THE ANNUAL DAN POPPER SYMPOSIUM
OF THE NATIONAL CENTER FOR MARICULTURE

March 21, 2002
Eilat, Israel

Gideon Hulata. Aquaculture in the 21st century

Avner Cnaani, Michael Ron, Gideon Hulata, Eyal Seroussi. Fishing in silico: searching for tilapia genes using sequences of microsatellite DNA markers

Serge Gorshkov, Hillel Gordin, Galina Gorshkova, Wayne Knibb. Genetic improvement of sea bass and seabream: practical applications

Itai Tzchori, Alisha Hurvitz, J. Vaya, Gad Degani, Boaz Moav. The influence of phytoestrogens and estradiol-17β on growth rates and sex determination in the European eel (Anguilla anguilla)

Jean-Christophe Avarre, Morad Khayat, Regina Michielis, Hiromishi Nagasawa, Alisa Tietz, Esther Lubzens. Regulation of protein synthesis in shrimp ovaries by neurohormones

Galit Lisaey, Yoav Gothilf, Benny Ron. Development of the pineal melatonin-generating system in gilthead seabream (Sparus aurata) embryos and larvae

Timor Katz, Noa Eden, Dror Angel, Anat Tsemel, Stephan Breitstein, Amir Yurman, Ehud Spanier. Use of solid substrates and biofiltration to reduce mariculture effluents to surrounding waters - preliminary results from the Gulf of Eilat

Ishasar Ben-Atia, Keren Bresler, Miri Gada, Hillel Gordin, Serge Gorshkov, Galina Gorshkova, Gilad Heinisch, William Koven, Sigal Lutzky, Iris Meiri, Adi Paduel, Benny Ron, Hanna Rosenfeld, Amos Tandler. The effect of rearing temperature on sex determination of European sea bass (Dicentrarchus labrax) at different rearing stages


Sigal Lutzky, William Koven, Rogier Van Anholt, Ilishar Ben-Atia, Ruth Weiss, Amos Tandler. The effect of dietary arachidonic acid on survival and cortisol level in gilthead seabream (Sparus aurata) larvae exposed to stress conditions

Tal Tzafrir-Prag, Ingrid Lupatsch, David Sklan. Functional and morphological aspects of the gastrointestinal tract of tilapia

Nutrit Gordon, Amir Neori, Sheenan Harpaz, Muki Shpigel. Nutritional requirements of postlarvae of the Japanese abalone Haliotis discus hannai

Iliya Gelfand, Eddie Cytryn, Jaap van Rijn. Sulfide oxidation in a zero-discharge marine recirculating system

Micha Eshchar, Michael Feduik, Noam Mozes. CO₂ and pH control in super-intensive marine fish culture of the seaweed

Flower E. Msuya, Lilach Shauli, Amir Neori. Biofilters of the seaweed Ulva lactuca in integrated mariculture biofilters: what aeration really does to nutrient uptake, yield and protein content

Daniel Conijeski, Noam Mozes. "RAS-potential": a tool for analyzing production and financial performance of recirculating fish culture systems

Yechiam Shapiro, Ayana Benet-Perlberg, Tanya Zak, Gideon Hulata, Berta Levavi-Sivan. Differences in resistance to Koi Herpes Virus and growth rate between strains of carp (Cyprinus carpio) and their hybrids

AQUACULTURE IN THE 21ST CENTURY
Gideon Hulata*
Department of Aquaculture, Institute of Animal Science, Agricultural Research Organization, P.O. Box 6, Beit Dagan 50250, Israel

A variety of genetic technologies are currently being applied worldwide to aquacultured organisms in an effort to overcome production challenges. These include classical breeding methods (e.g., selection, crossbreeding and hybridization), methods for gender and chromosome-set manipulations (e.g., polyploidy, gynogenesis and androgenesis) and more modern molecular technologies (e.g., DNA markers, genomics and gene manipulation). While having great potential for speeding-up genetic improvement in aquaculture (and agriculture in general), some of the latter technologies entail social or environmental problems. Only 5-10% of the world’s aquaculture production derives from the use of genetically improved stocks, most of which were developed using classical breeding methods. The challenges for the new century are to initiate more breeding programs for currently unimproved species and to decide which modern genetic improvement strategies are necessary, beneficial and acceptable in terms of social and environmental safety.

* Tel: 972-3-9683388; fax: 972-3-9605667; e-mail: vlaqua@volcani.agri.gov.il

FISHING IN SILICO: SEARCHING FOR TILAPIA GENES USING SEQUENCES OF MICROSATELITE DNA MARKERS
Avner Cnaani, Michael Ron, Gideon Hulata, Eyal Seroussi*
Institute of Animal Science, Agricultural Research Organization, P.O. Box 6, Beit Dagan 50250, Israel

Genetic linkage maps of some edible fish species consist of hundreds of DNA markers but few genes. Microsatellite DNA markers are short tandem repeats, with unique flanking sequences. They are highly abundant throughout the genome and appear in coding and non-coding regions. Therefore, it is likely that the flanking sequences may be part of a gene which can be identified by similarity searches of their sequences against the GenBank database. Microsatellite sequences were downloaded from GenBank and queried against the databases using Blastn and Blastx searches. Of 312 microsatellites compared to the databases, 17 loci significantly matched known genes. We were able to map nine of these genes in the tilapia linkage map, providing anchors for comparative mapping between tilapia and other vertebrates. The rapid in silico approach utilized in this study, previously used in mice and livestock, increased the number of genes in the tilapia linkage map from 14 to 23, and identified seven more genes which match unmapped microsatellites.

* Corresponding author. Tel.: 972-8-9484425; fax: 972-8-9470587; e-mail: seroussi@agri.huji.ac.il
GENETIC IMPROVEMENT OF SEA BASS AND SEABREAM: PRACTICAL APPLICATIONS
Serge Gorshkov*, Hillel Gordin1, Galina Gorshkova1, Wayne Knibb2
1 Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel
2 Bribie Island, Aquaculture Research Centre,144 North Street, Woorim P.O. Box 2066, Bribie Island, Queensland 4507, Australia

In Israel, the first systematic long-term selective breeding programs for seabream (Sparus aurata) and sea bass (Dicentrarchus labrax) were initiated at the National Center for Mariculture in the early 1990s. The aim of the program was to increase the efficiency of commercial production by using additive genetic variance for growth. In the first stage of the program, the performance of existing strains and wild populations of seabream and sea bass was tested. The best performing strains were identified as a basis for starting selective breeding.

Family selection was impractical due to specific reproductive constraints in seabream. Since market weight was moderately inherited and responded to selection, an industrial mass selection program was started (at the Ardag fish farm). Three lines of seabream were established to continue the long-term mass selection program and to develop commercial crossbreedings intended for sale of eggs and fingerlings. Industry records from sea cages indicate that the selection response was in the rank of 5-10% per generation. Splitting the broodstocks into several lines assisted in neutralizing inbreeding and provided a high level of security to guard against technical incidents and outbreaks of diseases. New strains of the first domesticated generations of sea bass were developed and released to local mariculture.

The development of other genetic improvement techniques involved interspecific hybridization, chromosome set manipulation, development of monosex stocks and genetic engineering. At present, traditional selective breeding is the major key to genetic improvement of marine fish in Israel and can easily be implemented at a relatively low cost.

* Corresponding author. E-mail: gorshkov@agri.huji.ac.il

THE INFLUENCE OF PHYTOESTROGENS AND ESTRADIOL-17ß ON GROWTH RATES AND SEX DETERMINATION IN THE EUROPEAN EEL (ANGUILLA ANGUILLA)
Itai Tzchori1,2,*, Gad Degani2,3, Ronit Elisha2, Rivka Eliyahu2, Avshalom Hurvitz2, Jacob Vaya2, Boaz Moav1
1 Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel
2 MIGAL, Galilee Technological Center, Kiryat Shmona 10200, Israel
3 School of Science and Technology, Tel Hai Academic College, Israel

The ratio between males and females of the European eel (Anguilla anguilla) raised in different environmental conditions seems to vary. In commercial aquaculture, the majority of European eels (raised from glass eels) develop male gonads, whereas females reach a higher weight and greater market value. To test the effect of estrogen on growth rate and sex determination, the following experiments were conducted: juvenile European eels (0.4-0.6 g) with undifferentiated gonads were fed a diet supplemented with estradiol-17ß (E2) or phytoestrogens for 100-150
days. Treatment with estradiol-17β resulted in an average body weight that was 50-61% higher than in the control groups. Estradiol and phytoestrogens significantly enhanced the percentage of females in eels. While the control group produced only 5-7% females, treatment with estradiol at 20 mg/kg resulted in 70% females and treatment at 2 mg/kg estradiol resulted in 30% females after 100 days of treatment. Treatment with estradiol at 10 mg/kg for 150 days instead of 100 days resulted in about 90% females. Soy extract 1 groups had a high percentage of females (55%) at 2 mg/kg but, at a higher dose of 20 mg/kg, the percentage of females decreased to 15%. Soy extract 2 and licorice, each at 20 mg/kg, resulted in 30% and 18% females, respectively, also significantly higher than the control group. Our preliminary results demonstrate that phytoestrogens can be used as an alternative to sex steroids for sex manipulation in eels.

* Corresponding author. E-mail: tzchori@post.tau.ac.il

REGULATION OF PROTEIN SYNTHESIS IN SHRIMP OVARIES BY NEUROHORMONES

Jean-Christophe Avarre¹, Morad Khayat¹, Regina Michelis¹, Regina Michelis¹, Regina Michelis¹, Hiromishi Nagasawa², Alisa Tietz³, Esther Lubzens¹

¹ Israel Oceanographic and Limnological Research, P. O. Box 8030, Haifa 81080, Israel
² Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
³ Department of Neurobiochemistry, Tel Aviv University, Tel Aviv 66978, Israel
⁴ Eliachar Research Laboratory, Western Galilee Hospital, Nahariya 22100, Israel

Mature penaeid oocytes possess extracellular cortical rods that contain precursor proteins of the jelly layer. The jelly layer forms a protective layer around eggs immediately after spawning and dissipates following the assembly of the hatching envelope. Among those precursors is the shrimp ovarian peritrophin protein (SOP). The temporal pattern of mRNA expression and protein synthesis of SOP in Penaeus semisulcatus ovaries was followed during vitellogenesis and the regulation by sinus gland extracts (SGE) and crustacean hyperglycemic hormone (CHH) family peptides was evaluated.

SOP transcripts were detected in all stages of ovarian development but were more abundant in pre-vitellogenic ovaries than in other stages. SOP was synthesized in ovarian explant fragments that were removed from vitellogenic ovaries and incubated in vitro, but synthesis was not detected in explants that were collected from pre-vitellogenic ovaries. De novo synthesis of SOP was inhibited by P. semisulcatus SGE and by CHH-family peptides that were purified from P. japonicus sinus glands. Sinus gland extracts, however, did not affect the steady state levels of SOP transcripts at any stage of ovarian development. These results suggest that SGE regulate SOP synthesis at the post-transcriptional level.

* Corresponding author. E-mail: esther@ocean.org.il
DEVELOPMENT OF THE PINEAL MELATONIN-GENERATING SYSTEM IN GILTHEAD SEABREAM (SPARUS AURATA) EMBRYOS AND LARVAE
Galit Lisaey1, Yoav Gothilf2, Benny Ron1*

1 Israel Oceanographic and Limnological Research, National Center for Mariculture, Fish Physiology Department, P.O. Box 1212, Eilat 88112, Israel
2 Department of Zoology, Tel Aviv University, Tel Aviv 69978, Israel

Melatonin production is directly controlled by the activity of the enzyme serotonin-N-acetyltransferase (AANAT) which, in turn, is regulated by the circadian clock. The circadian clock-regulation of AANAT activity differs from one species to another and could occur at the transcriptional, post-translational or both levels. In fish, the endogenous circadian clock is contained within the photoreceptive cells of the pineal gland and the retina. As a consequence, circadian rhythmicity of AANAT activity can be observed when these tissues are placed in culture. In this work, we investigated the very early development of seabream pineal photoreceptors and the circadian clock function by measuring melatonin, AANAT activity and sbAANAT-2 mRNA expression.

It has been shown that two AANAT genes, AANAT-1 and AANAT-2, with different expression patterns are present in the teleost species pike, trout, and zebrafish. We discovered that seabream also has two AANAT genes. Our investigation revealed that sbAANAT-1 is expressed only in the retina and sbAANAT-2 is expressed only in the pineal gland (data not shown). We then ascertained that the sbAANAT-2 mRNA is an effective marker for developmental studies of the pineal and retinal photoreceptor. The melatonin generating system is functional immediately following hatching in seabream larvae. There is a significant difference between “immediately after” (42 hpf), “before” (18 hpf) and “12 hours after” (54 hpf) hatching in the expression of the gene, enzyme activity and melatonin production. It is possible that the rhythms of sbAANAT expression are in different phases. Therefore, studying the differential regulation of these two related clock-controlled genes in seabream may provide valuable insights into the mechanism of the circadian clock.

* Corresponding author. Fax: 972-8-6375761, e-mail: ronbenny@agri.huji.ac.il

USE OF SOLID SUBSTRATES AND BIOFILTRATION TO REDUCE MARICULTURE EFFLUENTS TO SURROUNDING WATERS - PRELIMINARY RESULTS FROM THE GULF OF EILAT
Timor Katz1*, Noa Eden1, Ehud Spanier2, Anat Tsemel2, Stephan Breitstein2, Amir Yurman2, Dror Angel1

1 Israel Oceanographic and Limnological Research, The National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel
2 Leon Recanati Institute for Maritime Studies, University of Haifa

Eight biofiltration arrays (each consisting of 11 plastic mesh cylinders mounted on a PVC frame) were deployed in midwater (8 m below surface), four arrays adjacent to the Ardag fish farm and four arrays 300 m west of the farm (reference site). Identical biofiltration arrays were deployed and studied at net cage fish farms in Crete, Slovenia and Scotland as part of the EU-funded BIOFAQs (BIOFiltration and AQuaculture) project in June 2001. Every second month, one cylinder from each array was sampled and the community structure and distribution of organisms on the cylin-
ders were documented. Dry weight, ash, P, N, C and energy of the total biomass on the cylinders and of selected invertebrate species representing the major groups were determined.

Invertebrates were also sampled for stable isotope composition analysis. In addition, we carried out measurements to determine the ability of the biotic communities associated with the biofilters to remove phytoplankton from the ambient water.

We found that there was a constant increase in biomass of attached organisms and a higher phosphorus content in the biomass of these invertebrates on the biofilters near the farm than on the biofilters at the reference site. The biomass on the biofilters near the farm was sevenfold higher than at the reference site and the phosphorus enrichment ratio (calculated as a comparison between the two sites) was 9.7. The composition of the organisms differed both qualitatively and quantitatively between the sites. Some organisms were found only near the farm and some were found only at the reference site. On average, we found 3.3 stony coral colonies per biofilter unit (mainly on the PVC frame) and there was a similar settlement rate at the two sites. The phytoplankton filtration capacity of the biofilters was measured, but the results have not been analyzed yet. Preliminary measurements indicated that the community associated with the biofilter (100 g, dry weight) consumed $5.1 \times 10^7$ cyanobacteria per minute, suggesting rapid removal of phytoplankton biomass and a potentially important role in particle capture.

* Corresponding author. E-mail: timor@ocean.org.il

---

**THE EFFECT OF REARING TEMPERATURE ON SEX DETERMINATION OF EUROPEAN SEA BASS (DICENTRARCHUS LABRAX) AT DIFFERENT REARING STAGES**

Ishasar Ben-Atia*, Keren Bresler, Mirit Gada, Hillel Gordin, Sergei Gorshkov, Galina Gorshkova, Gilad Heinisch, William Koven, Sigal Lutzky, Iris Meiri, Adi Paduel, Benny Ron, Hanna Rosenfeld, Amos Tandler

*Israel Oceanographic and Limnological Research, The National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel*

Sex differentiation in fish is controlled by genes but it has recently become apparent that sexual development in teleosts has some degree of plasticity and can be influenced by environmental factors. The main environmental factor influencing sex in fish is temperature. Somatic growth in male sea bass is lower than in females, resulting in an 18-40% smaller body weight at two years of age for males. In many Mediterranean and European cultured sea bass stocks there is an undesirably high proportion of males. Therefore, efforts are being made to change the sex ratio in cultured sea bass to favor females.

The objective of this work was to study the effect of different temperature regimes during the first 100 days after hatching on sex differentiation in European sea bass and to identify when plasticity of gonadal sex differentiation occurs. As there are indications that the genetic makeup of the population may influence its response to temperature, we examined the influence of temperature on sex determination in two Mediterranean strains of European sea bass. The results for both strains demonstrate that exposure to a high temperature (21°C) during early development has a strong masculinizing effect. Conversely, exposure to a low temperature (13°C) results in a population with a significantly higher percentage of females. The present study indicated that sex differentiation is temperature-sensitive from 10 to 90 days after hatching.
We showed that androgenesis can be prevented in European sea bass by exposing them to low temperatures during the larvae and nursery stages. Our results suggest that the low temperature treatment may not have long-term negative effects on growth rates and, to the contrary, such effects may be beneficial.

* Corresponding author. E-mail: sbenatia@ocean.org.il

CAN GILTHEAD SEABREAM, SPARUS AURATA, BE CULTURED IN EIN TAMAR WATER? A MODEL FOR ASSESSING THE FEASIBILITY OF MARINE FISH CULTURE IN THE ARAVA’S BRACKISH WATER

Benny Ron1*, Adi Peduel1, Keren Bresler1, Daniel Conijesky1, Micha Eshchar1, Rami Alon2, Noam Mozes1

1 Israel Oceanographic and Limnological Research, National Center for Mariculture, Fish Physiology Department, P.O. Box 1212, Eilat 88112, Israel

2 Ein Tamar, Center for Research and Development, Tamar Municipality, Israel

As high quality water resources become increasingly limited, the use of marginal lands and water resources for food production is becoming a necessity. There is great interest in developing the technology to profitably produce high-priced fish with marginal resources. The chemical contents of water can significantly influence the growth and survival of fish, not only from the aspect of osmoregulation. A protocol was developed for assessing the feasibility of culturing gilthead seabream in brackish water from the well at Ein Tamar. Our regimen included the assessment of growth and survival potential by culturing fish for a short term in the test water, and in sea water for comparison. During long-term culture of the fish in both water sources, the cortisol level, innate immune system activity and tissue pathology (by histology) were monitored to determine the long-term effects of the specific conditions.

There was significant retardation in growth of fish that were raised in the brackish water, but there were no significant differences in survival rate, cortisol level or activity of the immune system. Fish grown in the brackish water had a higher level of contamination by Mycobacterium marinum after six months, but all the fish were found to carry the disease.

The closed water system with its multiple water supply system enables fish culture in abnormal conditions to be compared with fish culture in conventional conditions. Thus, the feasibility of a project can be assessed before large investments are made.

* Corresponding author. Fax: 972-8-6375761, e-mail: ronbenny@agri.huji.ac.il
THE EFFECT OF DIETARY ARACHIDONIC ACID ON SURVIVAL AND CORTISOL LEVEL IN GILTHEAD SEABREAM (SPARUS AURATA) LARVAE EXPOSED TO STRESS CONDITIONS

Sigal Lutzky1*, William Koven1, Rogier Van Anholt2, Isashar Ben-Atia1, Ruth Weiss1, Amos Tandler1

1 Israel Oceanographic and Limnological Research, The National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel
2 Department of Animal Physiology, University of Nijmegen, Toernooiveld, 6525 ED, Nijmegen, The Netherlands

This paper summarizes the effects of dietary arachidonic acid (20:4n-6, ArA) on survival in gilt-head seabream larvae exposed to handling, water turbulence, air exposure, crowding, temperature increase and salinity increase, and the possible metabolic pathways of ArA that are involved.

Initial studies demonstrated that dietary ArA markedly (p<0.05) improved survival of seabream larvae following handling stress, provided this essential fatty acid was fed prior to handling. Dietary ArA also promoted better survival of larvae stocked in aquaria at different densities (3, 6 and 12 larvae/l). Another feeding trial tested the effect of dietary ArA on the acute (handling) and chronic (daily fluctuating salinity) stress response of larvae during metamorphosis and postmetamorphosis. Results showed a clear correlation between dietary ArA, cortisol level and survival in larvae exposed to handling while a high level of dietary ArA fed to chronically stressed larvae led to increased mortality and markedly higher levels of cortisol. The data suggest that metamorphosing larvae are more sensitive to the effects of dietary ArA than postlarvae. This hypothesis was reinforced in further studies which investigated the effect of dietary ArA in larvae at different stages of development that were rapidly exposed to salinity increase, temperature increase, air exposure or water turbulence.

Accumulated results led to the hypothesis that the prostaglandin PGE2, derived from ArA, was involved in regulating cortisol synthesis through the hypothalamus-pituitary-interrenal (HPI) axis during a stress event. However, recent findings also suggest that free ArA may directly mediate the stress response as well.

* Corresponding author. Fax: 972-8-6375761, e-mail: gali@ocean.org.il

FUNCTIONAL AND MORPHOLOGICAL ASPECTS OF THE GASTROINTESTINAL TRACT OF TILAPIA

Tal Tzafrir-Prag1, Ingrid Lupatsch2, David Sklan1*

1 Faculty of Agriculture, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel
2 Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

Tilapia are widely grown in Israel since they can feed on low trophic levels and are opportunistic omnivores. Further, they survive well in low quality water and are tasty. This study reports on functional and morphological aspects of the digestive tract of Oreochromis niloticus x O. aureus. No correlation was found between the length of the gastrointestinal tract and body size, possibly because these fish are hybrids. Microscopic examination revealed different patterns of villi along the tract, with villi becoming more sparse and wider distally. Digestive enzymes were secreted by
the hepatopancreas and their activity decreased from the proximal small intestine distally. Diets of fishmeal or wheat-fishmeal resulted in maximal absorption of protein and fatty acids in the proximal small intestine, decreasing distally. Overall digestion of protein was 95.52% and 83.2% and of fat 94.5% and 77.5% for fishmeal and wheat-fishmeal, respectively. Digestion of energy was 85-90%.

* Corresponding author. E-mail: sklan@agri.huji.ac.il

**NUTRITIONAL REQUIREMENTS OF POSTLARVAE OF THE JAPANESE ABALONE HALIOTIS DISCUS HANNAI**

Nurit Gordon¹, Amir Neori¹*, Sheenan Harpaz², Muki Shpigel¹

¹ Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

² Department of Aquaculture, Volcani Agriculture Research Center, Beit Dagan, Israel

Diatoms were selected according to their attractiveness to veliger settlements and to postlarvae growth and survival in the Japanese abalone *Haliotis discus hannai*. Attractiveness was quantified as follows: Veligers were placed in Petri dishes and offered a choice of several diatom patches on solid media. The veligers that settled and metamorphosed on each patch were counted.

Several diatom species were examined. The three most attractive - *Nitzschia laevis*, *Navicula lenzi* and *Amphora luceae* - were selected for further experiments. The attraction by the diatoms was chemosensory and depended on their excreted compounds. Diatom extracts, their cell fragments and aspartic acid (the only free amino acid detected in the excretions of two of the tested diatoms) strongly attracted the veligers.

The three attractive diatoms were also nutritious for the abalone. They contained high levels of lipids and fatty acids, including polyunsaturated fatty acids n-3 (e.g., EPA- 20:5n-3) and n-6. Arginine, proline and glutamate dominated the free amino acids in extracts of the attractive diatoms. Mixtures rather than single species of algae provided the postlarvae a better balance of required nutrients, resulting in better growth and survival. The best mixture - *N. lenzi* and *A. lucea* - resulted in a survival rate of 50% and a shell growth rate of 36 microns shell per day, 30 days after settlement. This compares to 3-5% survival in nonselected diatoms. The results of this research enable commercial hatcheries to achieve greatly increased survival of abalone larvae.

We thank Prof. J. Lee and H. Krogliak for their help.

* Corresponding author. E-mail: neori@ocean.org.il
A unique treatment system allowing culture of marine fish without waste discharge has been operated for the past three years at our facilities in Rehovot. The system supports intensive culture of gilthead seabream (Sparus aurata) in a completely closed mode and comprises an aerobic and an anaerobic treatment stage. In the aerobic stage, ammonia is oxidized to nitrate by nitrifying bacteria situated in a trickling filter. In the anaerobic stage, consisting of a digestion basin and a fluidized bed reactor, organic waste from the fish tank is biologically degraded with a concomitant reduction of nitrate to nitrogen gas. Sulfide formation, from reduction of sulfate in the anaerobic treatment component, forms a potential threat to the successful cultivation of fish in this marine zero-discharge system.

In the present study, it was demonstrated that reduction of sulfate to sulfide takes place in organic-rich and nitrate-poor zones in the digestion basin. Sulfide concentrations in the effluent of the digestion basin were occasionally as high as 80 µM. However, due to rapid oxidation in the fluidized bed reactor situated between the digestion basin and the fish tank, the sulfide concentrations were lower than 0.2 µM in water leaving the anaerobic treatment stage. Laboratory incubations of crude cultures derived from the fluidized bed reactor demonstrated that sulfide oxidation coincided with a reduction of nitrate. Subsequent studies on denitrifying strains isolated from the fluidized bed reactor revealed the presence of heterotrophic as well as chemolithotrophic denitrifiers capable of reducing nitrate to nitrogen gas and oxidizing sulfide to elemental sulfur and sulfate. DGGE analysis of PCR-amplified 16S rDNA fragments showed a close affiliation between the microbial community of the fluidized bed and known marine sulfide-oxidizing denitrifiers.

*Corresponding author. Fax: 972-8-9465763; e-mail: vanrijn@agri.huji.ac.il

**CO2 AND pH CONTROL IN SUPER-INTENSIVE MARINE FISH CULTURE**

Micha Eshchar, Michael Fediuk, Noam Mozes*

Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

A super-intensive recirculating aquaculture system (RAS) was developed at the National Center for Mariculture in Eilat, Israel. Fish density, which reaches 70-90 kg/m³ in super-intensive systems, is limited by high toxic CO2 concentrations. In such a system (which is enriched with pure oxygen), CO2 accumulates, reducing the pH of the water. The decrease in pH could also be attributed to carbonate alkalinity consumption during nitrification, when a nonvolatile carbon species (bicarbonate) is changed into a volatile form (CO2). Further aeration in the biofilter removes CO2, reducing the total carbonate carbon C7. Water pH dominates the CO2 fraction of C7 in an inverse correlation.

In our system, paddlewheel aerators were used to strip CO2. The transfer coefficient (KLa)
for CO₂ stripping by a paddlewheel (1 kw) was measured. At an average CO₂ concentration of 8 mg/l, the stripping was 1170 g CO₂/h (28 kg/d). In seabream (Sparus aurata) culture, this enables a feeding rate of 40 kg feed/d. The cost of energy to remove 1 kg CO₂ is estimated at $0.06.

Mass budgets showed that the main CO₂ sink was stripping by paddlewheel aerators. The main carbonate alkalinity source was makeup sea water, while sinks were nitrification and water discharge. pH values were low (6.7-6.9). CO₂ concentrations were not accordingly high, due to the low C₇. 

* Corresponding author. E-mail: mozes@ocean.org.il

**BIOFILTERS OF THE SEAWEED ULVA LACTUCA IN INTEGRATED MARICULTURE: WHAT AERATION REALLY DOES TO NUTRIENT UPTAKE, YIELD AND PROTEIN CONTENT OF THE SEAWEED**

Flower E. Msuya¹,²*, Lilach Shauli¹, Amir Neori¹**

¹ Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel
² Department of Plant Sciences, Tel Aviv University, Tel Aviv 69987, Israel

Cultured seaweed performs better in air-agitated water. Aeration may affect vertical light fields, removal of excess oxygen or nutrient diffusion and uptake. To evaluate these possibilities, the seaweed Ulva lactuca was cultured in tanks (0.6 m³, 1 m²). Each tank received intensive fish-pond effluents at 0.85 m³/h and one of four water agitation regimes and two total ammonia nitrogen (TAN) fluxes. The agitation regimes were: standard aeration, siphon (varying water levels), combined aeration and siphon, and water exchange only (no aeration, no siphon). The TAN fluxes were high (37 g TAN/m²/day) and low (7 g TAN/m²/day).

With high TAN fluxes, TAN was removed at 6.2 g/m²/day with insignificant variation (p=0.11) between agitation treatments. Yields (268 g/m²/day) and protein contents (43% in dw) also varied insignificantly between agitation treatments. With low TAN fluxes, TAN was removed at 3.4 g/m²/day but the variation between agitation treatments was significant (p=0.04). The yield was higher (335 g/m²/day) and the protein content was lower (36% in dw), both with insignificant variation between agitation treatments.

Agitation enhances nutrient diffusion into the algae, when nutrients are limiting, by reducing the thickness of the boundary layer around the seaweed. The practical message from this study is that with a high nutrient supply, seaweed biofilters can function well with any form of water agitation.

* Corresponding author. E-mail: flowerem@hotmail.com or neori@ocean.org.il
“RAS-POTENTIAL”: A TOOL FOR ANALYZING PRODUCTION AND FINANCIAL PERFORMANCE OF RECIRCULATING FISH CULTURE SYSTEMS
Daniel Conijeski, Noam Mozes*
Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

Recirculating aquaculture systems (RAS) have generated much interest in recent years due to their ability to raise fish in a controlled environment. Further, fish density can be high in RAS, resulting in high yields and reducing the investment and extent of culture units and associated buildings. Because RAS conserve heat and water, the growing season of many commercial species can be extended, there is flexibility in locating the RAS and water costs are reduced.

Notwithstanding these advantages, the technology of RAS is still poorly understood by many farmers. The high investment, coupled with a lack of comprehensive information on the industrial and economic performance of these systems, has raised skepticism about the economic competitiveness of this technology.

The National Center for Mariculture, together with the Fisheries and Aquaculture Department of the Israeli Ministry of Agriculture, has developed a standard form for evaluating commercial RAS called “RAS-Potential”. RAS-Potential involves an official set of production and economical indicators to help both private parties and the government make investment decisions. These indicators provide the means to compare technologies, to benchmark fish production costs in RAS and to follow-up standard measurements of performance efficiency of RAS farms over time.

* Corresponding author. E-mail: mozes@ocean.org.il

DIFFERENCES IN RESISTANCE TO KOI HERPES VIRUS AND GROWTH RATE BETWEEN STRAINS OF CARP (CYPRINUS CARPIO) AND THEIR HYBRIDS
Yechiam Shapira1, Ayana Benet-Perlberg2, Tanya Zak2, Gideon Hulata3, Berta Levavi-Sivan1*

1 Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University, Rehovot 76100, Israel
2 Aquaculture Research Station Dor, M.P. Hot Hacarmel 30820, Israel
3 Department of Aquaculture, Institute of Animal Science, Agricultural Research Organization, Volcani Center, P.O. Box 6, Beit Dagan 50250, Israel

Following the temperature change in the beginning of spring 1998, an outbreak of unknown etiology caused mass mortality in koi and carp (Cyprinus carpio) on the northwestern coast of Israel. The disease resulted in 40-90% mortality of fingerlings and mature fish within three weeks. Preliminary bacteriological and virological studies revealed virus-like particles in the nuclei and cytoplasm of respiratory epithelial cells of the gills, identified as Koi Herpes Virus (KHV). To improve the genetic resistance of local carp strains, frozen sperm of a wild carp was brought from Czechoslovakia (Cyprinus carpio haematopterus; Sassan; S) to increase heterosis by producing hybrids.

We compared crosses and hybrids of three strains of local and wild carp (WxW, UxU, WxU, WxS, SxU) by challenging them with the virus using cohabitation. The hybrid WxS was the most
resistant (60% survival), while UxU was the most sensitive (8% survival). Differences in survival between the hybrids can be used to estimate the genetic mechanism of the resistance trait.

Other commercial performance factors (growth rate, feed consumption, survival) were tested in outdoor ponds to evaluate economic value. The WxS hybrid, due to its low fertility rate, had a small advantage in all the above parameters. No significant differences between the other hybrids were recorded.

* Corresponding author. E-mail: sivan@agri.huji.ac.il

BLUE-FIN TUNA - NEW DEVELOPMENTS:
REPORT ON THE “1ST INTERNATIONAL SYMPOSIUM ON THE DOMESTICATION OF BLUE-FIN TUNA THUNNUS THYNNUS THYNNUS – DOTT: STRATEGIES FOR EUROPEAN DEVELOPMENT IN THE CONTEXT OF A GLOBAL MARKET”
Hillel Gordin*

Israel Oceanographic and Limnological Research, National Center for Mariculture,
P.O. Box 1212, Eilat 88112, Israel

It took six long years to conceive, advocate, plan and run the first DOTT meeting, which was held in Cartagena, Spain, on February 3-8, 2002. The meeting was funded mainly by the EU Commission (~ 90,000 Euros) and by a grant given by the Marine Policy Center, Woods Hole Oceanographic Institution. There were over 200 participants from some 25 countries representing places and interests relevant to blue-fin tuna fishing and aquaculture. There were some fifty presentations covering issues such as the state of wild tuna populations and fishing pressures, fishermen’s views on the emerging blue-fin tuna farming, environmental aspects of blue-fin tuna farming, views of the EU and ICCAT on blue-fin tuna farming and the problems and vision of the Spanish Blue-Fin Tuna Farmers Association. There was a preliminary report on the socio-economic aspects of the blue-fin tuna farming industry. At the end of the first day, there was a panel discussion on the administrative, regulative and R&D requirements for the development of a sound and sustainable farming industry and a set of resolutions was proposed.

The second day was devoted to the biology of the blue-fin tuna with emphasis on identifying the gaps in knowledge that would hinder the domestication of the blue-fin tuna. Speakers from throughout the world (Australia, Japan, USA, Europe) presented talks on the basic biology and straddling nature of the blue-fin tuna, its reproductive cycle and control, larvae and juvenile rearing and mass production, nutrition and feeding, handling and manipulation, growout techniques and environmental impact. At the end of the day, a panel of representatives from the above disciplines gave their views about the subjects that R&D efforts should concentrate on. The conference heard from nine national representatives about their experience in establishing blue-fin tuna farming (all based on capture of adult fish at sea and fattening them for the Japanese market).

Resolutions called for an international effort, led by the EU, to domesticate the blue-fin tuna for the sake of establishing a sustainable farming industry and methods for restocking blue-fin tuna populations in nature. It also called for the establishment of a virtual blue-fin tuna R&D center which will include physical land-based facilities and enable careful scientific experimental work. The EU granted the first R&D funding for studying reproduction control of the blue-fin tuna to a consortium of nine partners (eight European partners and NCM-IOLR in Eilat). The project is scheduled to start work at the end of 2002.

* E-mail: gordin@agri.huji.ac.il