Monosex Prawns: Exploiting Androgenic Gland Function for Sex Reversal: Case Study 9

Research goal	Novel bio-technologies for monosex culture of prawns
Beneficiaries	Seafood companies and farmers
Activities conducted in order to achieve the objectives	Discovery of insulin-like androgenic hormones (IAGs) in decapod crustaceans. The first implementation of RNA-interference in aquaculture. Generation of genes transcriptomic and genomic libraries for IAGs.
Funding	2 BARD awards: QB-9308-06 and IS-4493-12: $0.455 million. Industry funds: $1.02 million
Publications	12 peer-reviewed journal publications and 1 book chapter
Research graduates involved	Six graduate students and one post-doctoral researcher. Current positions: 4 in academia of which 3 are in Israel and 1 in Australia, 3 are in the private sector of which 2 are in biotech industries (algae and pharmaceutical) and 1 is a patent attorney
Stakeholders' collaboration	Tiran shipping promoted R&D Collaborative agreements with Enzootic
Environmental impact	Use of monosex prawns as biocontrol agents against fish pathogens
Social impact	Use of monosex prawns as biocontrol agents in African rivers against freshwater snails that transfer bilharzia
Commercial engagement	License agreement with Tiran; Establishment of startup company - Enzootic
Patents	1 patent to BGU, 2 patent applications with Enzootic
Practical agricultural applications	According to Tiran's management, the all-male technology is applied in 3.5% of the global production with fast growth anticipation. Enzootics sales of the all-female technology started in 2017/18.
Economic impact	Net present value of BARD's investment is $38 million, thereof $4 already attained million. The Internal rate of return is 21%. Benefit cost ratio is 51, thereof 6 already attained.

Green- Academic information; Yellow - Social and environmental information; Blue - Economic information

1 Research Objective: Monosex Crustacean Aquaculture

The research objective was to develop a monosex culture for crustacean aquaculture, specifically for the commercially important giant freshwater prawn, *Macrobrachium rosenbergii*, and to improve production efficiencies.

2 Research Activities

In crustaceans, sexual differentiation is controlled by the androgenic gland (AG), an organ unique to males. Amir Sagi researched the prawn's sexual plasticity during his doctoral studies and showed that when neo-females (phenotypic females with male genotype) were crossed with males, the progeny was 100% male[1]. A. Sagi continued to post-doctoral research on crustacean endocrinology at Woods Hole Mass. with the support of a BARD post-doctoral fellowship (1989-1991).

Two BARD funds, QB-9308-06 and IS-4493-12 were awarded to A. Sagi (Ben-Gurion University), A. Elizur, C. Jones, W. Knibb (Dept. of Agriculture and Fisheries. Queensland), G. Hulata (Agr. Res. Org), S. Du and Y. Zohar (U. Maryland). See Appendix A for full details.

The research focused on identifying and characterizing the transcript encoding the insulin-like androgenic gland specific factor in decapod crustaceans; prawns, shrimps, crabs, lobsters and crayfish. Research's milestones:

- Use of the then new molecular approach to construct for the first time in decapods cDNA libraries of the insulin-like AG gene, termed IAG, in males.
- RNA interference was implemented for gene silencing for the first time in aquaculture.
- Additional AG-specific transcripts co-expressed with IAG or related to the insulin signaling pathway were discovered.
- The studies provided the first evidence that silencing an insulin-like gene leads to full sex reversal of males into ‘neo-females’

[1] 1) A. Sagi, D. Cohen, Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males, World Aquaculture 21 (1990) 87–90.; 2) A. Sagi, D. Cohen, Y. Milner, Effect of androgenic gland ablation on morphotypic differentiation and sexual characteristics of male freshwater prawns, *M. rosenbergii*, General and Comparative Endocrinology 77 (1990) 15–22.3) A small scale experiment in 1986 in Israel in hand segregated *M. rosenbergii* and the monosex populations
resulted with significantly higher yields when all-male populations were cultured

3 Academic Impact

3.1 Publications

12 peer-reviewed journal publications and 1 book chapter were published based on research from the 2 BARD awards.

3.2 Capacity Building

Seven post-graduate students were involved in the research projects in Israel. Currently, three of them have academic positions in Israel and one in Australia, two are in algae and pharmaceutical industries, and one is a patent attorney.

4 Stakeholder collaboration

The identification of the transcripts laid the foundation for identification of the IAG gene in around 20 additional decapods by other research groups.[2]

The Israel fish breeding association and Emek Hamaayanot regional R&D are supporting an ongoing follow-up project of A. Sagi that aims to utilize an all-female river prawn population for biocontrol of fish parasites.

5 Patents

RNA silencing of insulin-like gene in prawn and uses thereof, A. Sagi and T. Ventura, US20110010784A1, Granted 9-6-2015 to Ben Gurion University

Two patent applications on all-female are pending:

Functional sex-reversal of decapod crustacean females, A. Shechter, Ohad Rosen and Amir Sagi, EP3217785A4, Application: 2015-11-13 by Enzootic Holdings Ltd

A ww homogametic male decapod crustacean and methods of using the same, A. Shechter, Ohad Rosen and Amir Sagi, US20180317467A1, Application 2016-11-10 by Enzootic Holdings Ltd.

[2] Chung et al., (2011) Gen. and Comp. Endocrinology (173), Sroyraya et.al., (2010) Tissue Cell. (45). doi: 10.1016/j.tice.2010.07.003.
Levy T et al., (2017). PLoS ONE 12(12): e0189982. https://doi.org/10.1371/journal.pone.0189982
Ma et al., (2013) Gen. and Comp. Endocr. (185) https://doi.org/10.1016/j.ygcen.2013.01.018
Mareddy et al., (2011) Aquaculture, (318) https://doi.org/10.1016/j.aquaculture.2011.05.027, Banzai et al, (2011), Fisheries Science (77) doi: 10.1007/s12562-011-0337-8
Ventura et al., (2015) Gen. and Comp. Endocr. (214)

6 Commercial Engagement

The BARD research was the foundation for two separate engagement outcomes; a) all-male culture and b) all-female culture.

6.1 Tiran Aquaculture: All-Male Culture

In 2010, the IP for generation of neo-females was licensed to Tiran Aquaculture, who concurrently invested $1 million in A. Sagi's group for scale up and optimization of the all-male *M. rosenbergii* production. Tiran pays royalties to BGU from sales of products based on the IP.

6.2 Enzootic – All-Female Culture

Ben Gurion University (BGU) has engaged with the agro-biotech company "Enzootic", established in 2012, who develop all-female culture of *M. rosenbergii*. *A.* Sagi and A. Shechter, his former Ph.D student, co-founded Enzootic.

BGU provides research services to the company and its students conduct research using the infrastructure at Enzootic. Additionally, Enzootic partnered with NRGene who conducted the sequencing of the genome of *M. rosenbergii*, which is available for free to BGU researchers. Enzootics employs ~15 staff members from the Beer-Sheva vicinity and is in preliminary stages of setting up a commercial pilot facility for intensive aquaculture together with the Besor R&D station.

7 Practical Aquaculture Applications

For *M. rosenbergii*, a fraction of the males are much larger than females and all-male culture has been shown to improve production efficiencies and increase total production by 25% - 40% when compared to mixed culture ponds[3,4]. However, large dominant males are territorial and inhibit the growth performance of smaller males. This has motivated the development of all-female cultures in which the end-size of the females is smaller than the males: an average of 30 gr *vs* 75 gr, but stocking densities are greater: 15 females per m^2 *vs*. 4 males per m^2, and a higher size uniformity is attained. The grow-out cycle of female is decreased, 120 days *vs* 180 days for mixed cultures, allowing 2.5 - 3 growth cycles a year.

Advantageous to both approaches is that monosex culture is inherently nonbreeding, such that energy is diverted to growth and unwanted breeding is prevented, and that makes Food Conversion Ratio (FCR) more efficient. The production of "neo-females" and "Super-females" is conducted in Israel and shipped to markets in the far east. Vietnam, China, Thailand, Malaysia, Cambodia and Indonesia. Both products (all-male and all-female

[3] Aquaculture 256 (2006) 468–478
[4] Aquaculture Research, 2006, 37, 949 - 954 doi:10.1111/j.1365-2109.2006.01521.x

breeding stocks) are commercially in demand and are being adopted as the prawn aquaculture management system by distinct growers.

8 Social Impact

A. Sagi has led and been involved in a number of research projects with regard to the use of all-male prawns as a biocontrol agent for snails, which host a parasitic worm that causes Schistosomiasis (Bilharzia). Trials in Senegal, together with multiple international researchers, showed that re-introducing the African prawn to river sites led to reduced disease burden. The statistics could be improved greatly were the prawn population to be all-male and hence larger and more voracious predators. As the prawn cannot reproduce, non-local species can be introduced with no concern of the entrance of an invasive species. Follow up studies are being conducted also in Nigeria together with Tiran.

9 Economic Impact

9.1 Investment Cost

The economic evaluation is assessed separately for the two project outcomes. For the all-male production, BARD contributed $485,000 in research funds between 2006 to 2015, which is $580,000 in 2018 dollar-terms, including an initial grant of $40,000 to support the post-doctoral studies of A. Sagi between 1989-1991. The IP for all male progeny was licensed to Tiran Aquaculture, who invested $1 million between 2010-2013 to further R&D for the all-male *M. rosenbergii* production. In 2018 dollar-terms, the Tiran investment contributed $1.35 million.

Later, between 2013-2019, Enzootic received investments of $1.4 million from the Israel Innovation Authority and raised private funds of $6.5 million for the all-female project.

9.2 The Benefits of Monosex Culture

A study was conducted in 2006 in India, to compare the economics of all-male, mixed and all-female cultures, segregated manually[5]. The finding showed that in an extensive production system, all-male culture produced 1,532 kg/ha, 27% higher than mixed culture that reached 1,210 kg/ha. The study showed that almost all expenses are the same per pond, except of feed and seed cost. They found the FCR in the all-male culture to be more efficient by 20% compared to the mix culture.

Better FCRs were also found in a 2017 study conducted in Israel[6]: 20% improved FCR for all-female culture compared to mixed culture in a semi-intensive production system, and a 10% improvement in an extensive production system. The 2017 study found that an all-

[5]Aquaculture Research, 2006, 37, 949 – 954, doi:10.1111/j.1365-2109.2006.01521.x
[6] Aquaculture, 2017, 479, 857-862, doi.org/10.1016/j.aquaculture.2017.07.039

female extensive system produces 11% more kg/ha compared to a mixed culture, a similar result to the 2006 study. In a semi-intensive system, productivity per hectare was 22% higher for all-female culture when compared to a mixed culture.

These results indicate that an all-male culture yields benefits when used in extensive farming systems, and apparently, is also of benefit in semi-intensive systems. The all-female culture would be more profitable when applied to semi-intensive and intensive production, due to the ability to stock all females at higher densities and the shortening of the harvest time. In order to calculate the benefit of the all-male culture, we use the net benefit calculated in the 2006 study, \$0.98/kg for all-male production, compared to \$0.6/kg for mixed production. This equals a benefit of \$0.38/kg.

The evaluated benefit is corroborated by post-larvae prices. According to Tiran's management, the hatcheries sell an all-male post larva for 2-3 cents in Vietnam, which is 2 to 3 times the cost of regular post larvae[7]. This leads to a 1.5 cents/kg higher price. An all-male population will have a mean of 16 individuals per 1 kg produce[6], indicating that farmers are paying, on average, an additional 24 cents/kg when using the all-male culture. This is reasonable if the benefit is 38 cents/kg. We added to this premium 33%, which is the retail + wholesale share in the end-consumer prices of fish products. Thus, the benefit is calculated \$49 cents/kg (=38/0.77).

9.3 Global Production and the All-Male Culture Benefit

Global production of *M. rosenbergii* reached 234,000 tons in 2016, and during the last years it has been increasing annually by a rate of 3%[8]. The FAO predicts that the global aquaculture production annual growth rate will decrease from 5.7% for the years 2003–2016 to 2.1% between 2017–2030[9] and hence we assume a market growth of 2.1% for *M. rosenbergii* between 2017 – 2028.

According to Tiran's management, commercial sales began in 2014 and gradually increased to sales in 2018 of larvae accountable for the production of around 8,000 tons *M. rosenbergii*. Sales have commenced in China and are growing. Tiran expect to double their sales in 2019, and anticipate fast growth in the upcoming years.

For the purpose of a conservative calculation, we assume 10% annual growth of all-male sales between the years 2019 – 2028. This assumption leads to the company being

[7] Some prices for comparison from Giant prawn 2017 meetup, http://www.shrimpnews.com/FreeReportsFolder/FreshwaterPrawnFolder/AsiaTheRevivalOfFreshwaterPrawnFarming.html

[8] http://www.fao.org/3/i9540en/I9540EN.pdf

[9] http://www.fao.org/3/i9540en/I9540EN.pdf

responsible for production of 21,000 tons or 7% of the global production in 2028. We do not calculate any benefit after 2028.

9.4 All-Female Culture Benefit

Enzootic estimates that in 2018 it held ~ 1% of the global *M. rosenbergii* market and anticipates a 3% share by 2019. The all-female system targets intensive and super-intensive production systems, which are a developing global sector. Enzootic is establishing a pilot facility for intensive aquaculture of *M. rosenbergii* with the vision of providing such facilities to the US and Western markets. Enzootic intends to extend the technology to additional species such as the Pacific white shrimp *(Penaeus vannamei)* and the giant tiger prawn (*Penaeus monodon)*. For the all-female system, although clearly of economic impact, we do not have the ability to translate the market penetration of intensive systems to economic terms, and thus do not include this enterprise in the benefit calculation.

9.5 Economic Results

BARD invested in the initial and hence risky part of the project. According to the calculation described in the methodology section we attribute 52% of the benefit to BARD.

- Net present value of the BARD's investment is $38 million, thereof $4 million already attained.
- The Internal rate of return is 21%
- Benefit cost ratio is 51, thereof 6 already attained.

The US and Israel are importers of crustaceans. The US imports more than 600,000 ton annually but we do not know what fraction of this is *M. rosenbergii*. We assume that the share in the benefit to Israel is 1% and to the US 2%.

Benefits that were not attributed to the project in this calculation:

- Environmental impact and social impact, as detailed above, were not included in the benefit calculation
- It seems very convincing plausible that monosex female prawns' sector will have an economic success. We did not include it in the evaluation since sales have not yet begun.
- We did not include the benefit for the Israeli economy attributed to a new high-tech company which emerged from this project.

Table 1: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Other Countries
BARD's Share in the Cost	37%					
Share in the Benefit		52%				
Cost	2	1	1	0.4	0.4	
Benefit	75	39	4			
Net Present Value	73	38	4	0	0	38
Internal Rate of Return	23%	21%	14%	7%	3%	
Benefit Cost Ratio	36	51	6	1	0	

9.6 Sensitivity Analysis

The low and high alternative assumptions used in the sensitivity analysis were brought together to estimate results under pessimistic and optimistic scenarios. Table 2 displays the net present value sensitivity results, between the low result: $15 million, to the high result: $69 million.

Table 2: NPV - Sensitivity Analysis, 2018 Million Dollar-Terms

			BARD's Share in the Benefit		
			Low	Central	High
			42%	52%	62%
Change in Benefit	Low	50%	15	19	23
	Central	100%	31	38	46
	High	150%	47	58	69

10 Appendix A: BARD Awards

Table 3: List of BARD awards granted between 2006 - 2015

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
QB-9308-06	Improving Crustacean Aquaculture Production Efficiencies through Development of Monosex Populations Using Endocrine and Molecular Manipulations				
	Elizur, A. Sagi, A. Hulata, G. Jones, C. Knibb, W.	Department of Agriculture and Fisheries. Queensland, DPI&F Ben Gurion U ARO, Min. Ag. DPI&F DPI&F	$145,000	3 years	2006
IS-4493-12	Delivery of gene silencing agents for improving aquaculture production efficiencies				
	Sagi, A. Du, J.S-J Zohar, Y	Ben Gurion U U MD, Baltimore UMBC	$300,000	3 years	20012

11 Appendix B: Information providers: Personal communication

- Amir Sagi - PI and Co-PI for BARD grants, Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev.
- Eyal Avioz - Tiran Group, Tiran Aquaculture.
- Assaf Shechter - Founder & CEO, Enzootic Ltd.