fish oil more than doubled over the past decade to 68 percent and 88 percent respectively, observed the study. According to the lead author of the study Rosamund Naylor, in 2006, aquaculture production was 51.7 million harvested for fishmeal production. It can take up to 5 lb of wild fish to produce one lb of salmon.

One way to make salmon farming more environmentally sustainable, say the authors, is simply to lower the quantity of fish oil in the salmon diet rather than fishmeal. If fish oil is reduced by a mere 4 percent, they argue, the amount of wild fish needed to produce one lb of salmon can be reduced from 5 lb to just 3.9 percent would have very little environmental impact. Naylor and co-authors also point to several fish feed substitutes which are currently being investigated and which could take the pressure off wild fish in the future, including protein made from grain and livestock byproducts and long-cell microorganisms and genetically modified land plants ☯