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Full Life-Cycle Hatchery-Based Aquaculture
Case Study 7

Research goal	To artificially induce and control reproductive maturation and spawning in farmed fish, such as Sea Bream, Stripe Bass, Sea Bass, Grouper, Salmon and many others, that suffer from sexual dysfunction when raised in captivity.
Beneficiaries	Aquaculture industries and consumers.
Activities conducted in order to achieve the objectives	Investigation of Gonadotropin-releasing hormone (GnRH) regulation and receptor affinity and additional neuropeptides involved in sexual maturation, ovulation and spawning. Optimization of sustained release delivery systems for GnRH analogs.
Funding	2 BARD awards between 1985- 2002 $452,000 Other academic funds: $45,000
Publications	5 book chapters and 32 publications
Students involved	3 graduate students. Current position: Two in academia, of which 1 in Israel and 1 in Greece.
Stakeholders' collaboration	Fields tests in cooperation with commercial broodstock farms (Domsea Broodstock Inc., Riverence, Maritime Culture, and Iceland Salmon Ltd, Iceland, Kibbutz Ma'agan Michael, Ha'Maapil) Distribution of the GnRHa implants to tens if not hundreds of hatcheries and academic groups worldwide.
Environmental impact	Conservation of overexploited marine species. Applied tool for gene rescue and population amplification of threatened and endangered species.
Social impact	Generation of local US European sea bass and sea bream market that are currently imported to the U.S. market
Commercial engagement	No official commercialization although widespread application in commercial facilities worldwide.
Patents	3 patents.
Practical agricultural applications	The developed polymer-based GnRHa delivery systems have provided the global aquaculture industry with a tool to induce fish to spawn in captivity. The technique is used in fish hatcheries around the world to induce spawning and egg/juvenile production in scores of fish species (Sea Bream, Sea Bass, Salmon and recently Blue Fin Tuna).
Economic impact	Net present value of BARD's investment is $12 billion, thereof $7.5 billion already attained. The Internal rate of return is 143%. Benefit cost ratio is 5,800, thereof 3,600 already attained.

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

1 Objective: Techniques to Induce Fish Reproduction in Captivity

Fish in captivity typically demonstrate reproductive dysfunction due to stress factors in their non-wild environment. Therefore, they do not spawn spontaneously in captivity and their eggs and juveniles ("seeds") cannot be produced. The research aimed to establish tools to control reproductive processes and their timing in captivity, and to acquire high-quality gametes, i.e. eggs and sperm, fertilized eggs and larvae.

2 Research Activities

Between 1985 and 2002 two BARD awards were granted to Yonathan Zohar (then Isr. Ocean Res., now at U of Maryland) in collaboration with Yitzhak Koch (Weizmann Inst), Martin P. Schreibman (Brooklyn College), Robert S. Langer (MIT), W.W. Dickhoff (Washington U) and additional researchers from the Weizmann Institute and the Israel Ocean Research Center.

These 2 awards were the first of 8 BARD awards with Y. Zohar as PI with many US and Israeli collaborators that studied fish reproductive biology and endocrinology in the wild and in captivity. See Appendix A for full details of the awards.

The first BARD study (IS-772-85) researched the bioactivity of the Hypothalamic Gonadotropin Releasing Hormone (GnRH1), the key hormone in the control of gametogenesis (egg and sperm generation), gonadal growth and spawning in fish. Ovulation and spawning of female Sea Bream and many other aquaculture important fish are impaired in captivity and the research showed that this lack of reproduction in captivity is the result of a failure of the brain to produce/release the main reproductive hormone called GnRH. The researchers then injected different known GnRH forms to the fish in order to induce ovulation and spawning but found that native GnRHs of different origins (fish or mammals) undergo rapid degradation by enzymes present in different tissues and therefore are rendered inactive. The researchers identified the cleavage sites, and studied the enzymatic pathways and kinetics of the degradation. They then designed and synthesized GnRH analogs that were more resistant to degradation and had high affinity for GnRH binding sites. These longer-lived and highly bioactive synthetic GnRH analogs were shown to induce oocyte maturation, ovulation and successful spawning in farmed fish. Through in-vitro and in-vivo bioactivity and dose response studies, the optimal analogs were established.

The effects of a single administration of even the potent GnRH analogs can still be too short-lived, and the follow-up BARD study (IS-1468-89) focused on development of delivery systems that would enable sustained release of GnRH and long-term consistent spawning. These two studies were conducted on Sea-Bream (*Sparus aurata*), Sea Bass *(Dicentrarchus labrax)* and various Salmon species.

The research outcomes from these two first awards have had an immense impact on marine aquaculture, as detailed in Section 5, and are the focus of this evaluation. We acknowledge that Y. Zohar and collaborators received 6 additional BARD awards focusing on GnRH and farmed fish reproduction.

- Two BARD awards (IS-2149-93 and IS-2634-96C) focused on the late maturing (6-7 years) freshwater black carp and investigated the endocrine elements along the primary tissues (hypothalamus, pituitary gland, and gonads) involved in the hormonal cascade leading to reproduction. This laid the foundation to manipulate and advance puberty in captive-fish.
- Two BARD awards (IS-3214-01F and IS-3428-03C) in which the researchers created a novel approach for inducing reproductive sterility in farmed fish, which has economic and environmental benefits. Researchers used GABA receptor ligands to alter the early development of the GnRH neuronal system.
- Two BARD awards (MB-8719-08 and IS-4499-12R) in which the researchers investigated the role of additional neuropeptides (NKB and NKF) in the regulation of GnRH neurons and sexual maturation in fish.

3 Academic Impact

3.1 Publications

5 book chapters and 32 peer-reviewed publications on GnRH bioactivity and cleavage and on the polymer-based GnRHa delivery technology emerged from the 2 studies and were published between 1989- 2016.

3.2 Capacity Building

3 graduate students were involved in the BARD supported research. Currently, two have positions in academia, of which 1 is in Israel and 1 is in Greece.

4 Stakeholder's Collaboration

Some of the US and Israeli industry collaborators involved in the field trials are listed below. Many more industry collaborators worldwide, in Chile, Mexico, Greece, Spain, Australia, France, Norway, Japan, Iceland as well as other countries, were also involved in the field trials.

- US: Domsea Broodstock Inc and Riverence, LCC, Washington; Mariculture Products Ltd., Maine,
- Israel: Madan Hatchery, Kibbutz Ma’agan Michael, Fish Hatchery, Kibbutz Ha’Maapil. Latimeria LTD.

The group of Y. Zohar at the University of Maryland regularly provide thousands of GnRHa implants to research groups around the world, including groups in the US, Canada, Mexico, several European countries, Japan, Australia, New Zealand, Tasmania, Chile, Ecuador, Brazil, Argentina as well as others.

Y. Zohar has had long-standing collaborations with his Ph.D. student of the second BARD award, Constantinos C. Mylonas, who is now director at the Hellenic Centre for Marine Research, Crete. The two have continued research together on the productive endocrinology of various cultured fish including application of GnRH1 delivery systems for Atlantic salmon and Atlantic Bluefin Tuna.

Efficient and competitive production of farmed sea bream and seabass was enabled by planned broodstock management programs that were researched and practiced at the National Center for Mariculture (NCM), Eilat. The research on induction of spawning was led by Y. Zohar, then affiliated with the Institute and in parallel, research led by Amos Tandler focused on increasing the survival rate of fertilized eggs and young larvae. The complementary research projects led to the development of spawning and rearing protocols for various marine commercial fish species. The collaboration with NCM continues to this day with joint studies on broodstock management of the Atlantic Bluefin Tuna.

5 Commercial Engagement

The widespread implementation of the GnRH analogs and delivery systems has never been officially commercialized by the involved researchers.

Implants provided to industry groups are sold through the University of Maryland to cover their production costs. Amongst others, implants are sold to industry in Australia where they are used for bluefin tuna and for kingfish, and to industry in Japan for their local salmon species.

Following the newer implementation of the delivery system for Atlantic Blue Fin tuna, the commercial company, TUNAsource, was founded by Y. Zohar in partnership with Kali Tuna, a Croatian tuna farming company. TUNAsource supplies Bluefin tuna eggs to industrial and academic groups around the world. These eggs are produced by broodstock tuna farmed off the coast of Croatia and induced to spawn using the GnRHa implants.

5.1 Patents

1. *Manipulation of ovulation and spawning in fish.* Yonathan Zohar, US5288705A, Granted 22-02-94 to Israel Oceanographic and Limnological Research Ltd
2. *Compounds comprising gonadotropin releasing hormone (GnRH) and methods for controlling reproduction in fish.* Yonathan Zohar, Nancy M. Sherwood, Jean Rivier, Jim Powell, Yoav Gothilf, US5643877A. Granted 01-07-1997 to Victoria

University of Innovation and Development Corp., University of Maryland Biotechnology Institute (UMBI), Salk Institute for Biological Studies.

3. *Inducing sterility in fish by disrupting the development of the GnRH system* Yonathan Zohar, Yoav Gothilf, Susan Wray; US7194978B2, Granted 23-03-2007, to Tel Aviv University Ltd, University of Maryland, Baltimore County (UMBC), US Government

6 Practical Agricultural Applications

The generation of highly effective GnRH analogs (GnRHa) and the polymer-based GnRHa delivery systems provided the global aquaculture industry with a low-cost tool to induce fish to spawn in captivity. Thus, opening the spawning bottleneck and enabling hatchery-based aquaculture. Following equally important research on fish larval growth (not BARD supported), the practical GnRH analogs and delivery systems were immediately adopted and implemented; leading to the establishment of widespread aquaculture of European Sea Bream and Sea Bass in the Mediterranean basin. The main production countries are Greece, Spain, France and Turkey.

The hormonal analogs and delivery systems have been used in fish hatcheries around the world to induce spawning and egg/juvenile production in scores of fish species. A list of farmed fish species which have benefited from the use of the analogs can be found in Appendix B.

Today, after many generations of domestication in captivity, Sea Bream and Sea Bass spawn relatively readily in captivity. Hormones are used almost exclusively in breeding programs, when geneticists aim to induce specific individuals, or to synchronize the spawning of a large group within 1-2 days. Additionally, it is implemented for species whose aquaculture is being newly developed, such as Greater Amberjack, Meagre and Grouper in the Mediterranean basin. In the US, Norway and Chile, GnRHa delivery systems are routinely used in Salmon hatcheries as well as in the US in several new marine aquaculture species such as Snook, Pompano, Red Snapper and others.

In the BARD studies, the agonist determined to be most effective is used both as a liquid injection (dissolved in saline) and *via* the slow release implants. Today, the aquaculture industry also uses other analogs. Other commercial slow release implants are also available (from Canada and Poland) and alternative analogs have been shown to be as or more effective for some other fish species.

However, all these developments are based upon the foundational work achieved during the BARD awards. i) the discovery that spawning in captivity fails due to the lack of brain GnRHs and thus spawning should be optimally induced through administering this hormone to fish, and ii) the studies of the native GnRH degradation and development of the potent GnRH analogs and their delivery systems.

Today, 90% of the hormonal therapies used in Mediterranean aquaculture are based on these GnRH analogs and 50% of this is in the form of slow release delivery systems[1].

Recently, the collaboration with the Crete research group has led to successful development of advanced land-based broodstock operations of the Atlantic (or Northern) Bluefin Tuna (*Thunnus thynnus*, ABFT), one of the tuna species with the greatest commercial interest for fisheries. The GnRHa delivery systems were optimized and are routinely used to induce spawning in ABFT and thus opened the spawning bottleneck for this important fish too.

7 Environmental Impact

Fish production in captivity can lead to the conservation of important marine species whose wild stocks have collapsed due to overexploitation.

Additionally, the controlled release delivery systems for GnRH1 have proven to be an important broodstock management tool critical to the success of many federal, state, private, and tribal fisheries programs. Programs that involve the restoration of threatened and endangered species are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning. Appendix B lists some US endangered fish species for which GnRH analogs have or are being applied as part of conservation efforts in captive broodstock programs.

Protein for human consumption, produced from livestock, requires huge amounts of feed, exerting great pressure on t global agriculture and the environment. Fish has a very efficient FCR (Feed Conversion Ratio) and are the preferred source of protein from livestock with respect to maintaining a sustainable environment.

8 Economic Impact

8.1 Investment Cost

BARD contributed $452,000 in research funds between 1985 and 1990.

Additional funding: Maryland Sea Grant (NA46RG0091) and Maryland Agricultural Experiment Station (MBI-93-29) Massachusetts Sea Grant (NA86AA-D-SG089), USA.

8.2 Benefits

This implementation of the GnRH analogs and the sustained-delivery system allows the reliable, predictable, and consistent production of eggs and larvae for hatchery and nursery operations; and allows the extension of the reproductive season, significantly enhancing the economic feasibility of aquaculture.

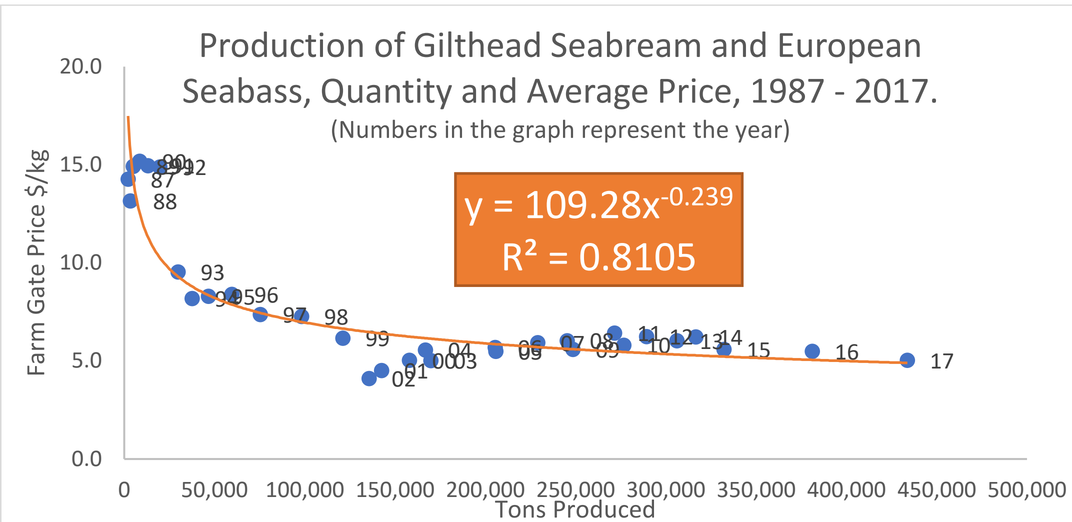
[1] Constantinos C. Mylonas, Director at the Hellenic Centre for Marine Research, Crete

One of the main markets which were influenced by the novel application is the Mediterranean production of Sea Bass and Sea Bream. In 1990 the aquaculture production of seabream and seabass in the Mediterranean was around 8,500 tons and farm gate prices were around \$15/kg. Today this market produces around 433,000 tons annually and farm gate prices are around \$5/kg.[2] Annual production and price is listed in Appendix C, and expressed as a graph in Figure 1.

The production increase occurred when the hatcheries were able to produce eggs in captivity using the new spawning technique funded by BARD, and by rearing the fish through the larval phase to provide juveniles to the farmers, using the know-how that was developed in parallel to the BARD project by other research groups.

The consequence of the ability to farm fish in captivity through the full life cycle is that high-quality fish are today available to consumers in the Mediterranean as well as globally, and can be purchased at a reasonable price, whilst 30 years ago the consumer was limited by availability and price. The farmed seabream and seabass are almost a new product.

Figure 1: Production and Farm Gate Price of Gilthead Seabream and European Seabass



From knowledge of the annual production and price we derived the trend-line as shown in Figure 1, and its formula: $y=109.28X^{-0.239}$. This generalized formula enabled the calculation of the buyer's welfare at each point, as shown in Figure 2 and illustrated in the explanation below.

2 http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY

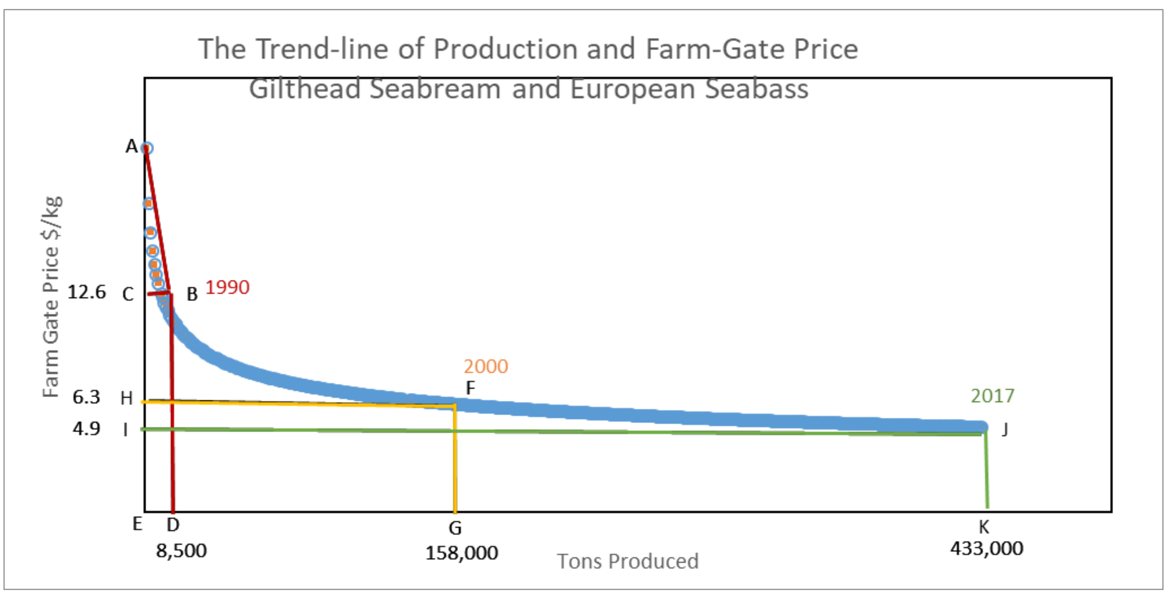
1990 (year 0): Buyers paid $12.6/kg in order to buy 8,500 tons of fish. The sum they paid at the farm-gate is represented by the area CBDE. However, we know that most of the buyers were willing to pay more for those fish, based on the above formula. Their welfare increased by the area under the curve ABC. This is the amount they were willing to pay, but did not have to pay, since the price was lower.

2000: Buyers paid $6.3/kg in order to buy 158,000 tons. The sum they paid in the farm-gate is represented by the area HFGE. Their welfare increased by the area under the curve AFH.

2017: Buyers payed $4.9/kg in order to buy 433,000 tons. The sum they paid in the farm-gate is represented by the area IJKE. Their welfare increased by the area under the curve AJI.

We calculated using 1990 as the zero year, in which the buyer's welfare was calculated as $34M. Every year, the result was calculated relative to 1990. For example, in 2000 the welfare was calculated at $310M. Therefore, the extra welfare for the year 2000 is $276M ($310 – $34 M). In 2017, the welfare was calculated at $669M and the extra welfare is $635M ($669 – $34M). These numbers reflect the extra welfare generated by the new technology to the Mediterranean population and the population of importing countries.

Figure 2: Production and Farm Gate Price Trend-line



With respect to the farmer's benefit, a cost analysis of production of these fish for several Turkish farms showed that the net benefit for the farmers is 11% of the farm gate price[3].

[3] http://www.trjfas.org/uploads/pdf_226.pdf

Therefore, we assumed an 11% profit for the farmers throughout the years. The total benefit includes the farmers benefit and the buyer's welfare, as calculated in farm gate terms.

We calculated future annual growth of the production for the years 2018 – 2028 as 4.4%, the same as the average growth during the period 2008 – 2017. We allowed benefit attribution 38 years after first implementation because the project created a new product and a market that is ongoing, and for which the research outcome provided the foundation and is a dominant contributing factor to its existence.

8.3 Economic Results

The BARD project pioneered the domestication of these and other fish, enabled also by the parallel advances made in juvenile production from the larval phase. Therefore, 40% of the benefit was attributed to BARD.

- Net present value of the BARD's investment is $12 billion, thereof $7.5 billion already attained.
- The Internal rate of return is 143%.
- Benefit cost ratio is 5,800, thereof 3,600 already attained.

In Israel, there is only a small-scale production, of these fish, 3,000 tons annually. Israel and the US both import several thousands of tons annually and benefit dominantly from the consumer surplus rather than by production.

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

- Implant and analog use in aquaculture of additional fish species such as salmon, catfish, amberjack and more…
- The recently developed techniques for production of Atlantic Bluefin Tuna fingerlings and stock in grow-out cages based on the BARD research outcomes.
- The positive ecological benefit of conservation and restoration of important or threatened and endangered marine species.

Table 1: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Rest of the WORLD
BARD's Share in the Cost	90%					
Share in the Benefit		40%				
Cost	2.0	2.0	2.0	1.0	1.0	
Benefit	30,130	12,052	7,524			
Net Present Value	30,128	12,050	7,522	156	240	11,654
Internal Rate of Return	179%	143%	143%	49%	57%	
Benefit Cost Ratio	14,707	5,883	3,673	152	234	

8.4 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: $4.5 billion, to the high result: $22 billion.

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

			BARD's Share in the Benefit		
			Low	Central	High
			30%	40%	50%
Change in Benefit	Low	50%	4,517	6,024	7,530
	Central	100%	9,037	12,050	15,063
	High	150%	13,556	18,076	22,595

9 Appendix A: BARD Awards

Table 3: The 2 BARD awards from 1985-2002

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
IS-772-85	Development of Improved GNH Analogs for Controlled Reproduction of *Sparus aurata* in Farming Conditions				
	Koch, Y. Schreibman, M.P. Zohar, Y. Kohen, F. Salomon, Y. Fridkin, M.	Weizmann Inst. City College NY Isr. Ocean. Res. Weizmann Inst. Weizmann Inst. Weizmann Inst.	$180,000	3 years	1985
IS-1468-89	Novel Technology for the Manipulation of Fish Reproductive Cycles: Controlled Release of Gonadotropin Releasing Hormones				
	Zohar, Y. Langer, R.S. Hassin, S. Dickhoff, W.W.	Isr. Ocean. Res. MIT Isr. Ocean. Res. U Washington	$272,000	3 years	1989

	Elizur, A. Gothilf, Y.	Isr. Ocean. Res. Isr. Ocean. Res.			

10 Appendix B: Application of GnRHa Sustained-Release Technology (Reproboost) Around the World

Food Fish Species in the US	**Food Fish Species in International Markets**	**Ornamental / Baitfish Species (US)**	**Conservation / Threatened Species:**
Striped bass	Gilthead seabream	Pinfish	American shad (US)
White bass	European Seabass	High hats	Arapaima (Brazil)
Cobia	Atlantic Bluefin tuna	Smallmouth grunt	Yaqui catfish (US)
Nassau grouper	Southern Pacific Bluefin tuna (Australia)	Seahorse	Redfish Lake sockeye salmon (US)
Yellowtail flounder	Red seabream	Sea dragon	
Summer flounder	Red porgy (Pagrus)		
Atlantic sturgeon	Dentex		
Common snook	Grey mullet		
Black snook	Dusky grouper		
Pompano	White grouper		
Red snapper	Turbot		
Mutton snapper	Plaice		
Atlantic salmon	Sole		
Coho salmon	Sturgeon		
Sockeye salmon	Pacu		
Chinook salmon	Meagre (Corvina)		
Chum salmon	Shi drum		
Pink salmon	Wreckfish		
Rainbow trout	Common carp		
Brown trout	Greater amberjack		
Arctic charr			
Catfish (hybrid)			
Blackfin tuna			

Amberjack			
Yellowtail			
Tripletail			
Puffer fish			

11 Appendix C: Gilthead Seabream and European Seabass: Production and Average Price, 1987 - 2017

Year	Tons	Price $/kg
1987	2,124	14.3
1988	3,452	13.2
1989	5,090	14.9
1990	8,491	15.2
1991	13,221	15.0
1992	19,796	14.9
1993	29,843	9.5
1994	37,662	8.2
1995	46,744	8.3
1996	59,533	8.4
1997	75,319	7.4
1998	98,207	7.3
1999	121,117	6.2
2000	157,997	5.0
2001	142,552	4.5
2002	135,643	4.1
2003	169,811	5.0
2004	166,782	5.6
2005	205,799	5.5
2006	205,526	5.7
2007	229,111	5.9
2008	245,380	6.0
2009	248,601	5.6
2010	276,634	5.8
2011	271,613	6.4
2012	289,359	6.2
2013	306,076	6.0
2014	316,658	6.2
2015	332,230	5.6
2016	381,018	5.5
2017	433,735	5.0

Source:
http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Aquaculture&tb_mode=TABLE&tb_act=SELECT&tb_grp=COUNTRY

12 Appendix D: Information providers: Personal communication

Yonathan Zohar – PI of BARD grants, Professor and Chair of marine biotechnology, UMBC-IMET.

Constantinos C. Mylonas, - Director, Hellenic Centre for Marine Research, Crete.

Amos Tandler - Scientific adviser to the General Director and Chief Scientist at Ministry of Agriculture.

Berta Levavi-Sivan – Fish Reproduction, Department of Animal Sciences, Vice Rector - Hebrew University of Jerusalem.

Hanna Rosenfeld – Director, National Center for Mariculture, Eilat, Israel Oceanographic and Limnological Research Institute.

Noam Mozes - Head of Mariculture Division at the Ministry of Agriculture