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Marine Hydrodynamics, Coastal & Ocean Engineering

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Dear Drs. Jacobsen and Ayalon:

I am please to submit the enclosed copies of the final report of "Culture of Grey Mullet, *Mugil Cephalus*, in Egypt and Israel." This report encompasses work carried out during the period 2003-2007, under Grant Agreement No. TA-MOU-99-M!8-062.

First, my colleagues and I want to thank you for permitting the no-cost extension, and second for accepting delay in submitting this report. Both were occasioned by unforeseen occurrences and problems that developed during the research process. The cooperative research which led to solution was directed mainly from the joint meetings when ongoing results were compared and mid-point corrections suggested therefrom.

The final months of this project appear to marked, also, a felicitous relaxation in intra-country politics which bodes well for the environment in which future work can be conducted. This has particular reference to meetings, training, and other personal interactions.

Finally, at the risk of sounding like a broken record, I need, once again, to invite your attention to the benefits of a meeting of project directors, to compare notes re planning and operating, to share results, and to aggrandize contacts.

Again, we all express our thanks for your wonderful support of this project.

Sincerely,

S/ RBA

Robert B. Abel
Principal Investigator

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FINAL REPORT, COVERING PERIOD 2003 - 2007–05–01

SUBMITTED TO THE U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT, BUREAU FOR GLOBAL PROGRAMS, FIELD SUPPORT AND RESEARCH, CENTER FOR ECONOMIC GROWTH AND AGRICULTURAL DEVELOPMENT

CULTURE OF GREY MULLET, *MUGIL CEPHALUS*, IN EGYPT AND ISRAEL

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Grant Agreement No: TA–MOU-99-M18-062

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EXECUTIVE SUMMARY

The Israelis, and the Egyptians at their laboratories at Eilat and Fayoum/Alexandria respectively, have collaborated on a project to culture the Grey Mullet, a fish which bears promise of an outstanding food resource throughout the entire Middle East but which has historically been difficult to domesticate.

The project's complimentarily of need/opportunity was obvious from the beginning. The Israelis, who have traditionally given other stocks (e.g. tilapia, carp, seabream and sea bass) higher priority, possess some of the world's outstanding fish farming facilities. The Egyptians, who have always placed enormous value on the mullet as a a food resource, have previously been limited to "fishing in the wild." Their Research and Development facilities had been less developed.

Accordingly, am outstanding accomplishment of the project must be considered the Egyptians excellent capacity building program. As illustrated herein, by the project's end they had designed and constructed and now possess, an outstanding facility for research and fisheries production. Promulgation of the designs and operations of their hatchery and earthen ponds should be of material assistance to ministries and laboratories throughout the Middle East.

The two countries decided – by agreement – on three research subjects: Reproduction, Larval-Rearing and Nutrition. They:

1. Developed successful out-of-season reproduction using photoperiod and water quality control.
2. Defined the daily protein and energy needs of the mullet from juvenile to market size for their maintenance and predicted growth at varying water temperatures.
3. Translated these requirements into fed formulas to allow production of practical feeds.
4. Developed an effective protocol for the larval rearing of the grey mullet.
5. Stimulated interest in both Agriculture Ministries to increase grey mullet production through expanded farming of mullets in monoculture.

There is now a clearer perception of future practical research avenues to further improve grey mullet larval rearing. These areas of continuing interest include determining the developmental stage at which eggs need to be stocked for optimal hatchability; the role of essential fatty acids in larval nutrition; reduction of deformities; and shortening the time of acclimation to various salinities.

Research direction and progress in both laboratories was originally determined during joint preparation of the proposal; mid-course corrections were applied during annual meetings among all project scientists. Five technical papers were published regarding the ongoing work, three Egyptian and one Israel student obtained their masters' degrees as a result of the project.

Both Agriculture Ministries are encouraging continuing research. An unforeseen impact of the project, has been to expand production, not only for the meat itself, but for the roe, which has achieved marketability as the "poor man's caviar". As a result, a joint research project has been proposed to MERC by the team (M27-966) and was

recently accepted for possible funding contingent on meeting certain provisos set out by MERC.

General Introduction

Grey Mullet, *Mugil cephalus,* which are farmed in both Egypt and Israel for human consumption, are considered a high quality fish.

This statement is critical to the economics and general welfare to the Egyptians and Israelis, as well as to most of their Middle East neighbors. It is emblematic of the huge irony in fisheries research, i.e. the inverse relationship between the **need** for a fish species and the **level of research on that same species**.

It is an unfortunate (if logical) fact that the major laboratories among the wealthiest countries devote their research talents and resources to what we called "Tiffany stocks" which are highly prized and profitable. By definition, they are also unavailable to the great mass of the world's population. On the other hand, the stocks, which reproduce at higher rates and could theoretically satiate and benefit large third world populations, are given short shift in first line laboratories.

There is no stock more representative of this thesis than mullet. The magnificent Special Report of the National Geographic of April 2007, predicts terrifying clarity, an anticipated dry-up of the sea's bounty, with particular reference to the Mediterranean, where man is busily draining all available sea-derived food resources. In this regard, the Geographic implies that the most likely solution to the problem is aquaculture, i.e. fish farming.

Accentuating this critical irony is the enormous bonus provided by Mediterranean fish that represent the only food source on earth not requiring fresh water for survival and growth, in a region where water deprivation is a major cultural, economic, and political consideration.

The Egyptians and Israelis, who have been working together in close harmony for a quarter century under the MERC program, have long recognized the potential of aquaculture to meet nutritional needs in the region. An efficient farming process is essential to properly exploit this outstanding resource. Unfortunately, the stock is fighting back. Up to now, they have refused to reproduce in captivity, with any degree of regularity.

Solution of this problem may never win a Nobel Prize, but its utilization importance to the Middle East cannot be overvalued. Accordingly, the reader is cautioned to understand that production figures notwithstanding, the supply of fingerlings is almost exclusively from the wild with Egypt alone harvesting over 300 million fry and Israel (with much less fresh water acreage) in the region of 7-10 million. Egyptian production has reached 173,000 tons, representing 20% of domestic fish production, while Israeli production has been limited to 2000 tons. It must be reemphasized that reliance on natural sources of fish for maintaining the domestic production in both countries is **unsustainable** as the fingerling supply is vulnerable to natural fluctuations in fry production and the massive fishing of the fry in the coastal waters in the region.

Proceeding from a series of intensive conferences, the Egyptians and Israelis agreed to a coordinated line of research, divided into three basic sectors: Reproduction, Larval Rearing, and Nutrition, and this report will be sectioned accordingly.

I – Reproduction Research

Introduction

A guide to reproduction of grey mullets in captivity in Hawaii was published in 1974 (Kuo et.al,) but the main source of fingerlings today is still from the wild. The spawning season of the Mediterranean *Mugil cephalus* (grey mullet) ranges from July to December. However, in captivity, mullets do not spawn naturally with both genders displaying reproductive dysfunctions. Gonadal development in females usually halts at early stages of vitellogenesis and does not proceed with final oocyte maturation, while spermiating males are rarely observed and in most cases the produced milt is highly viscous and fails to fertilize the eggs. The inability of fish in captivity to complete its reproductive cycle is most likely due to a failure at one or more sites along the hypothalamo-hypophyseal-gonadal axis. In addition, with mullet it is difficult to establish the right sex ratio among broodstock, as this species does not exhibit any sexual dimorphism. Moreover, the conventional oviduct catheterization technique is feasible only at the more advanced developmental stages of gonadal growth.

Objectives

With the intention of inducing successful breeding in captive Mediterranean grey mullet this study addressed the following objectives:

(a) To develop a rapid tool for determining the sex of young brooders,
(b) To determine and identify the concentrations of amino acids and fatty acids in hormone-injected and non-injected females and compare them with spawned ova.
(c) Investigate the important aspects of the biological, histological, histochemical and fine structures of gonads and pituitary glands, and generally synchronize gonadal development in both males and females.
(d) To induce spawning: continuing successful spawning trials with varying hormones and doses under varying environmental conditions in order to ultimately standardize an optimum procedure to induce spawning.

Materials and Methods- NCM

Grey mullet broodstock used for this study consisted of 5-8 year old females (BW 2.3±0.08 kg), and 3-5 year old males (BW 1.1±0.06 kg). Fish were maintained outdoors in 5m3 covered fiberglass tanks and were subjected to either natural or 6-month shifted conditions of light and water temperature. The experimental tanks were stocked equally at a density of 4.0±0.174 kg/m3, consisting of a 7:4 (female: male) sex ratio. Fish were fed daily at the rate of 1.5% of their body weight using a 30% crude protein and 6% lipid feed formulated and prepared at NCM.

Hormones & treatments. The hormonal treatments used to accelerate gonadal growth were carried out in early July. The hormones: GnRH analogue (GnRHa), and 17α methyltestosterone (MT), loaded on Ethylene-vinyl acetate copolymer (EVAc) slow-release implants, and dopamine antagonists (i.e. domperidone [Dom] or metaclopramide [MET]) dissolved in a vehicle, were injected intramuscularly.

Control fish were injected with saline only. The state of ovarian development as determined by oocycte growth was examined bi-weekly from biopsies using polyethylene cannuli. In parallel, male mullets were checked for the presence of milt by applying gentle abdominal pressure.

Female mullets were considered fully mature once their oocytes reached an averaged diameter of 500μm. A mature female was stocked in a $1m^3$ tank with two running males in which seawater at 24-28^{0}C was provided. The spawning induction treatments consisted of GnRHa and MET, given as two injections (24 h apart).

Steroid hormone determination. Blood samples of mullet broodstock were obtained from July to October. The blood was centrifuged (1500g for 15 min at 4°C) and plasma was collected. Steroids were extracted twice with ethyl ether and following evaporation of the organic phase, solubilized in the appropriate buffer for ELISA. Both steroids, 17α-estradiol (E2) and 11-Ketotestosterone (11-KT), were measured by specific ELISAs.

Vitellogenin dot-blot immunoassay. Plasma samples were applied directly to a nitro-cellulose membrane. Nonspecific binding sites were blocked with 5% skimmed milk in PBS (137 mM NaCl, 8 mM Na2PO4, 1.4 mM KH2PO4, and 6.6 mM KCl, pH 7.5). For detection of Vtg, the membrane was incubated with anti-sb-Vtg (polyclonal antibodies raised in rabbit against seabream Vtg, diluted 1:5,000, for 1 h at room temperature, washed five times with PBS-T (PBS plus 0.05% Tween-20), and then incubated with horse-raddish peroxidase conjugated to goat ani-rabbit-IgG. After 1 h of incubation, the membrane was washed as described above and the signal was visualized by an ECL detection system (Amersham-Pharmacia-Biotech).

Results

Rapid tool for determining the sex of young brooders. Vitellogenin, the precursor of the yolk proteins, was used as a marker protein, in a dot-blot immunoassay, for distinguishing sexual mature females from males (Fig. 1). This simple method enabled us to obtain with a high degree of certainty, 4 months ahead of spawning season, the sex of more than 98% of newly integrated brooders.

Synchronized gonadal development in females and males. The hormonal treatments used to accelerate gonadal growth indicate that, a single injection of Dom induces synchronized ovarian development, while the GnRHa implant has no additive effect (Fig. 2). The Dom treatment, seems to disrupt the female social hierarchy, and liberates suppressed females, allowing them to reach sexual maturity. In addition, our results indicate that MT is a potent spermiating agent, which enhances milt production in all treated males (Fig. 3). A positive correlation between 11-KT levels in male plasma along with the presence of fully mature females, suggest an additive effect probably associated with female pheromones, which highlights the importance of the female-male hormone relationship in this species.

Induced spawning. Our spawning induction trials indicate that the combined treatment (GnRHa & MET) is more effective in inducing ovulation and spawning as compared to GnRHa alone; 85% and 12% spawning success, respectively (Table 1). An accurate latency period (21-22 h) was recorded in all spawning induction trials during three

successive natural as well as artificially 6-month shifted reproductive seasons, demonstrating consistent effectiveness of the protocol.

Conclusions

This part of the project enabled the establishment of a potent hormonal therapy, which synchronized gonadal development in mullet females and males, and sequentially induced their spawning. The results indicate that dopaminergic inhibition has a role in two different phases of the reproductive cycle of grey mullet, i.e. at the early stages of vitellogenesis and at the stage of final oocyte maturation and ovulation. A single injection of dopamine antagonist, Domperidone (Dom), helps females complete vitellogenesis and reach the stage of final oocyte maturation, thus overcoming the first reproductive block. Further intervention with hormone-therapy based on GnRHa and another form of dopamine antagonist, metaclopramide (MET), induces mature females to spawn, overcoming the second reproductive block. In addition, by using artificial thermo- and photoperiod regimes the natural spawning season was shifted, making a year-round supply of mullet fingerlings feasible.

Materials and Methods- NIOF

All of the females needed for the Egyptian spawning trials were obtained from the brood stock holding facilities in Mallahet Port Fouad, Sinai, Egypt.

Induction of spawning in 2005 and 2006. All All fish were maintained at marine hatchery in National Institute of Oceanography & Fisheries at Alexandria and Fayoum as follows:

a) Broodstock: Grey mullet brood stock (0.67 to 1.86 Kg body weight), used in the spawning trials were maintained in $3m^3$, fiberglass tanks with stocking density of 2 fish / m^3, manipulated light exposure at the shortest day (14D/10L) during September, October and November.

b) Females: The state of ovarian maturity for each female was assessed prior to each spawning attempt by using polyethylene cannula each sample was fixed in 10% formalin in seawater. In this study, two different strategies for spawning mullet were attempted, the first strategy called for a "Priming" dose of human chorionic gonadotropin hormone or carp pituitary homogenate. After twenty four hours later a second "resolving" injection was giving consisting of LHRH-a. The appropriate dosage was dissolved in 0.9% saline and injected into the dorsal musculature.

c) Males: Males with body weight of 0.63 – 0.87 kg were placed with each treated female, examined for the presence of milt by applying slight pressure to the abdomen, and only running ripe males were used in each spawning attempt. Spawned eggs were fertilized naturally, collected and incubated in 1000 L open system fiber glass tanks. Fertilization rates, average spawned egg diameters, hatching rates and larval developmental stages were recorded.

d) System preparation: A flow through system supplied seawater 34ppt salinity. The water temperature ranged about 25°C ± 1 throughout the experiment. Fish were fed diet containing 30% crude protein and 6 % lipid.

*Total lipid and fatty acid extraction.*Lipids were extracted from the samples by using chloroform-methanol-water (2:2:1.8) according to the method of Bligh and Dyer (1959).Lipid fractions were methylated to obtain fatty acids methyl esters according to Gallagher *et al.* (1984). Fatty acids methyl esters were analyzed by using Shimadzu gas chromatography 4 CM equipped with a flame ionization detector.

Total protein extraction. Total protein was determined using micro-Kjeldahl method (Association of Official Agricultural Chemists,1980).

Amino acid extraction. Extracts of amino acids were prepared according to Moore *et al,.* (1958). Twenty micro liters of each prepared filtrate were injected in the instrument capsule of Beckman 116 GI amino acids analyzer apparatus. All amino acids were expressed as gram amino acid /100 gm. fresh weight of each sample.

Results

Induced spawning. Induced spawning trials were carried out in September, October and November in NIOF Facilities in Shakshouk (Fayoum) and Alexandria. Spawners were injected with various doses of CPE, HCG and LH-Rh-a hormone doses. The best results were obtained using the following procedure: priming injection was 10.000 I.U. of HCG and 30 mg/kg carp pituitary extract. After 24h from the first injection, the cannulated ova samples have diameters of 580 ± 30 µm. The resolving injection was given after 24h from the priming one, with 200 µg /kg LHRH-a. Twelve males of (+2) and (+3) were placed with injected females. Sex ratio was 3 males: 1 female. After 24 hours from the second injection the females spawned fertilized eggs. The egg diameter was about 680 ± 10 µm. The number of ova spawned ranged between 1.360.000 and1.950.000 eggs / spawned fish. The percent of fertilization was about 79% from the total number of ova. Table (2) shows the results of one of the successful trials for the spawned females.

Total lipid content of ripe ovary : The maximum content of total lipid was recorded in ripe ovary. Gradual decrease was recorded in total lipid content in relation to hormonal injection. After first injection highly significant decrease was noticed in total lipid ($P< 0.01$); whereas, no significant difference in total lipid of ovary was noticed after second injection. However, sharp decrease in total lipid content was recorded for ovary after third injection and egg spawning as indicated in Table (3). This decrease in total lipid for spawned eggs indicates direct fat transfer from fat storage into reproduction involvement.

Fatty acids content in ripe, injected ovaries and spawned eggs of female Mugil cephalus:

a) Saturated fatty acids: Highly significant increase were recorded between normal ovary and spawned egg for each of carpoic acid ($C_{6:0}$) and carpylic acid A ($C_{8:0}$) at $p < 0.01$, whereas insignificant correlation for injected ovary at $p > 0.01$. Three saturated fatty acids (C_{11}, $_{12}$ and $_{15}$) disappeared in spawned eggs, while more or less detected in injected ovary. Significant correlation were recorded between spawned egg and normal ovary for each of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) at $p<0.05$.

b) Poly-unsaturated fatty acids: Highly significant decrease correlation were noticed between ripe ovary and spawned egg for each of the four Poly-unsaturated fatty acids Linolenic ($C_{18:3}$), Eicosapentadecanoic ($C_{20:5}$), Arachidonic acid ($C_{20:4}$) and ($C_{22:}$

$_2$) at P<0.01. Significant correlation between ripe ovary and spawned egg for ecosatrienoic acid ($C_{20:3}w_6$) at P<0.05, while insignificant correlation for the injected ovary for two and three injections at P>0.01. Highly significant correlation between ripe ovary and injected ovary for one and two injections for arachidonic acid at P<0.01, whereas insignificant correlation were recorded between ripe ovary and injected ovary for three injections at P<0.05 . The results suggest the role of arachidonic acid in injected and spawned *Mugil cephalus*. Arachidonic acid is an important precursor of prostaglandins which have important regulatory roles in the body including vasodilatation, platelet aggregation and perhaps osmoregulation".

Total protein content of female Mugil cephalus: The total protein content reached a maximum value Table (4) in the injected ovary with second injections (38.63 ± 1.25). The minimum value of total protein content was recorded in spawned egg (24.49 ± 1.53) with highly insignificant decrease correlation in relation to ripe ovary at P>0.001.

Amino acid content of female Mugil cephalus:
a) Essential amino acids: Valine, leucine and lysine were the highest essential acids in the ripe ovary of *Mugil cephalus*, while histidine was the lowest. The present results showed that, eight essential amino acids (Threonin, Valine, Methionine, Isoleucine, Leucine, Phenylalanin, Histidine and Arginine) exhibited the same trend, being increased in the ripe ovary, which was followed by a significant decrease in spawned eggs. This increase in the ripe ovary may be related to their importance in the development of a ripe ovary, while decreasing after spawning, fertilization and formation of the embryo. Amino acids seemed to be mobilized for energy in the last part of the yolk sac stage.

b) Non essential amino acids (NEAA): Aspartic, glutamic and alanine had the greatest value in the ripe ovary for the NEAA, while cystine the lowest value. The maximum content for each of the eight NEAA (Aspartic, Serine, Glutamic, Proline, Glycine, Alanine, cystine and Tyrosine) were recorded in ripe ovary. Then fluctuated after hormone injections with decrease in concentration then reached to the minimum content in the spawned egg. Highly significant decrease was recorded between concentration of NEAA in ripe ovary of female *Mugil cephalus* and spawned egg at P< 0.01. The results showed that glutamic acid content reached a maximum value in ripe ovary, and then fluctuated in injected ovary with insignificant correlation with the injected ovary. This information will be used as a guideline for development of appropriate feeds for use in larval rearing and/or broodstoock diets.

The histological, histochemical and ultrastructure of gonads and pituitary gland:
a) Ovaries : Immature, maturing, and nearly ripe ovaries were developed with a normal shape, while the ripe ovaries had abnormal and atretic ovae. The fine structure of the wall in nearly ripe ovae appeared with five different normal layers (thecal, basement membrane, follicular epithelial, zona radiata externa, zona radiata interna and the absence of a cortical alveoli layer). The fine structure of the wall of ova of ripe ovaries was characterized by atretic and deformed layers with damaged thecal layer, basement membrane and follicular epithelial layer. The fine structure of the wall of ova in the resorbed ovaries appeared atretic and abnormal.

b) Testes : Investigation of testes of *M. cephalus* indicated that the interlobular connective tissue between the lobules at all stages of maturity were abnormal and were enlarged with collagenous tissue. This abnormal tissue is considered a sign of infertility. All the spermatozoa fine structure appeared with deformed head, tail and mitochondria. The chromatin material were atretic and deformed in their distribution surrounding the head and tail of spermatozoa.

c) Pituitary : The pituitary gland of *M. cephalus* showed damaged and atretic gonadotropic cells which are responsible for secretion of the sex steroid hormone and give a signal for hormonal control for sex maturation, differentiation and spawning. The fine structure of the gonadotropic cells at all stages of maturity indicated that the granulation of the cells were few in number and small in size.

II – Larval Rearing

Introduction

There are only a few papers in the literature dealing with the larval rearing of grey mullet (Murashige et al. 1991; Yoshimatsu et al. 1995; Harel et al. 1998). However, there is general consensus that a major factor limiting the commercial rearing of the grey mullet is the high mortality occurring during early larval development when the fish are feeding on rotifers (*Brachionus spp.*). Although little is known of the nutritional requirements of mullet larvae, preliminary studies have shown that enriching the rotifers with the n-3 highly unsaturated fatty acids (HUFA); eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) improved survival, growth and stress resistance (Eda et al. 1990, Tamaru et al. 1992). These fatty acids play a critical role in membrane function and structure in the tissues of marine fish larvae in general and must be supplied in the diet. (Koven et al. 1990; Sargent et al. 1993). Another fatty acid considered essential for the larvae of a number of marine species is arachidonic acid (ArA, 20:4n-6), which has been associated with improved larval survival and resistance to acute stress in the gilthead seabream (Koven et al.2001, 2003). Although a number of authors have addressed the problem of stress in larviculture, the efficacy of dietary ArA has not been examined in larvae of this species. Finally, use of vitamin C has received little exploration.

By agreement, the laboratories at both Alexandria and Eilat were to investigate the requirements of mullet larvae for highly unsaturated fatty acids (HUFA). Rotifers and artemia were the food of choice. The objectives were to determine the optimal conditions for stimulating larval growth and determining the basic nutritional requirements which, when coupled with effective environmental conditions, will form the basis of a practical protocol to commercially rear mullet larvae from hatching to 35 days post hatching (DPH). The Israelis at Eilat were to test growth under a large range of environmental conditions, and at different levels of n-3 HUFA having a high DHA/EPA ratio. In addition, the Egyptians at Alexandria tested the addition of vitamin C to the food mixtures.

Methods

Preliminary study – effect of enrichment on growth, survival and stress:

Prior to conducting the main study in 27 l aquaria, a preliminary trial was run in Eilat on mullet larvae reared in six 400 l V-tanks to determine which stressors (salinity, air exposure, handling) would be tested in the primary aquarium experiment and the

protocols for carrying them out. In each set of three 400 l tanks, larvae from 3 to 17 days post hatching (DPH) were reared at 25 ‰ and fed rotifers previously enriched at 29 °C in 200 l V-*tanks* on low or high *n-3* HUFA emulsions whose compositions are listed in Table 5 The *n*-3 HUFA are comprised almost entirely of the essential fatty acids; eicosapentaenoic acid (EPA, 20:5*n-3*) and docosahexaenoic acid (DHA). After 17 DPH Artemia similarly enriched on these two emulsion treatments were fed together with the rotifers to the end of larval rearing at 35 DPH. The 400 l tanks during rotifer feeding were "greened" with *Nannochloropsis* sp., which has been reported to enhance mullet larval rearing (Yoshimatsu et al. 1995). This alga, although rich in EPA and deficient in DHA, was added at relatively low concentrations (2 x 10^5 cells ml^{-1}), which would not significantly influence rotifer fatty acid composition. Larval samples were taken on 8 and 17 DPH to determine the average dry weight (DW) in fish fed the *n-3* HUFA treatments. On 23 DPH, nine groups of 10 larvae from each selected treatment V-tank were transferred to three sets of three 300 ml plastic cups where each set contained 0, 25 or 40 ‰ water at rearing temperature. By monitoring accumulated larval mortality over 60 minutes, the effect of salinity challenge, as a function of *n*-3 HUFA level, was tested in triplicate replicates. The larvae transferred from 25 ‰ ambient seawater in the 400 l tanks to 25 ‰ water in the cups were considered exposed to handling stress only.

Main aquarium study: On 17 DPH, mullet larvae were transferred to twenty-five 27 l aquaria and stocked at 300 larvae $aquarium^{-1}$. Ten and fifteen aquaria were stocked with similarly sized fish that were reared on rotifers having a low or high *n-3* HUFA content, respectively. The larvae were then fed Artemia nauplii previously enriched on one of the two treatment emulsions in order to test in replicates of 5 aquaria the separate and combined effect of rotifers and Artemia containing different levels of *n-3* HUFA (Table 6) on growth and stress resistance. An additional Artemia treatment was tested on larvae previously fed high *n-3* HUFA rotifers, which were enriched with an emulsion containing both high *n-3* HUFA and arachidonic acid (ArA, 20:4*n*-6).

Acclimation study- The effect of dietary n-3 HUFA on low salinity acclimation: In this study, 12 aquaria (27 L) were stocked with 350 (23 DPH) larvae $aquarium^{-1}$ where in each set of 6 aquaria, larvae from 3 aquaria were fed either a commercial weaning diet (Coppens, the Netherlands) which is relatively low in *n*-3 HUFA or the NCM prepared microdiet (FeedPro, Israel) which is high in *n*-3 HUFA. The salinity was gradually decreased in three aquaria from each set of 6 aquaria over 10 days from 25 to 8 ‰ (Fig. 4) and the survival monitored.

Acclimation study- The effect of larval size on low salinity acclimation: In this trial, 18 aquaria (27 L) were stocked with 350 (23 DPH) larvae $aquarium^{-1}$ where in each set of 9 aquaria, large larvae (ca 16 mg) were stocked in 5 aquaria while small larvae (ca 2 mg) (Fig. 5) were stocked in 4 aquaria. The salinity was gradually decreased in one set of 9 aquaria over 14 days from 25 to 0 ‰ (Fig. 5). Samples were taken for cortisol and Na^+-K^+ATPase determinations to evaluate the stress response in these treatments. These samples are presently being prepared for analyses.

Vitamin C trial-In actuality, precise coordination between Eilat and Alexandria laboratories during this phase of the experiments was somewhat inhibited owing to the Egyptians' need to design, construct, and furnish laboratories comparable to those

of the Israelis. This was, in fact accomplished, as will be described in later sections of this report. These laboratories were, in fact, completed nearly three years into the project, and with permission of USAID the Egyptians occupied an additional year of experimentation for this purpose.

Eggs of *Mugil cephalus* were obtained from females spawned in October 2005 in NIOF. Hatched larvae were stocked in 120 L conical fiberglass tanks at a density of 60 larvae per liter. Rearing conditions were as follows: water flow = 2 L/min; salinity = 32 ± 2 ppt; water temperature = 16-18° C; tanks kept in shaded natural light conditions. Fatty acid rich oils delivered to Alexandria from Japan were emulsified with lecithin and mixed with water then added to rotifers for 12 hr at 0.2 g emulsion for one million rotifers. Vitamin C (ascorbyl palmitate) was added at 20% W/V to the oil emulsion.

Results and Discussion

Preliminary study- The average dry weights (DW) on 8 and 17 DPH of larvae fed the dietary treatments are shown in Fig. 8. Although there was no treatment effect in 8 DPH larvae, fish fed rotifers containing high levels of *n-3* HUFA grew markedly better by 17 DPH than their counterparts ingesting the low *n-3* HUFA treatment. This attests to the importance of dietary *n-3* HUFA in early mullet larval growth and supports earlier findings of similar studies on the *n-3* HUFA requirement of marine fish larvae. On 23 DPH, the effect of two types of salinity stress (0 and 40 ‰), handling and exposure to air (30 and 60 seconds) on larval mortality were tested. The preliminary studies showed that the larvae were stress resistant to air exposure, independent of duration tested, as well as handling stress carried out at ambient salinity (25‰). In contrast, larvae exhibited low resistance, which was *n-3* HUFA dependent, to rapid exposure to 0 ‰ although better resistance was shown to 40 ‰ (Figs. 6a,b).

Main aquarium study- Fish reared on high *n-3* HUFA rotifers and Artemia (high-high, Fig. 7) clearly showed reduced accumulated mortality when immersed in 0 ‰ water compared to larvae fed both rotifers and Artemia containing low *n-3* HUFA (low-low). This suggested that a nutritional history of high EPA and DHA consumption imparts enhanced stress resistance in fish exposed to a severe drop in salinity.

In Fig. 8 supplementing ArA to the emulsion fed to Artemia resulted in a non-significant tendency to increase accumulated mortality in fish exposed to 0 ‰. This could have resulted in an increase in the membrane ArA/EPA ratio, which would have increased ArA derived eicosanoids and possibly reduced osmoregulatory compensation during the stress of rapid salinity drop.

Accumulated mortality was very similar in larvae fed high *n-3* HUFA in rotifers only or in both rotifers and Artemia. In comparison, larvae fed the increased levels of *n-3* HUFA only during Artemia feeding exhibited increased, although not significantly, accumulated mortality (Fig.9). This reinforced our earlier observation in the 400 l tanks that feeding high levels of *n-3* HUFA during rotifer feeding, which would increase the endogenous store of these essential fatty acids, tends to promote better survival in older mullet larvae.

In summary, fish fed rotifers containing high levels of *n-3* HUFA grew significantly better than larvae fed rotifers containing low levels of these essential fatty acids. In contrast, dietary *n-3* HUFA appeared to promote growth less well during Artemia feeding attesting to the importance of DHA in early larval pre-metamorphic development. Mullet larvae demonstrated high stress resistance when exposed to handling as well as 30 and 60 seconds of air exposure, independent of dietary supplementation of EPA and DHA. On the other hand, the larvae were sensitive to rapid salinity change that was dietary *n-3* HUFA dependent, particularly a decrease in salinity from the ambient 25‰ to 0 ‰ . Mullet larvae ingesting high *n-3* HUFA during only rotifer or rotifer and Artemia feeding exhibited enhanced stress resistance and reduced mortality during low salinity challenge compared to fish ingesting increased EPA and DHA levels only during Artemia feeding. On the other hand, supplementing ArA and high *n-3*HUFA together in the diet tended to increase mortality during exposure to 0 ‰. The results from the stress tests supported earlier findings that suggested *n-3* HUFA ingestion during rotifer feeding might influence late larval and juvenile quality more than during Artemia feeding.

As a result of these studies, as well as others on abiotic factors (not reported here), a larval rearing protocol for mullet was developed that included detailed guidelines for salinity, water exchange, algal tank concentration, temperature, aeration, enrichment of live food, schedule for weaning on to dry particulate diets. The successful application of this protocol was clearly shown in the fall of 2006 where over 60,000 fry were produced 3 months after a high quality spawn.

Acclimation study- Fig. 10 shows a non-significant increase in survival in fish fed the high *n*-3 HUFA diet regardless of salinity treatment. More importantly, the mullet larvae.exposed to a reduction of salinity from 25 to 6 ‰ and fed a high *n*-3 HUFA diet survived well (>75%). In the second trial, large mullet larvae showed significantly ($P<0.05$) higher survival than their smaller cohorts (Fig. 10), which was independent of their saline environment. Moreover, these larger fish survived similarly to the first study (ca 75%) when salinity was decreased from 25 ‰ to that of freshwater. This suggests that freshwater acclimation could be incorporated into the larval rearing protocol in fast growing larvae previously fed a high *n*-3 HUFA diet leading to a dependable supply of mullet fry already pre-adapted to freshwater.

Vitamin C trial- The recent results obtained at Alexandria are illustrated in Figs. 11 & 12. Optimal growth at that laboratory, as evidenced by total length, dry weight and survival was achieved when a mixture of DHA and EPA was added to rotifers as feed for mullet larvae. The addition of arachidonic acid (ArA) to DHA only improved survival of larvae but less than a combination of DHA and EPA. The addition of vitamin C to HUFA improved the survival of mullet larvae.

Overall conclusions

a) Dietary *n*-3 HUFA during rotifer feeding significantly improved growth while these fatty acids were less effective during *Artemia* feeding.
b) Mullet larvae demonstrated high stress resistance to handling a well as air exposure independent of *n*-3 HUFA supplementation.
c) Conversely, larvae were sensitive to rapid salinity change that was dietary *n*-3 HUFA dependent particularly from 25-0 ‰.

d) Larvae ingesting high n-3 HUFA during only rotifer or rotifer and Artemia feeding exhibited enhanced stress resistance, in terms of accumulated mortality, compared to larvae ingesting high n-3 HUFA only during Artemia feeding.
e) Possibly both dietary *n*-3 HUFA and algal level play roles in pigmentation and metamorphic success.
f) Preliminary results suggest that *n*-3 HUFA are also effective in growth, survival and reduced deformities during post metamorphosis.
g) Preliminary results suggest that during larval rearing (23 DPH) ambient salinity can be successfully reduced to 0 without markedly affecting survival.
h) DHA, EPA, and vitamin C enriched rotifers has a positive effect on survival and growth of mullet larvae.

III – Nutrition Research

Introduction

A modest program of mullet monoculture is being conducted in Taiwan using formulated feeds, almost nothing, however, has been published about nutritional requirements.

The Egyptian and Israeli scientists agreed that the most important route to follow would relate to quantifying protein and energy needs of mullet to ensure optimal growth. These are recognized as the major nutritional needs of fish for maintenance and growth. This methodology, derived from terrestrial animal husbandry, has already been applied to a number of marine cultured species, including gilthead seabream, European sea bass, and white grouper (Lupatsch et al 1998, 2001, 2003).

The Eilat and Alexandria laboratories pursed somewhat parallel lines of research, adding measured amounts of feed on a time-controlled basis, keeping careful track of environmental changes (e.g. temperature, salinity etc.) trading their data along the way.

Objectives

The agreed-upon goals of both laboratories included:

(a) Development of an optimal growth curve for mullet growth in tanks (Israel) and earthen ponds (Egypt) which can be used to predict growth of the fish along the whole range of sizes from juvenile to market size (400g).
(b) Determination of digestible protein and energy requirements for maintenance and growth of mullets at different sizes from juvenile to market size. Use of four protein levels for growth trials in Egypt.
(c) Gross body composition changes across the weight range from juvenile to market size.
(d) Determination of metabolic body weights for protein and energy from the starvation treatments in the short-term growth trials (primarily in Israel).
(e) Determination of the efficiency of utilization of protein and energy from data of the short term feeding trials, involving formulas developed at both laboratories.
(f) Development of a database for daily protein and energy necessary for different sizes of mullets to achieve their growth potential at specific water temperatures.

Methods- NCM

a- Fish were grown out in experimental tanks to market size on a surplus of feed (fed to satiation) in experimental 700 L tanks with constant sea water exchange and aeration..

b- Daily feed intake of fish was monitored during the growout of fish to market size.

c- Mathematical expressions were developed to describe weight gain and feed intake of fish grown to market size.

d- Periodic sampling was carried out at different sizes of fish during the development of the optimal growth curve for analysis of body composition changes that are occurring.

e- Laboratory analysis of feeds and mullet for proximate composition: protein, lipid, moisture, and ash using standard methods AOAC were carried out on samples. Energy content of feeds and mullet was determined using a Parr bomb calorimeter with benezoic acid as the standard.

f- Development of mathematical expressions defining metabolic body weight for protein and energy from the cumulative data of the starvation trials were made.

g- Short term feeding trials (30 – 45 days) with increasing levels of feed from starvation to excess to determine protein and energy retention as a function of protein and energy intake were carried out.

h- Mathematical expressions to describe the relationship between nutrient intake (protein or energy) and their retention and the efficiency with which the fish utilizes these nutrients were developed.

i- Calculation of daily protein and energy needs for different size of mullets to achieve their growth potential was made from the mathematical expressions developed.

j- Use was made of the mullet's daily protein and energy requirements to show how we set the feed formula criteria to meet these needs for fish of different sizes.

Results

a- The following equation describes the formal approach used in calculations of protein and energy requirements of mullets for maintenance and growth:

$$\textbf{Energy or protein requirement} = a \times BW\,(kg)^{b} + c \times \textbf{growth}$$

Where $BW\,(kg)^{b}$ = metabolic body weight

a = maintenance requirement

c = cost in units of energy or protein to deposit energy or protein as growth.

b- The equation developed for defining daily weight gain at specific fish sizes (Figure 13) is:

$$\textbf{Weight gain (g / day)} = 0.00226 \times BW\,(g)^{0.557} \exp^{(0.140 \times T)} \qquad r^2 = 0.89$$

for W = Weight (g) of fish for weights between 1 and 400 g.

T = Temperature between 20 - 27^{0}C.

c- The equation describing feed intake at certain fish sizes and water temperatures (Fig. 13) is:

$$\textbf{Feed intake (g / day)} = 0.0029 \times \textbf{Weight (g)}^{0.653} \times \exp^{0.135 \times Temp} \qquad r^2 = 0.93$$

d- Changes in body composition as a function of fish size are defined by the following three equations and the changes are illustrated in Fig. 14:

$$\textbf{Energy (kJ / g)} = 5.25 \times BW\,(g)^{0.131} \qquad r^2 = 0.91$$

$$\textbf{Lipid (\%)} = 6.12 \times BW\,(g)^{0.196} \qquad r^2 = 0.89$$

Moisture (%) = **$75.96 \times BW\ (g)^{-0.038}$** **$r^2 = 0.92$**

e- Metabolic body weights for protein and energy are defined from the short term growth trials using the starvation data:

Energy loss (kJ fish^{-1} day^{-1}) = $46.2\ (\pm 1.84) \times BW(kg)^{0.80}$ **$r^2 = 0.97$**

Protein loss (g fish^{-1} day^{-1}) = $0.38 \times BW(kg)^{0.70}$ **$r^2 = 0.96$**

The exponents of $(kg)^{0.80}$ and $(kg)^{0.70}$ can thus be described for mullet as the metabolic body weights for energy and protein, respectively (Fig. 15).

f- Figure (16) which illustrates the relationship between digestible protein intake ($x=g/kg^{0.70}$) and protein gain ($y=g/kg^{0.70}$) in mullets at two different temperatures and indicates linear relationships defined by the equations:

Trial at 23^{0}C $y = -0.20 + 0.42\ x$ **$r^2 = 0.96$**

Trial at 26.5^{0}C $y = -0.31 + 0.41\ x$ **$r^2 = 0.95$**

What is clear from these expressions and the Figure is that at 0 protein gain the maintenance requirement for protein increases with water temperature, being 0.48g $(kg)^{-0.70}$ at 23^{0}C and 0.75g $(kg)^{-0.70}$ at 26.5^{0}C. But, as the slopes of both expressions, which refer to the efficiency of use of protein for growth, are the same (0.42) we know that efficiency is not affected by water temperature.

g- The relationship between digestible energy intake ($x=g/kg^{0.80}$) and energy gain ($y=g/kg^{0.80}$) at the same temperatures (Figure not shown) are also linear and described by the equations:

Trial at 23^{0}C: $y = -27.3 + 0.54\ x$ **$r^2 = 0.97$**

Trial at 26.5^{0}C $y = -39.0 + 0.55\ x$ **$r^2 = 0.97$**

As with protein, energy needed for maintenance (0 growth) also increases with temperature being 50.7 kJ $(kg)^{-0.80}$ at 23^{0}C and 70.9 kJ $(kg)^{-0.80}$ at 26.5^{0}C. Slopes of both expressions are parallel and equal to 0.55, the efficiency of energy use or growth in mullets so as in protein they are not effected by temperature.

h- Using the various mathematical expressions developed in this study, we calculated the required feed intake and it's digestible protein and energy content for mullets of four sizes that is needed for them to obtain their predicted weight gain at 23°C. The following table provides this information:

Body weight, per fish	5g	50g	250g	750g
Predicted wt. gain, g day^{-1}	0.14	0.50	1.23	2.26
Feed intake, g day^{-1}	0.21	0.93	2.67	5.47
DE need, kJ fish^{-1}day^{-1}	2.38	12.61	40.95	91.90
DP need, g fish^{-1} day^{-1}	0.066	0.257	0.670	1.298

i- The following table shows how we use the information provided in h- above to set criteria for feeds to meet the fish's protein and energy needs at these weights:

Body weight, per fish	**5g**	**50g**	**250g**	**750g**

Using a predetermined DE content of feed of 12 or 15 MJ per kg								
DE content of feed	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*
Required feed intake, g $fish^{-1}$ day^{-1}	0.20	0.16	1.05	0.84	3.41	2.73	7.66	6.13
Resulting DP content of feed, g kg^{-1}	330	412	245	306	196	245	169	212
Predicted FCR	1.43	1.14	2.10	1.68	2.77	2.22	3.39	2.71
DP/DE ratio, g MJ^{-1}	27.5	27.5	20.4	20.4	16.3	16.3	14.1	14.1
Using a predetermined DP content of feed of 250 or 350g per kg								
DP content of feed	*250g*	*350g*	*250g*	*350g*	*250g*	*350g*	*250g*	*350g*
Required feed intake, g $fish^{-1}$ day^{-1}	0.26	0.19	1.03	0.73	2.68	1.91	5.19	3.71
Resulting DE content of feed, MJ kg^{-1}	9.10	12.73	12.24	17.27	15.27	21.43	17.70	24.77
Predicted FCR	1.86	1.36	2.06	1.46	2.18	1.55	2.29	1.64
DP/DE ratio, g MJ^{-1}	27.5	27.5	20.4	20.4	16.3	16.3	14.1	14.1

Discussion and Conclusions

The mathematical expressions developed in the course of this study allow us to accurately formulate feeds for the rearing of grey mullet. Feeds that provide sufficient nutrients (protein and energy the major nutrient needs of fish) for both maintenance and predicted growth while having a minimal effect on environmental enrichment. Such feeds are more efficient hence, less costly and are more environmentally friendly, very desirable characteristics for fish farming enterprises.

Methods - NIOF

a- Average growth performance of grey mullet M. cephalus, reared in earthen ponds was determined for fish fed at four dietary protein levels (30, 40, 50 and 60%).
b- Digestibility coefficients of the four dietary protein levels were obtained using the stripping method to collect feces.
c- Gross body composition of grey mullet flesh (initial and final body weight) was determined for the reared fish.
d - Nutrient retention efficiencies of protein lipid, and energy were obtained using the formula:

NRE = [Nutrient gain in carcass/(Nutrient intake x Digestibility of nutrient)].

e- Economic evaluation was applied to the experimental diets.

Actual experimental procedures

(a) Three ponds with water volume of about $90m^3$ and a fourth pond with about $290m^3$were used.
(b) Stocking density was one fish/m^3 in all ponds.
(c)The experimental period lasted for three months.
(d) The amount of daily ration (3% from live body weight) was divided into two equal meals at 10 a.m. and 2 p.m. daily .
(e) Hand feeding was applied to ensure that the feeds are available to all the fish in the ponds for consumption .
(f) The chemical analyses were carried out to estimate moisture, crude protein, ether extract, crude fiber and ash content according to the methods of AOAC (1984), and

nitrogen-free extract was calculated by difference. Gross energy were measured using Oxygen Bomb Calorimeter.
(g) Digestibility trial was conducted for ten days. 0.5% chromic oxide was added to the diets .The feces were collected by stripping.
(h) Water samples were taken every two weeks for analysis of dissolved oxygen, ammonia, and salinity, using the American Public Health Association (APHA, 1992) methodology. The pH values were measured by digital pH meter (model 68 of "Systems and Engineered Designs").

Calculations:
*** Specific growth rate (%/day) =**
[(Ln Final body weight – Ln Initial body weight)/Period (day)] x 100

***Feed conversion ratio (g./g.)=Feed (g.)/Gain (g.)**

Digestibility coefficient,% = 100-100[(Ii / If) x (Nf / Ni)],
Where I=concentration of chromic oxide,I = ingest, N=concentration of nutrient (%,demand f = feces.

Nutrient retention efficiency = NRE=[Nutrient gain carcass/(Nutrient intake X Digestibility of nutrient)].

Results
a- Dietary protein level requirements were decreased to 30% for grey mullet *M. cephalus* reared in earthen ponds as compared with 60% in tanks.

b- Digestibility coefficient of protein was higher for grey mullet, *M. cephalus* fed at 30% protein level compared to those fed at 40, 50 and 60%.

c- Nutrient retention efficiency of protein was higher for grey mullet *M. cephalus* fed at 30 % protein than 40, 50 and 60 %.

d-The net revenue percentage of grey mullet *M. cephalus* fed at 30% protein level has increased by about 76%, higher than those 40, 50 and 60% level .

e-The artificial feed could be considered as supplementary feed beside the natural feed.

Conclusions:

The effectiveness of diets formulated on the basis of digestible energy and nutrients can be evaluated by measuring weight gain ,feed efficiency (gain ,g./feed g.) or feed conversion (feed, g./gain ,g.) and body composition of fish receiving the diets under particular culture regimes.

The efficiency of each feedstuff in the diets can be evaluated using the following scheme:-
(a) Analyzing feed ingredients for their chemical composition.
(b) Measuring digestibility of feed ingredients.

(c) Formulating and reformulating balanced diets in combination with several ingredients or substitution with other ingredients.
(d) Observing the levels of productivity supported by these diets.
(e) Measuring the feed intake and weight gain and calculating the feed efficiency.
(f) Calculating energy and nutrient retention efficiencies by analyzing fish carcasses. NRE can be calculated using the formulae: **NRE = [Nutrient gain in carcass / (Nutrient intake x digestibility of nutrient)].**

IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

With the obvious exception of political unrest, there can be no subjects of greater importance to Middle East cultural and economic well-being than food resource expansion and water conservation.

Prior to the advent of MERC a quarter of a century ago, technology transfer in the Middle East was hopeless. Although the Israelis had perhaps the best technology in the region, external contact was zero. The dramatically expanding influence of MERC itself, aided by Egyptian leadership, has already and will undoubtedly continue to, develop relationships, as already demonstrated, with Lebanon, Jordan, Tunisia, Morocco, and the Palestinians. The author's personal contacts with Jordanians and Palestinians brought assurances of desire for collaboration nearly two decades ago. Several projects between them and Israelis have already matured.

At one time, the Saudi Government actually signed an agreement to collaborate with the Egyptians on a cooperative Egypt/Israel mariculture project; this was unfortunately curtailed. A very high level American consultant team, which has recently been contracted by the Saudis to review their Red Sea facilities, has been advised to explore the possibilities of enlarging their mariculture program, in which grey mullet might be featured.

As referred to above, both laboratories intend carrying the project further. The Egyptian government is already providing assistance and the Israeli Ministry of Agriculture is already sponsoring preliminary studies designed to expand monoculture of mullets. It is difficult to state how much future expansion will occur but it is fairly obvious that mullet production will expand in both countries. Of two projects submitted to MERC by Egypt and Israel which involve grey mullet, one is in the last stages of contract negotiation and the other is in the review process. Both studies are expected to advance mullet culture significantly in the region in the years to come.

By their own admission, all of the project scientists have learned much about the mullet; their level of knowledge is world class. Clearly, were it not for the maddeningly frustrating political tensions, mullet culture would become a win-win proposition.

PROJECT ACTIVITIES/OUTPUTS

The project scientists all met together at the newly developing Cyprus Research Foundation at Larnaca, Cyprus. The Cypriots, with assistance from MIT, are in the

process of forming a Research Enterprise with a formidable facility and an ambitious program plan. Their mariculture activity is already impressive.

The Foundation's Board of Trustees actively seeks a strong and productive relationship with our MERC scientists, as a means of furthering their own marine-oriented aspirations. Accordingly, they assumed responsibility for all costs associated with the meetings except transportation. This was particularly appealing to the author who was, and is, paying all of his own expenses on the project. These meetings took place in March 2004, 2005, and 2006.

In addition to the intensive work sessions at the annual meetings, the project scientists maintained electronic mail contact through out the project. This facilitated moving the project research forward and allowed the development of two additional grant proposals which are in various stages of negotiation or review for eventual funding. Both proposals are natural outcomes of the grey mullet work done under this project and should help in the expansion of regional mullet culture and the development of regional land-based polyculture systems that are environmentally friend.

The project produced the following publications:

- Aizen, J., Meiri, I., Levani-Sivan, B. and Rosenfeld, H. 2005. Enhancing spawning in Grey mullet by removal of dopaminergic inhibition. *General and Comparative Endocrinology*, 143: 212-221.
- El-Garabawy, M.M. and Assem, S.S. 2006. Spawning induction in the Mediterranean grey mullet *Mugil cephalus* and larval developmental stages. *African Journal of Biotechnology,* vol. 5(19): 1836-1845.
- El-Garabawy, M.M, Assem, S.S. and ismail, R.F. 2006. Steroid hormone in serum of female *Mugil cephalus* in relation to steroidogenic tissue (ultrastructure) in Lake Quaron. *Egypt. J. Aquat. Bil. & Fish.* vol. 30.
- El-Garabawy, M.M, Assem, S.S. and Fahmy, A.F. 2007. Steroid hormone in serum of male *Mugil cephalus* in relation to steroidogenic tissue (ultrastructure) in Lake Quaron. In press.
- Abdel Rahman, S.H. and Helal, A.M. 2007. Evaluation of various feeding regimes for optimal growth and survival of early stages of grey mullet, *Mugil cephalus. Egypt. J. Aquat. Bil. & Fish.* vol. 30.

A number of students, and technicians took part in this project in Israel and Egypt. Some of the participants were doing research dealing with grey mullets towards meeting their master's thesis requirement, while others were undergoing training in aquaculture research methodologies.

Israeli participants: M.Sc students: a) Y. Aizen – 'Spawning induction of grey mullet (*Mugil cephalus)'* b) R. Solomonovich – 'Moleculat characterization of sex reversal in grey mullet (*Mugil cephalus*).

Reseach associates & technicians: a) Dr. Iris Meiri, b) Dr. I. Lupatsch, c)G. Heinisch, d) E. Toledano, e) S. Lutzky, f) O. Nixon-Stupler, g) I. Geva, h) B. Ginsberg, i) Y. Chernov and j) R. Kastel.

Egyptian participants: M.Sc students: a) Ranai Fahmy Ismael (Masters thesis- Alexandria University, b) Hasan Ez El Din Mohammed (Masters thesis – Asyout University).

Reseach associates & technicians: a) Dr. Saffa Mohammed Abdel Kawy, b) Dr. Samira Shehata Asem, c) Suzan Mohammed Fadda, d) Mohammed Abdel Hamid El Okby, e) Mohammed Ata Allah, f) Abdallah Saad Mahmoud and g) Mahmoud Gaber Mohammed.

PROJECT PRODUCTIVITY

Did the project accomplish all of its goals? Candor requires the response: Technically – very much; socially – only partly.

On the plus side, the Egyptians have completed an excellent building program and have made wonderful progress in their R&D start up. The Israelis have made outstanding inroads in mullet culture and with the Egyptians, have solved many of the reproduction, larval rearing and nutrition problems.

Mid-East politics continues to dominate an otherwise marvelously effective program. The scientists learned how to culture mullet. They will continue to learn more about this invaluable food resource as time goes on. The technology is fostered by both governments and business interests in Israel. Many stomachs will be satiated by mullet and money will be made.

While one's instincts may argue that the U.S. Government might, itself, do more to ease both official and interpersonal contacts, political considerations are beyond the ken of this program and its principal investigators, including this author. At the minimum, however, it must be pointed out that travel by Egyptians to Israel and Israeli to Egypt was permitted by neither of their governments, for ost of the period of the project.

Regardless of the reasons, honesty compels the admission that interpersonal contact during the course of this project was disappointing and could have been helpful. The three annual meetings were among the most productive and impressive within this author's memory. More personal travel by the Principal Investigator (this author) might have been useful, but he had personally spent far beyond his means on the project, as it was, and simply could not afford further costs.

FUTURE WORK

One of the most promising achievements of this project is its stimulation of future work. The project has occasioned interest, not only with its own technical community, but broadly through the concerned societies in both countries. Work is already underway in both Egypt and Israel in all three of the major research subdivisions: reproduction, larval rearing and nutrition. The scientists believe in common that their goals are much closer than when the project began, and can be achieved in the

immediate future. The ultimate result will be possibly the most completely desirable food source in the region.

The Egyptians have already demonstrated an insatiable market for mullets as a food resource, and Israeli kibbutzim have begun to consider monoculture of mullets to expand annual production. The development and expansion of by-products of mullet culture (Batarekh/Karasumi) are also been investigated.

The mullet project, however, is only one of a catalog of excellent projects of MERC, possibly the most imaginative, productive, and socially enhancing programs that this country ever formulated for the Middle East. Properly developed, MERC can provide outstanding leadership for American government and business interests in that troublesome but vitally important region. In this regard, however, USAID has failed to capitalize its potential.

The fact is that MERC's Achilles heel is its internal communications failure. Each of the Principal Investigators has developed, individually, both technical, administrative, and diplomatic techniques ranging from successful to highly successful. Each year, we try to persuade USAID to call together all of its imaginative PIs to trade ideas, techniques, problems, and opportunities. And (at risk of sounding like a broken record) this author must admit total failure.

Sixty-five years of working in-or-with Government has failed to provide this P.I. with any degree of rationale as to why these potentially invaluable advisors cannot be convened at least twice per decade, to further advance a cooperative food-oriented program in the Middle East on which the U.S. Government spends one twentieth of one percent of what it spends on military assistance.

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Appendix: Tables and Figures

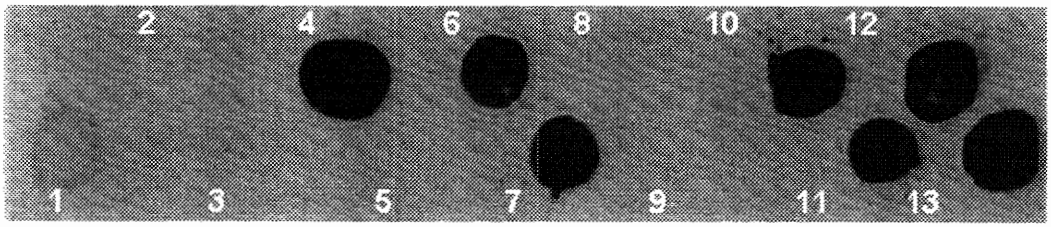

Fig. 1 Dot blot immunoassay for the detection of mullet Vtg by heterologous antibody rose against seabream vitellogenin (anti-sb-Vtg). Mullet plasma samples (Dots 1-13) were applied on a nitrocellulose membrane and incubated with anti-sb-Vtg, which was pre-absorbed on an equivalent membrane loaded with plasma of mature mullet males.

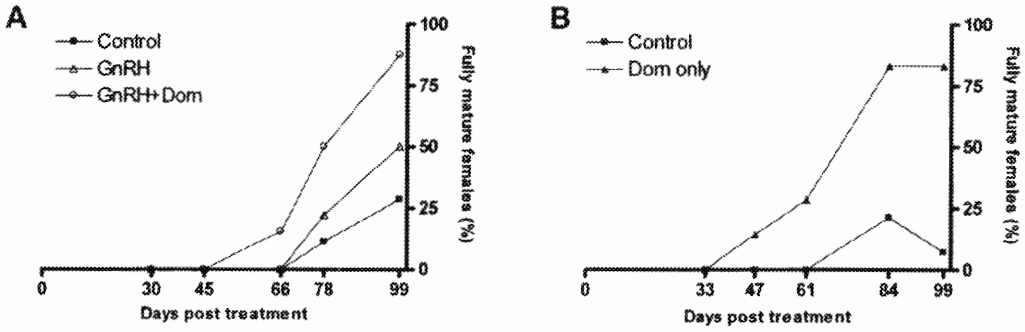

Fig. 2 Effects of various hormonal treatments on the percentages of fully mature *M. cephalus* females in July-October 2002 (A) and 2003 (B). Females were injected intramuscularly with EVAc implants loaded with GnRHa (4 □g/kg BW) only, or in addition to Dom (5 mg/ kg BW). Control fish were injected with saline. Values are presented as mean ± SEM (n=14). Statistically significant differences from control are marked by asterisks (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

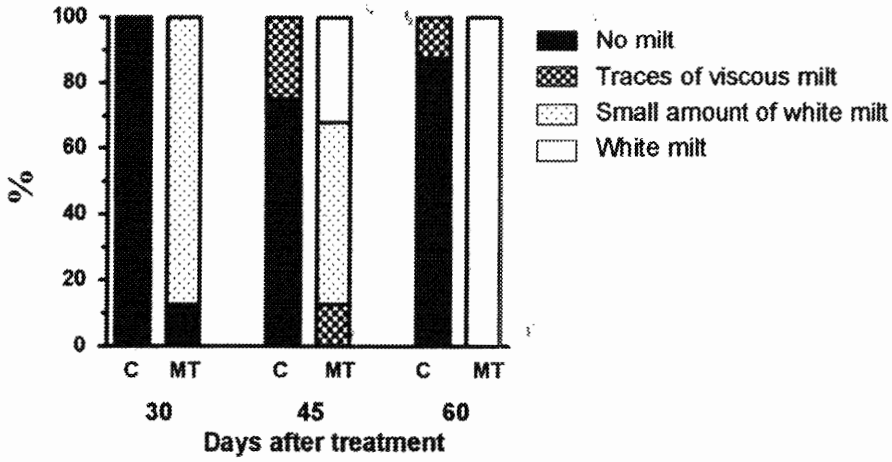

Fig. 3 The effect of MT- implantation on mullet spermiation.

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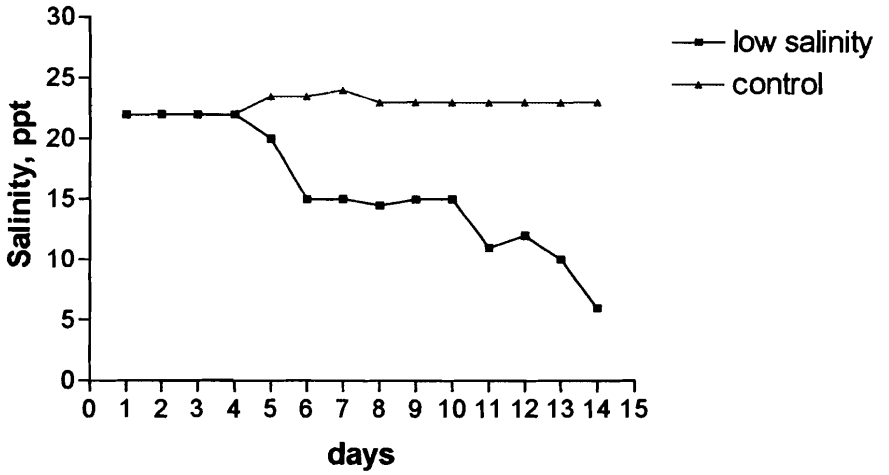

Fig. 4 The rate of salinity change in the treatment aquaria of the first study from ca 23 ‰ to 6 ‰ over 10 days compared to the relatively constant salinity in the control aquaria.

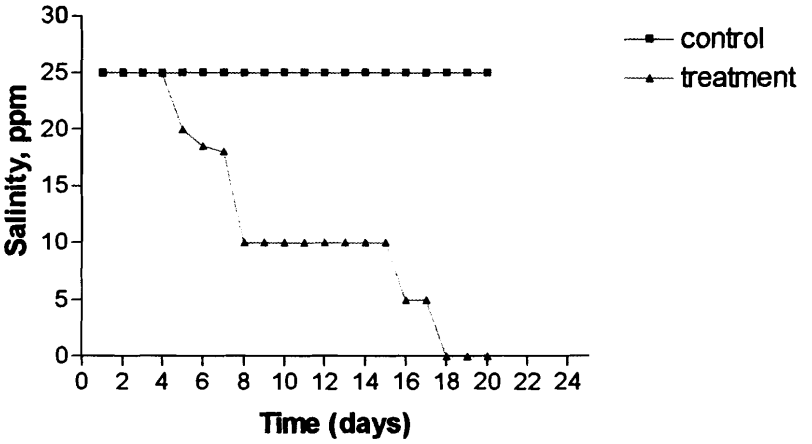

Fig. 5 The rate of salinity change in the treatment aquaria in the second trial from ca 25 ‰ to 0 ‰ over 14 days compared to the relatively constant salinity in the control aquaria.

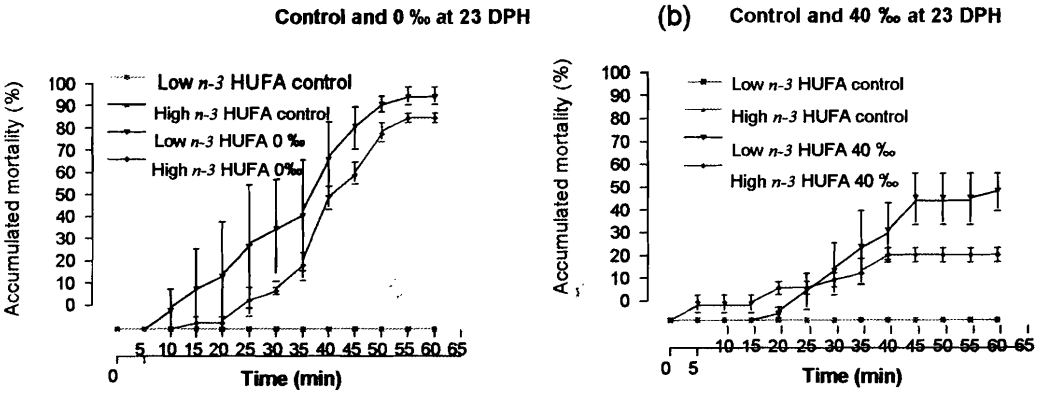

Fig. 6a,b The effect of dietary *n-3* HUFA on accumulated mortality over 60 minutes when larvae were exposed to (a) 25 ‰ (control) or 0 ‰; (b) 25 ‰ (control) or 40 ‰

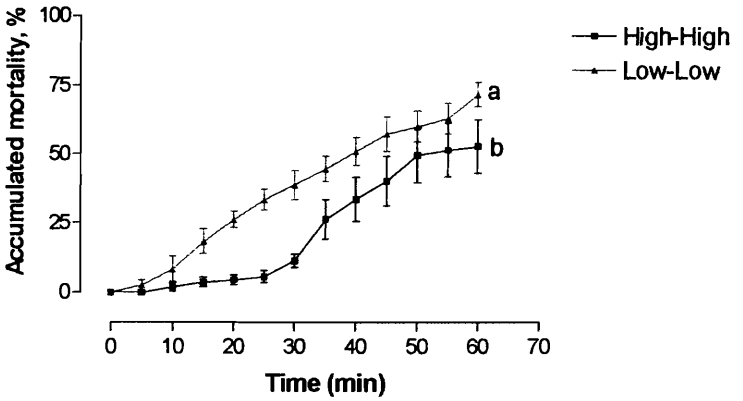

Fig. 7 The effect of rotifer - Artemia combinations of high or low *n*-3 HUFA on the accumulated mortality after a rapid drop from 25 to 0 ‰ in 34 DPH larvae. Mortality percent values at the end of 60 minutes having different letters were significantly (P<0.05) different

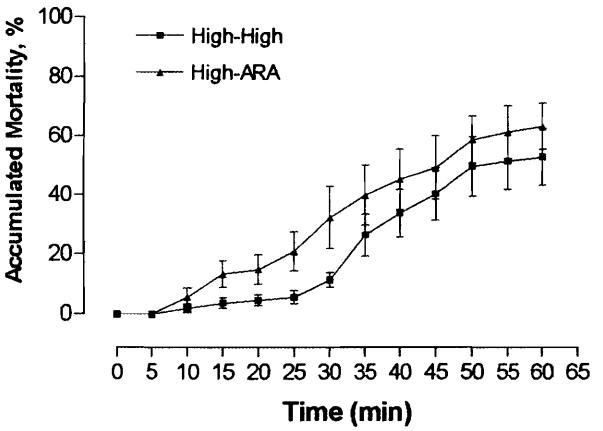

Fig. 8 The effect of feeding rotifer - Artemia combinations of high *n*-3 HUFA or high *n*-3 HUFA with ArA on the accumulated mortality after a rapid drop from 25 to 0 ‰ in 34 DPH larvae

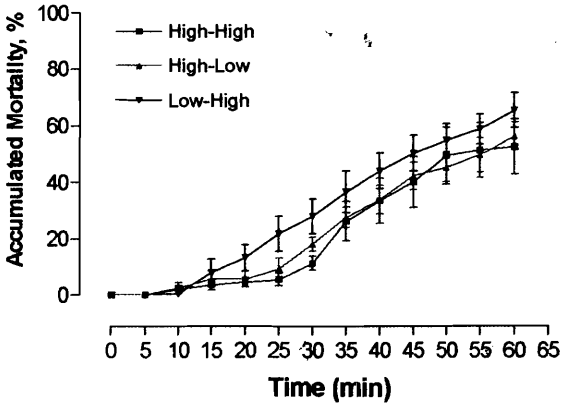

Fig. 9 The effect of selected rotifer - Artemia combinations of high or low *n*-3 HUFA on the accumulated mortality after a rapid drop from 25 to 0 ‰ in 34 DPH larvae.

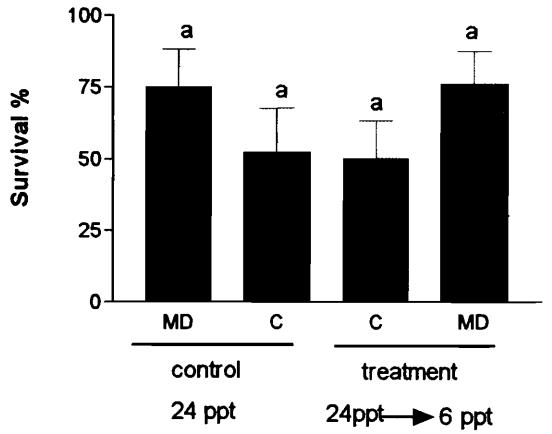

Fig. 10 The effect of constant (control) or salinity change (treatment) on survival in larvae fed the low *n*-3 HUFA starter diet (Coppens, C) or the high *n*-3 HUFA FeedPro microdiet (MD). Values within a salinity regime having the same letter were not significant (P>0.05)

Fig. 1 Survival of grey mullet larvae grown on different fatty acid mixtures

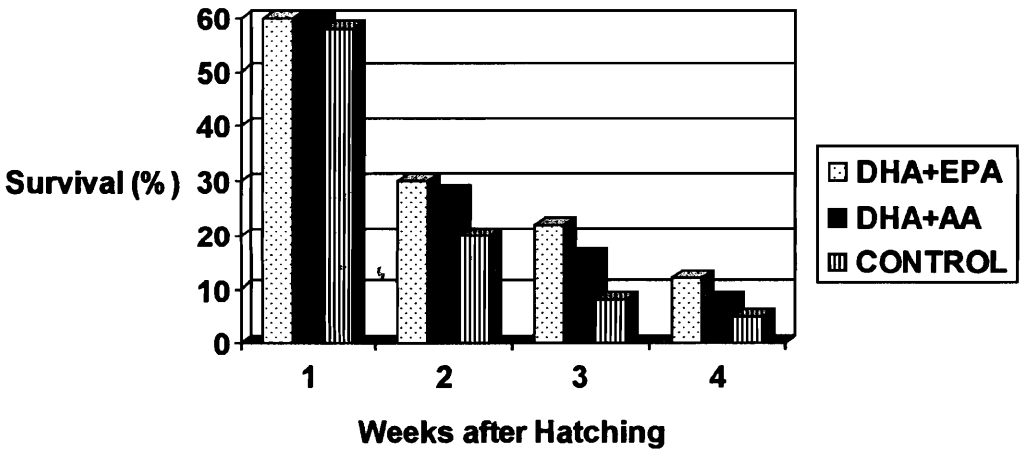

Fig. 12 Survival of grey mullet larvae grown on rotifers enriched with Vitamin C

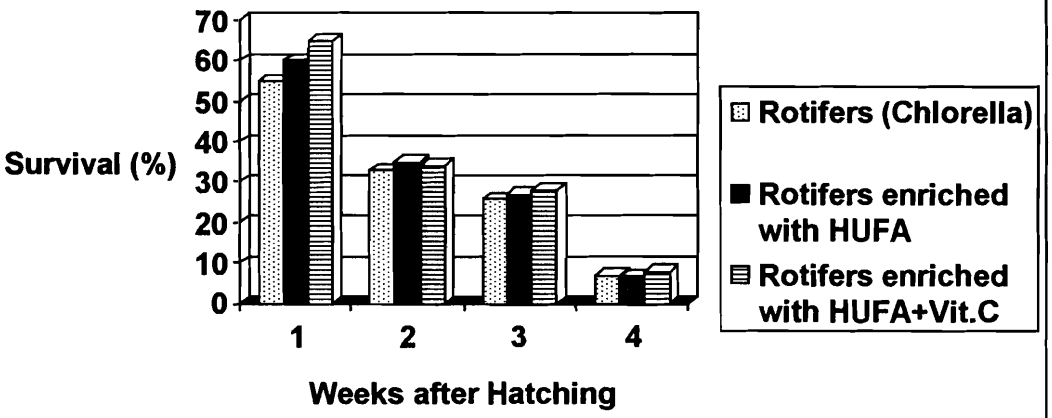

Table 1. Summary of the spawning data obtained from hormonally induced females during 2002 and 2003

ɜatment	No. of injected females	No. of spawned females	Female body weight (g)	Oocyte diameter (□m)	Egg diameter (□m)	Latency period (h)	Spawning ratio[1] (%)	Viable egg volume (ml/kg BW)	Relative fecundity[2] (x 10^3)
ıRHa	8	1	2398.13±197.70	570	755	24	12.50	407.40	1066
ıRHa+MET)02)	6	5	2586.66±109.00	573.33±7.53	735±8.16	21.21±0.42[a]	83.33	452.16±121.60	1236±303.4[a]
ıRHa+MET)03)	7	6	2635.83±167.60	600.83±7.01	720±4.47	20.54±1.51[a]	85.71	566.86±61.27	1649±174.0[a]

Female body weight, oocyte diameter, egg diameter, latency period, viable egg volume, relative fecundity are expressed as mean ± SEM. Means designated by the same letter do not differ significantly from each other (p>0.05).

[1] Spawning ratio: the number of females, which ovulated after injection, divided by the total number of injected females.

[2] Relative fecundity: total number of eggs/kg body weight of treated females.

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Table. 2- Represent individual data of female *Mugil cephalus* induced to spawn in October by priming injection of carp pituitary extract (CPE) or human chorionic Gonadatropin (HCG) and resolving injection of LHRH-a given 24 Later.

Fish no.	Body weight (g)	Total length (cm)	CPE (mg/kg)	Dose HCG I.U/ fish	Initial egg diameter (µm)	Egg diameter after 1st injection (µm)	2nd injection LHRH-a dose (µm kg	Egg diameter after 2nd injection	Total no. of spawned ova
1	1100	42.3	-	10.000	580	605	200	628	1.800.000
2	980	39.7	-	10.000	510	530	200	555	1.600.000
3	680	35.5	30	-	535	558	200	621	1.360.000
4	1090	42.1	30	-	580	603	200	625	1.950.000

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Table 3 Average total lipid content (gm/100gm) ± SD in ripe ovary, injected ovary after received one, two and three injections and spawned eggs of female Mugil cephalus during spawning season from September to November 2005.

Organ	Total lipid ± SD (gm/100gm FW)
Ripe ovary	16.316 ± 1.32
Ripe Ovary after 1st injection	12.174** ± 1.03
Ripe Ovary after 2 nd injection	12.152** ± 2.51
Ripe Ovary after 3 rd injection	5.812** ± 2.93
Spawned egg	5.654** ± 1.19

** Highly significant correlation (P< 0.01) compared with ripe ovary.

Table 4 Total protein content (gram / 100gm FW) in the ripe ovary, injected ovary after received one, two and three injections and spawned eggs for *Mugil cephalus* through the spawning season from October to November 2005.

Organ	Total protein ± S D
Ripe ovary	31.18 ± 0.92
Ovary after 1st injection	32.7* ± 1.03
Ovary after 2 nd injection	38.63** ± 1.25
Ovary after 3 rd injection	27.28** ± 1.34
Spawned egg	24.49** ± 1.53

** Highly significant correlation P< 0.01 compared with ripe ovary.
* Significant correlation P< 0.05 compared with ripe ovary.

Table 5 Composition of the test emulsions used to enrich rotifers and Artemia with different levels of DHA.

	High *n-3* HUFA mg/g emulsion	Low *n-3* HUFA mg/g emulsion
Capelin fish oil*	660	660
EPAX oil**	220	0
Olive oil***	0	220
Soy lecithin	50	50
Tween 80	30	30
Alginic acid	20	20
Vitamin C	10	10
Vitamin E	10	10

*Commercial fish oil, with varying purity, which contains low to moderate levels of DHA and EPA with a DHA/EPA ratio of <1
** Highly purified marine oil containing 50% DHA and 20% EPA with a DHA/EPA ratio of 2:5
*** A purified vegetable oil containing no EPA and DHA

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Table 6 Different combinations of low and high *n-3* HUFA in the rotifer and Artemia enrichment treatments to test the effect of separate and combined effect of these essential fatty acids at different developmental stages.

Rotifer Treatment in 400 l tanks	Artemia Treatment in 27 l aquaria
High *n-3* HUFA	High *n-3* HUFA
High *n-3* HUFA	Low *n-3* HUFA
Low *n-3* HUFA	Low *n-3* HUFA
Low *n-3* HUFA	High *n-3* HUFA
High *n-3* HUFA	High *n-3* HUFA + ARA

Fig 13 Weight gain and feed intake of mullets from juvenile to market size.

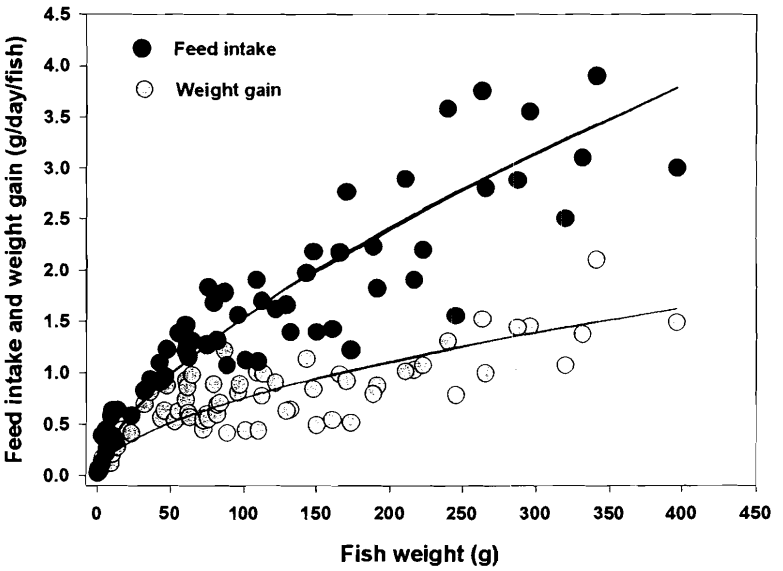

Fig. 14: Changes in body composition of growing mullets.

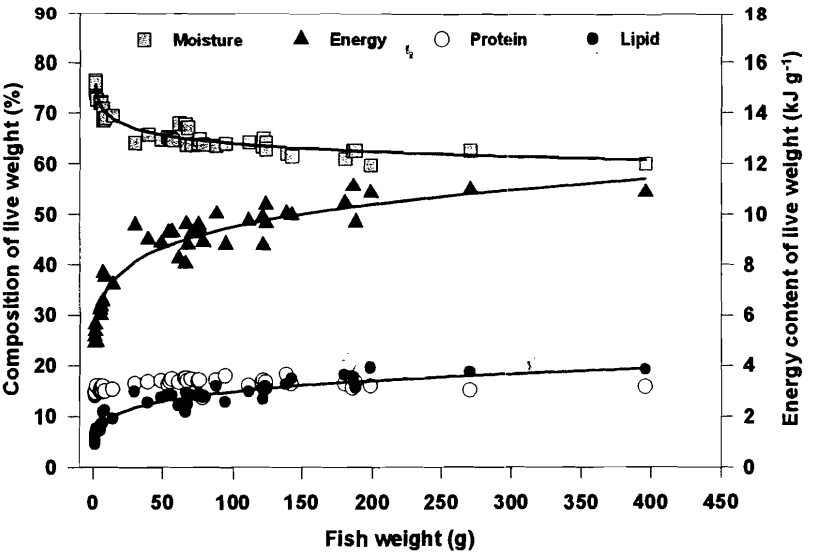

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Fig. 15 Energy and protein loss of mullets under starvation

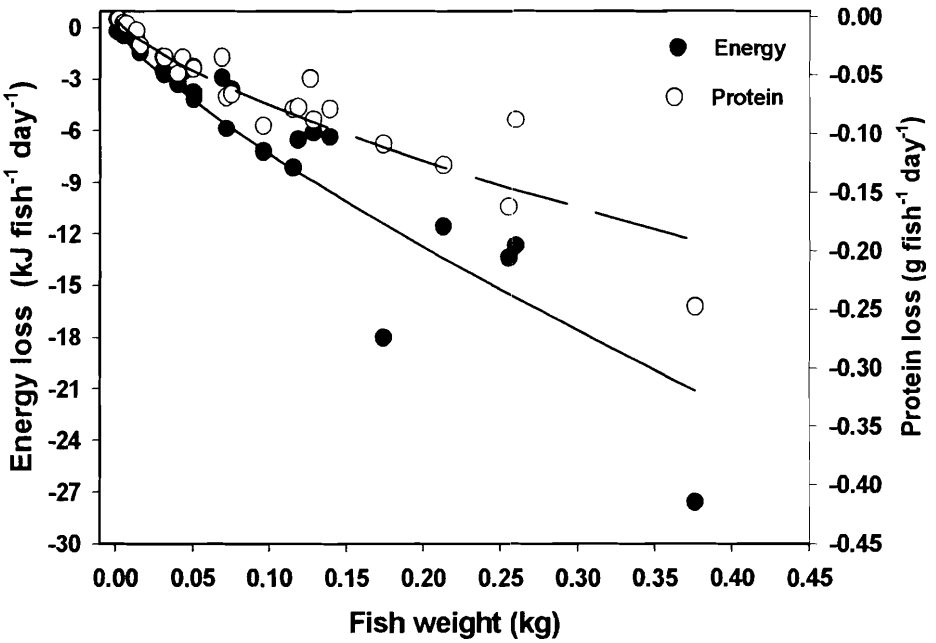
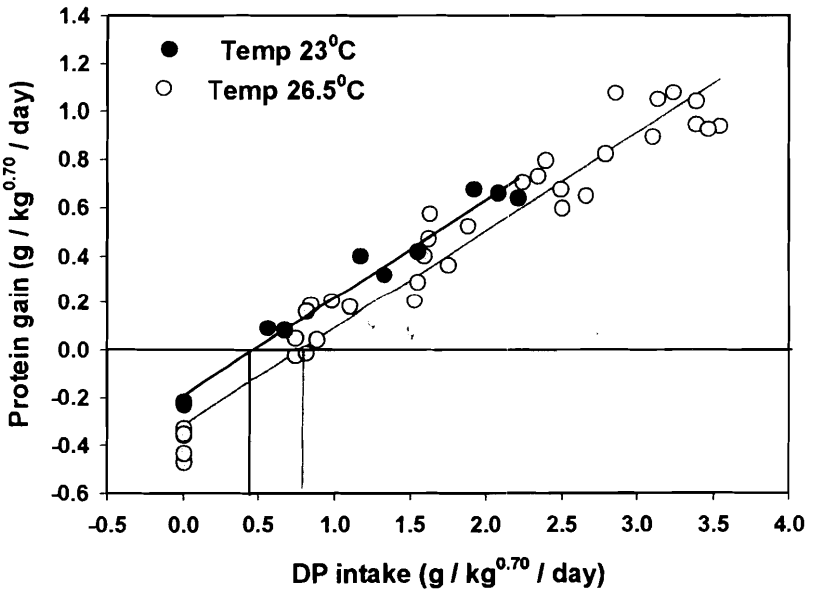


Fig. 16 Protein gain as a function of digestible protein intake in mullets.



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FINAL REPORT, COVERING PERIOD 2003 - 2007–05–01

SUBMITTED TO THE U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT, BUREAU FOR GLOBAL PROGRAMS, FIELD SUPPORT AND RESEARCH, CENTER FOR ECONOMIC GROWTH AND AGRICULTURAL DEVELOPMENT

CULTURE OF GREY MULLET, *MUGIL CEPHALUS*, IN EGYPT AND ISRAEL

Principal Investigator: Dr. Robert B. Abel, Stevens Institute of Technology
Co-Principal Investigator: Dr. Soliman Hamid Abdel Rahman, President and Director, National Institute of Oceanography and Fisheries, Egypt
Co-Principal Investigator: Dr. Amos Tandler, Director, National Center for Mariculture, IOLR, Eilat, Israel

Co-Investigators NIOF: Dr. M.M. El-Gharabawy, Dr. T. Shehada
Co-Investigators NCM: Dr. H. Rosenfeld, Dr. W.M. Koven & Dr. G.Wm. Kissil

Grant Agreement No: TA–MOU-99-M18-062

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EXECUTIVE SUMMARY

The Israelis, and the Egyptians at their laboratories at Eilat and Fayoum/Alexandria respectively, have collaborated on a project to culture the Grey Mullet, a fish which bears promise of an outstanding food resource throughout the entire Middle East but which has historically been difficult to domesticate.

The project's complimentarily of need/opportunity was obvious from the beginning. The Israelis, who have traditionally given other stocks (e.g. tilapia, carp, seabream and sea bass) higher priority, possess some of the world's outstanding fish farming facilities. The Egyptians, who have always placed enormous value on the mullet as a a food resource, have previously been limited to "fishing in the wild." Their Research and Development facilities had been less developed.

Accordingly, am outstanding accomplishment of the project must be considered the Egyptians excellent capacity building program. As illustrated herein, by the project's end they had designed and constructed and now possess, an outstanding facility for research and fisheries production. Promulgation of the designs and operations of their hatchery and earthen ponds should be of material assistance to ministries and laboratories throughout the Middle East.

The two countries decided – by agreement – on three research subjects: Reproduction, Larval-Rearing and Nutrition. They:

1. Developed successful out-of-season reproduction using photoperiod and water quality control.
2. Defined the daily protein and energy needs of the mullet from juvenile to market size for their maintenance and predicted growth at varying water temperatures.
3. Translated these requirements into fed formulas to allow production of practical feeds.
4. Developed an effective protocol for the larval rearing of the grey mullet.
5. Stimulated interest in both Agriculture Ministries to increase grey mullet production through expanded farming of mullets in monoculture.

There is now a clearer perception of future practical research avenues to further improve grey mullet larval rearing. These areas of continuing interest include determining the developmental stage at which eggs need to be stocked for optimal hatchability; the role of essential fatty acids in larval nutrition; reduction of deformities; and shortening the time of acclimation to various salinities.

Research direction and progress in both laboratories was originally determined during joint preparation of the proposal; mid-course corrections were applied during annual meetings among all project scientists. Five technical papers were published regarding the ongoing work, three Egyptian and one Israel student obtained their masters' degrees as a result of the project.

Both Agriculture Ministries are encouraging continuing research. An unforeseen impact of the project, has been to expand production, not only for the meat itself, but for the roe, which has achieved marketability as the "poor man's caviar". As a result, a joint research project has been proposed to MERC by the team (M27-966) and was

recently accepted for possible funding contingent on meeting certain provisos set out by MERC.

General Introduction

Grey Mullet, *Mugil cephalus,* which are farmed in both Egypt and Israel for human consumption, are considered a high quality fish.

This statement is critical to the economics and general welfare to the Egyptians and Israelis, as well as to most of their Middle East neighbors. It is emblematic of the huge irony in fisheries research, i.e. the inverse relationship between the **need** for a fish species and the **level of research on that same species**.

It is an unfortunate (if logical) fact that the major laboratories among the wealthiest countries devote their research talents and resources to what we called "Tiffany stocks" which are highly prized and profitable. By definition, they are also unavailable to the great mass of the world's population. On the other hand, the stocks, which reproduce at higher rates and could theoretically satiate and benefit large third world populations, are given short shift in first line laboratories.

There is no stock more representative of this thesis than mullet. The magnificent Special Report of the National Geographic of April 2007, predicts terrifying clarity, an anticipated dry-up of the sea's bounty, with particular reference to the Mediterranean, where man is busily draining all available sea-derived food resources. In this regard, the Geographic implies that the most likely solution to the problem is aquaculture, i.e. fish farming.

Accentuating this critical irony is the enormous bonus provided by Mediterranean fish that represent the only food source on earth not requiring fresh water for survival and growth, in a region where water deprivation is a major cultural, economic, and political consideration.

The Egyptians and Israelis, who have been working together in close harmony for a quarter century under the MERC program, have long recognized the potential of aquaculture to meet nutritional needs in the region. An efficient farming process is essential to properly exploit this outstanding resource. Unfortunately, the stock is fighting back. Up to now, they have refused to reproduce in captivity, with any degree of regularity.

Solution of this problem may never win a Nobel Prize, but its utilization importance to the Middle East cannot be overvalued. Accordingly, the reader is cautioned to understand that production figures notwithstanding, the supply of fingerlings is almost exclusively from the wild with Egypt alone harvesting over 300 million fry and Israel (with much less fresh water acreage) in the region of 7-10 million. Egyptian production has reached 173,000 tons, representing 20% of domestic fish production, while Israeli production has been limited to 2000 tons. It must be reemphasized that reliance on natural sources of fish for maintaining the domestic production in both countries is **unsustainable** as the fingerling supply is vulnerable to natural fluctuations in fry production and the massive fishing of the fry in the coastal waters in the region.

Proceeding from a series of intensive conferences, the Egyptians and Israelis agreed to a coordinated line of research, divided into three basic sectors: Reproduction, Larval Rearing, and Nutrition, and this report will be sectioned accordingly.

I – Reproduction Research

Introduction

A guide to reproduction of grey mullets in captivity in Hawaii was published in 1974 (Kuo et.al,) but the main source of fingerlings today is still from the wild. The spawning season of the Mediterranean *Mugil cephalus* (grey mullet) ranges from July to December. However, in captivity, mullets do not spawn naturally with both genders displaying reproductive dysfunctions. Gonadal development in females usually halts at early stages of vitellogenesis and does not proceed with final oocyte maturation, while spermiating males are rarely observed and in most cases the produced milt is highly viscous and fails to fertilize the eggs. The inability of fish in captivity to complete its reproductive cycle is most likely due to a failure at one or more sites along the hypothalamo-hypophyseal-gonadal axis. In addition, with mullet it is difficult to establish the right sex ratio among broodstock, as this species does not exhibit any sexual dimorphism. Moreover, the conventional oviduct catheterization technique is feasible only at the more advanced developmental stages of gonadal growth.

Objectives

With the intention of inducing successful breeding in captive Mediterranean grey mullet this study addressed the following objectives:

(a) To develop a rapid tool for determining the sex of young brooders,
(b) To determine and identify the concentrations of amino acids and fatty acids in hormone-injected and non-injected females and compare them with spawned ova.
(c) Investigate the important aspects of the biological, histological, histochemical and fine structures of gonads and pituitary glands, and generally synchronize gonadal development in both males and females.
(d) To induce spawning: continuing successful spawning trials with varying hormones and doses under varying environmental conditions in order to ultimately standardize an optimum procedure to induce spawning.

Materials and Methods- NCM

Grey mullet broodstock used for this study consisted of 5-8 year old females (BW 2.3±0.08 kg), and 3-5 year old males (BW 1.1±0.06 kg). Fish were maintained outdoors in 5m3 covered fiberglass tanks and were subjected to either natural or 6-month shifted conditions of light and water temperature. The experimental tanks were stocked equally at a density of 4.0±0.174 kg/m3, consisting of a 7:4 (female: male) sex ratio. Fish were fed daily at the rate of 1.5% of their body weight using a 30% crude protein and 6% lipid feed formulated and prepared at NCM.

Hormones & treatments. The hormonal treatments used to accelerate gonadal growth were carried out in early July. The hormones: GnRH analogue (GnRHa), and 17α methyltestosterone (MT), loaded on Ethylene-vinyl acetate copolymer (EVAc) slow-release implants, and dopamine antagonists (i.e. domperidone [Dom] or metaclopramide [MET]) dissolved in a vehicle, were injected intramuscularly.

Control fish were injected with saline only. The state of ovarian development as determined by oocycte growth was examined bi-weekly from biopsies using polyethylene cannuli. In parallel, male mullets were checked for the presence of milt by applying gentle abdominal pressure.

Female mullets were considered fully mature once their oocytes reached an averaged diameter of 500μm. A mature female was stocked in a $1m^3$ tank with two running males in which seawater at 24-28^{0}C was provided. The spawning induction treatments consisted of GnRHa and MET, given as two injections (24 h apart).

Steroid hormone determination. Blood samples of mullet broodstock were obtained from July to October. The blood was centrifuged (1500g for 15 min at 4°C) and plasma was collected. Steroids were extracted twice with ethyl ether and following evaporation of the organic phase, solubilized in the appropriate buffer for ELISA. Both steroids, 17α-estradiol (E2) and 11-Ketotestosterone (11-KT), were measured by specific ELISAs.

Vitellogenin dot-blot immunoassay. Plasma samples were applied directly to a nitro-cellulose membrane. Nonspecific binding sites were blocked with 5% skimmed milk in PBS (137 mM NaCl, 8 mM Na2PO4, 1.4 mM KH2PO4, and 6.6 mM KCl, pH 7.5). For detection of Vtg, the membrane was incubated with anti-sb-Vtg (polyclonal antibodies raised in rabbit against seabream Vtg, diluted 1:5,000, for 1 h at room temperature, washed five times with PBS-T (PBS plus 0.05% Tween-20), and then incubated with horse-raddish peroxidase conjugated to goat ani-rabbit-IgG. After 1 h of incubation, the membrane was washed as described above and the signal was visualized by an ECL detection system (Amersham-Pharmacia-Biotech).

Results

Rapid tool for determining the sex of young brooders. Vitellogenin, the precursor of the yolk proteins, was used as a marker protein, in a dot-blot immunoassay, for distinguishing sexual mature females from males (Fig. 1). This simple method enabled us to obtain with a high degree of certainty, 4 months ahead of spawning season, the sex of more than 98% of newly integrated brooders.

Synchronized gonadal development in females and males. The hormonal treatments used to accelerate gonadal growth indicate that, a single injection of Dom induces synchronized ovarian development, while the GnRHa implant has no additive effect (Fig. 2). The Dom treatment, seems to disrupt the female social hierarchy, and liberates suppressed females, allowing them to reach sexual maturity. In addition, our results indicate that MT is a potent spermiating agent, which enhances milt production in all treated males (Fig. 3). A positive correlation between 11-KT levels in male plasma along with the presence of fully mature females, suggest an additive effect probably associated with female pheromones, which highlights the importance of the female-male hormone relationship in this species.

Induced spawning. Our spawning induction trials indicate that the combined treatment (GnRHa & MET) is more effective in inducing ovulation and spawning as compared to GnRHa alone; 85% and 12% spawning success, respectively (Table 1). An accurate latency period (21-22 h) was recorded in all spawning induction trials during three

successive natural as well as artificially 6-month shifted reproductive seasons, demonstrating consistent effectiveness of the protocol.

Conclusions

This part of the project enabled the establishment of a potent hormonal therapy, which synchronized gonadal development in mullet females and males, and sequentially induced their spawning. The results indicate that dopaminergic inhibition has a role in two different phases of the reproductive cycle of grey mullet, i.e. at the early stages of vitellogenesis and at the stage of final oocyte maturation and ovulation. A single injection of dopamine antagonist, Domperidone (Dom), helps females complete vitellogenesis and reach the stage of final oocyte maturation, thus overcoming the first reproductive block. Further intervention with hormone-therapy based on GnRHa and another form of dopamine antagonist, metaclopramide (MET), induces mature females to spawn, overcoming the second reproductive block. In addition, by using artificial thermo- and photoperiod regimes the natural spawning season was shifted, making a year-round supply of mullet fingerlings feasible.

Materials and Methods- NIOF

All of the females needed for the Egyptian spawning trials were obtained from the brood stock holding facilities in Mallahet Port Fouad, Sinai, Egypt.

Induction of spawning in 2005 and 2006. All All fish were maintained at marine hatchery in National Institute of Oceanography & Fisheries at Alexandria and Fayoum as follows:

a) Broodstock: Grey mullet brood stock (0.67 to 1.86 Kg body weight), used in the spawning trials were maintained in $3m^3$, fiberglass tanks with stocking density of 2 fish / m^3, manipulated light exposure at the shortest day (14D/10L) during September, October and November.

b) Females: The state of ovarian maturity for each female was assessed prior to each spawning attempt by using polyethylene cannula each sample was fixed in 10% formalin in seawater. In this study, two different strategies for spawning mullet were attempted, the first strategy called for a "Priming" dose of human chorionic gonadotropin hormone or carp pituitary homogenate. After twenty four hours later a second "resolving" injection was giving consisting of LHRH-a. The appropriate dosage was dissolved in 0.9% saline and injected into the dorsal musculature.

c) Males: Males with body weight of 0.63 – 0.87 kg were placed with each treated female, examined for the presence of milt by applying slight pressure to the abdomen, and only running ripe males were used in each spawning attempt. Spawned eggs were fertilized naturally, collected and incubated in 1000 L open system fiber glass tanks. Fertilization rates, average spawned egg diameters, hatching rates and larval developmental stages were recorded.

d) System preparation: A flow through system supplied seawater 34ppt salinity. The water temperature ranged about 25°C ± 1 throughout the experiment. Fish were fed diet containing 30% crude protein and 6 % lipid.

*Total lipid and fatty acid extraction.*Lipids were extracted from the samples by using chloroform-methanol-water (2:2:1.8) according to the method of Bligh and Dyer (1959).Lipid fractions were methylated to obtain fatty acids methyl esters according to Gallagher *et al.* (1984). Fatty acids methyl esters were analyzed by using Shimadzu gas chromatography 4 CM equipped with a flame ionization detector.

Total protein extraction. Total protein was determined using micro-Kjeldahl method (Association of Official Agricultural Chemists,1980).

Amino acid extraction. Extracts of amino acids were prepared according to Moore *et al,.* (1958). Twenty micro liters of each prepared filtrate were injected in the instrument capsule of Beckman 116 GI amino acids analyzer apparatus. All amino acids were expressed as gram amino acid /100 gm. fresh weight of each sample.

Results

Induced spawning. Induced spawning trials were carried out in September, October and November in NIOF Facilities in Shakshouk (Fayoum) and Alexandria. Spawners were injected with various doses of CPE, HCG and LH-Rh-a hormone doses. The best results were obtained using the following procedure: priming injection was 10.000 I.U. of HCG and 30 mg/kg carp pituitary extract. After 24h from the first injection, the cannulated ova samples have diameters of 580 ± 30 µm. The resolving injection was given after 24h from the priming one, with 200 µg /kg LHRH-a. Twelve males of (+2) and (+3) were placed with injected females. Sex ratio was 3 males: 1 female. After 24 hours from the second injection the females spawned fertilized eggs. The egg diameter was about 680 ± 10 µm. The number of ova spawned ranged between 1.360.000 and1.950.000 eggs / spawned fish. The percent of fertilization was about 79% from the total number of ova. Table (2) shows the results of one of the successful trials for the spawned females.

Total lipid content of ripe ovary : The maximum content of total lipid was recorded in ripe ovary. Gradual decrease was recorded in total lipid content in relation to hormonal injection. After first injection highly significant decrease was noticed in total lipid ($P< 0.01$); whereas, no significant difference in total lipid of ovary was noticed after second injection. However, sharp decrease in total lipid content was recorded for ovary after third injection and egg spawning as indicated in Table (3). This decrease in total lipid for spawned eggs indicates direct fat transfer from fat storage into reproduction involvement.

Fatty acids content in ripe, injected ovaries and spawned eggs of female Mugil cephalus:

a) Saturated fatty acids: Highly significant increase were recorded between normal ovary and spawned egg for each of carpoic acid ($C_{6:0}$) and carpylic acid A ($C_{8:0}$) at $p < 0.01$, whereas insignificant correlation for injected ovary at $p > 0.01$. Three saturated fatty acids (C_{11}, $_{12}$ and $_{15}$) disappeared in spawned eggs, while more or less detected in injected ovary. Significant correlation were recorded between spawned egg and normal ovary for each of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) at $p<0.05$.

b) Poly-unsaturated fatty acids: Highly significant decrease correlation were noticed between ripe ovary and spawned egg for each of the four Poly-unsaturated fatty acids Linolenic ($C_{18:3}$), Eicosapentadecanoic ($C_{20:5}$), Arachidonic acid ($C_{20:4}$) and ($C_{22:}$

$_2$) at P<0.01. Significant correlation between ripe ovary and spawned egg for ecosatrienoic acid ($C_{20:3}w_6$) at P<0.05, while insignificant correlation for the injected ovary for two and three injections at P>0.01. Highly significant correlation between ripe ovary and injected ovary for one and two injections for arachidonic acid at P<0.01, whereas insignificant correlation were recorded between ripe ovary and injected ovary for three injections at P<0.05 . The results suggest the role of arachidonic acid in injected and spawned *Mugil cephalus*. Arachidonic acid is an important precursor of prostaglandins which have important regulatory roles in the body including vasodilatation, platelet aggregation and perhaps osmoregulation".

Total protein content of female Mugil cephalus: The total protein content reached a maximum value Table (4) in the injected ovary with second injections (38.63 ± 1.25). The minimum value of total protein content was recorded in spawned egg (24.49 ± 1.53) with highly insignificant decrease correlation in relation to ripe ovary at P>0.001.

Amino acid content of female Mugil cephalus:
a) Essential amino acids: Valine, leucine and lysine were the highest essential acids in the ripe ovary of *Mugil cephalus*, while histidine was the lowest. The present results showed that, eight essential amino acids (Threonin, Valine, Methionine, Isoleucine, Leucine, Phenylalanin, Histidine and Arginine) exhibited the same trend, being increased in the ripe ovary, which was followed by a significant decrease in spawned eggs. This increase in the ripe ovary may be related to their importance in the development of a ripe ovary, while decreasing after spawning, fertilization and formation of the embryo. Amino acids seemed to be mobilized for energy in the last part of the yolk sac stage.

b) Non essential amino acids (NEAA): Aspartic, glutamic and alanine had the greatest value in the ripe ovary for the NEAA, while cystine the lowest value. The maximum content for each of the eight NEAA (Aspartic, Serine, Glutamic, Proline, Glycine, Alanine, cystine and Tyrosine) were recorded in ripe ovary. Then fluctuated after hormone injections with decrease in concentration then reached to the minimum content in the spawned egg. Highly significant decrease was recorded between concentration of NEAA in ripe ovary of female *Mugil cephalus* and spawned egg at P< 0.01. The results showed that glutamic acid content reached a maximum value in ripe ovary, and then fluctuated in injected ovary with insignificant correlation with the injected ovary. This information will be used as a guideline for development of appropriate feeds for use in larval rearing and/or broodstoock diets.

The histological, histochemical and ultrastructure of gonads and pituitary gland:
a) Ovaries : Immature, maturing, and nearly ripe ovaries were developed with a normal shape, while the ripe ovaries had abnormal and atretic ovae. The fine structure of the wall in nearly ripe ovae appeared with five different normal layers (thecal, basement membrane, follicular epithelial, zona radiata externa, zona radiata interna and the absence of a cortical alveoli layer). The fine structure of the wall of ova of ripe ovaries was characterized by atretic and deformed layers with damaged thecal layer, basement membrane and follicular epithelial layer. The fine structure of the wall of ova in the resorbed ovaries appeared atretic and abnormal.

b) Testes : Investigation of testes of *M. cephalus* indicated that the interlobular connective tissue between the lobules at all stages of maturity were abnormal and were enlarged with collagenous tissue. This abnormal tissue is considered a sign of infertility. All the spermatozoa fine structure appeared with deformed head, tail and mitochondria. The chromatin material were atretic and deformed in their distribution surrounding the head and tail of spermatozoa.

c) Pituitary : The pituitary gland of *M. cephalus* showed damaged and atretic gonadotropic cells which are responsible for secretion of the sex steroid hormone and give a signal for hormonal control for sex maturation, differentiation and spawning. The fine structure of the gonadotropic cells at all stages of maturity indicated that the granulation of the cells were few in number and small in size.

II – Larval Rearing

Introduction

There are only a few papers in the literature dealing with the larval rearing of grey mullet (Murashige et al. 1991; Yoshimatsu et al. 1995; Harel et al. 1998). However, there is general consensus that a major factor limiting the commercial rearing of the grey mullet is the high mortality occurring during early larval development when the fish are feeding on rotifers (*Brachionus spp.*). Although little is known of the nutritional requirements of mullet larvae, preliminary studies have shown that enriching the rotifers with the n-3 highly unsaturated fatty acids (HUFA); eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) improved survival, growth and stress resistance (Eda et al. 1990, Tamaru et al. 1992). These fatty acids play a critical role in membrane function and structure in the tissues of marine fish larvae in general and must be supplied in the diet. (Koven et al. 1990; Sargent et al. 1993). Another fatty acid considered essential for the larvae of a number of marine species is arachidonic acid (ArA, 20:4n-6), which has been associated with improved larval survival and resistance to acute stress in the gilthead seabream (Koven et al.2001, 2003). Although a number of authors have addressed the problem of stress in larviculture, the efficacy of dietary ArA has not been examined in larvae of this species. Finally, use of vitamin C has received little exploration.

By agreement, the laboratories at both Alexandria and Eilat were to investigate the requirements of mullet larvae for highly unsaturated fatty acids (HUFA). Rotifers and artemia were the food of choice. The objectives were to determine the optimal conditions for stimulating larval growth and determining the basic nutritional requirements which, when coupled with effective environmental conditions, will form the basis of a practical protocol to commercially rear mullet larvae from hatching to 35 days post hatching (DPH). The Israelis at Eilat were to test growth under a large range of environmental conditions, and at different levels of n-3 HUFA having a high DHA/EPA ratio. In addition, the Egyptians at Alexandria tested the addition of vitamin C to the food mixtures.

Methods

Preliminary study – effect of enrichment on growth, survival and stress:

Prior to conducting the main study in 27 l aquaria, a preliminary trial was run in Eilat on mullet larvae reared in six 400 l V-tanks to determine which stressors (salinity, air exposure, handling) would be tested in the primary aquarium experiment and the

protocols for carrying them out. In each set of three 400 l tanks, larvae from 3 to 17 days post hatching (DPH) were reared at 25 ‰ and fed rotifers previously enriched at 29 °C in 200 l V-*tanks* on low or high *n-3* HUFA emulsions whose compositions are listed in Table 5 The *n*-3 HUFA are comprised almost entirely of the essential fatty acids; eicosapentaenoic acid (EPA, 20:5*n-3*) and docosahexaenoic acid (DHA). After 17 DPH Artemia similarly enriched on these two emulsion treatments were fed together with the rotifers to the end of larval rearing at 35 DPH. The 400 l tanks during rotifer feeding were "greened" with *Nannochloropsis* sp., which has been reported to enhance mullet larval rearing (Yoshimatsu et al. 1995). This alga, although rich in EPA and deficient in DHA, was added at relatively low concentrations (2 x 10^5 cells ml^{-1}), which would not significantly influence rotifer fatty acid composition. Larval samples were taken on 8 and 17 DPH to determine the average dry weight (DW) in fish fed the *n-3* HUFA treatments. On 23 DPH, nine groups of 10 larvae from each selected treatment V-tank were transferred to three sets of three 300 ml plastic cups where each set contained 0, 25 or 40 ‰ water at rearing temperature. By monitoring accumulated larval mortality over 60 minutes, the effect of salinity challenge, as a function of *n*-3 HUFA level, was tested in triplicate replicates. The larvae transferred from 25 ‰ ambient seawater in the 400 l tanks to 25 ‰ water in the cups were considered exposed to handling stress only.

Main aquarium study: On 17 DPH, mullet larvae were transferred to twenty-five 27 l aquaria and stocked at 300 larvae $aquarium^{-1}$. Ten and fifteen aquaria were stocked with similarly sized fish that were reared on rotifers having a low or high *n-3* HUFA content, respectively. The larvae were then fed Artemia nauplii previously enriched on one of the two treatment emulsions in order to test in replicates of 5 aquaria the separate and combined effect of rotifers and Artemia containing different levels of *n-3* HUFA (Table 6) on growth and stress resistance. An additional Artemia treatment was tested on larvae previously fed high *n-3* HUFA rotifers, which were enriched with an emulsion containing both high *n-3* HUFA and arachidonic acid (ArA, 20:4*n*-6).

Acclimation study- The effect of dietary n-3 HUFA on low salinity acclimation: In this study, 12 aquaria (27 L) were stocked with 350 (23 DPH) larvae $aquarium^{-1}$ where in each set of 6 aquaria, larvae from 3 aquaria were fed either a commercial weaning diet (Coppens, the Netherlands) which is relatively low in *n*-3 HUFA or the NCM prepared microdiet (FeedPro, Israel) which is high in *n*-3 HUFA. The salinity was gradually decreased in three aquaria from each set of 6 aquaria over 10 days from 25 to 8 ‰ (Fig. 4) and the survival monitored.

Acclimation study- The effect of larval size on low salinity acclimation: In this trial, 18 aquaria (27 L) were stocked with 350 (23 DPH) larvae $aquarium^{-1}$ where in each set of 9 aquaria, large larvae (ca 16 mg) were stocked in 5 aquaria while small larvae (ca 2 mg) (Fig. 5) were stocked in 4 aquaria. The salinity was gradually decreased in one set of 9 aquaria over 14 days from 25 to 0 ‰ (Fig. 5). Samples were taken for cortisol and Na^+-K^+ATPase determinations to evaluate the stress response in these treatments. These samples are presently being prepared for analyses.

Vitamin C trial-In actuality, precise coordination between Eilat and Alexandria laboratories during this phase of the experiments was somewhat inhibited owing to the Egyptians' need to design, construct, and furnish laboratories comparable to those

of the Israelis. This was, in fact accomplished, as will be described in later sections of this report. These laboratories were, in fact, completed nearly three years into the project, and with permission of USAID the Egyptians occupied an additional year of experimentation for this purpose.

Eggs of *Mugil cephalus* were obtained from females spawned in October 2005 in NIOF. Hatched larvae were stocked in 120 L conical fiberglass tanks at a density of 60 larvae per liter. Rearing conditions were as follows: water flow = 2 L/min; salinity = 32 ± 2 ppt; water temperature = 16-18° C; tanks kept in shaded natural light conditions. Fatty acid rich oils delivered to Alexandria from Japan were emulsified with lecithin and mixed with water then added to rotifers for 12 hr at 0.2 g emulsion for one million rotifers. Vitamin C (ascorbyl palmitate) was added at 20% W/V to the oil emulsion.

Results and Discussion

Preliminary study- The average dry weights (DW) on 8 and 17 DPH of larvae fed the dietary treatments are shown in Fig. 8. Although there was no treatment effect in 8 DPH larvae, fish fed rotifers containing high levels of *n-3* HUFA grew markedly better by 17 DPH than their counterparts ingesting the low *n-3* HUFA treatment. This attests to the importance of dietary *n-3* HUFA in early mullet larval growth and supports earlier findings of similar studies on the *n-3* HUFA requirement of marine fish larvae. On 23 DPH, the effect of two types of salinity stress (0 and 40 ‰), handling and exposure to air (30 and 60 seconds) on larval mortality were tested. The preliminary studies showed that the larvae were stress resistant to air exposure, independent of duration tested, as well as handling stress carried out at ambient salinity (25‰). In contrast, larvae exhibited low resistance, which was *n-3* HUFA dependent, to rapid exposure to 0 ‰ although better resistance was shown to 40 ‰ (Figs. 6a,b).

Main aquarium study- Fish reared on high *n-3* HUFA rotifers and Artemia (high-high, Fig. 7) clearly showed reduced accumulated mortality when immersed in 0 ‰ water compared to larvae fed both rotifers and Artemia containing low *n-3* HUFA (low-low). This suggested that a nutritional history of high EPA and DHA consumption imparts enhanced stress resistance in fish exposed to a severe drop in salinity.

In Fig. 8 supplementing ArA to the emulsion fed to Artemia resulted in a non-significant tendency to increase accumulated mortality in fish exposed to 0 ‰. This could have resulted in an increase in the membrane ArA/EPA ratio, which would have increased ArA derived eicosanoids and possibly reduced osmoregulatory compensation during the stress of rapid salinity drop.

Accumulated mortality was very similar in larvae fed high *n-3* HUFA in rotifers only or in both rotifers and Artemia. In comparison, larvae fed the increased levels of *n-3* HUFA only during Artemia feeding exhibited increased, although not significantly, accumulated mortality (Fig.9). This reinforced our earlier observation in the 400 l tanks that feeding high levels of *n-3* HUFA during rotifer feeding, which would increase the endogenous store of these essential fatty acids, tends to promote better survival in older mullet larvae.

In summary, fish fed rotifers containing high levels of *n-3* HUFA grew significantly better than larvae fed rotifers containing low levels of these essential fatty acids. In contrast, dietary *n-3* HUFA appeared to promote growth less well during Artemia feeding attesting to the importance of DHA in early larval pre-metamorphic development. Mullet larvae demonstrated high stress resistance when exposed to handling as well as 30 and 60 seconds of air exposure, independent of dietary supplementation of EPA and DHA. On the other hand, the larvae were sensitive to rapid salinity change that was dietary *n-3* HUFA dependent, particularly a decrease in salinity from the ambient 25‰ to 0 ‰ . Mullet larvae ingesting high *n-3* HUFA during only rotifer or rotifer and Artemia feeding exhibited enhanced stress resistance and reduced mortality during low salinity challenge compared to fish ingesting increased EPA and DHA levels only during Artemia feeding. On the other hand, supplementing ArA and high *n-3*HUFA together in the diet tended to increase mortality during exposure to 0 ‰. The results from the stress tests supported earlier findings that suggested *n-3* HUFA ingestion during rotifer feeding might influence late larval and juvenile quality more than during Artemia feeding.

As a result of these studies, as well as others on abiotic factors (not reported here), a larval rearing protocol for mullet was developed that included detailed guidelines for salinity, water exchange, algal tank concentration, temperature, aeration, enrichment of live food, schedule for weaning on to dry particulate diets. The successful application of this protocol was clearly shown in the fall of 2006 where over 60,000 fry were produced 3 months after a high quality spawn.

Acclimation study- Fig. 10 shows a non-significant increase in survival in fish fed the high *n*-3 HUFA diet regardless of salinity treatment. More importantly, the mullet larvae.exposed to a reduction of salinity from 25 to 6 ‰ and fed a high *n*-3 HUFA diet survived well (>75%). In the second trial, large mullet larvae showed significantly ($P<0.05$) higher survival than their smaller cohorts (Fig. 10), which was independent of their saline environment. Moreover, these larger fish survived similarly to the first study (ca 75%) when salinity was decreased from 25 ‰ to that of freshwater. This suggests that freshwater acclimation could be incorporated into the larval rearing protocol in fast growing larvae previously fed a high *n*-3 HUFA diet leading to a dependable supply of mullet fry already pre-adapted to freshwater.

Vitamin C trial- The recent results obtained at Alexandria are illustrated in Figs. 11 & 12. Optimal growth at that laboratory, as evidenced by total length, dry weight and survival was achieved when a mixture of DHA and EPA was added to rotifers as feed for mullet larvae. The addition of arachidonic acid (ArA) to DHA only improved survival of larvae but less than a combination of DHA and EPA. The addition of vitamin C to HUFA improved the survival of mullet larvae.

Overall conclusions

a) Dietary *n*-3 HUFA during rotifer feeding significantly improved growth while these fatty acids were less effective during *Artemia* feeding.

b) Mullet larvae demonstrated high stress resistance to handling a well as air exposure independent of *n*-3 HUFA supplementation.

c) Conversely, larvae were sensitive to rapid salinity change that was dietary *n*-3 HUFA dependent particularly from 25-0 ‰.

d) Larvae ingesting high n-3 HUFA during only rotifer or rotifer and Artemia feeding exhibited enhanced stress resistance, in terms of accumulated mortality, compared to larvae ingesting high n-3 HUFA only during Artemia feeding.
e) Possibly both dietary *n*-3 HUFA and algal level play roles in pigmentation and metamorphic success.
f) Preliminary results suggest that *n*-3 HUFA are also effective in growth, survival and reduced deformities during post metamorphosis.
g) Preliminary results suggest that during larval rearing (23 DPH) ambient salinity can be successfully reduced to 0 without markedly affecting survival.
h) DHA, EPA, and vitamin C enriched rotifers has a positive effect on survival and growth of mullet larvae.

III – Nutrition Research

Introduction
A modest program of mullet monoculture is being conducted in Taiwan using formulated feeds, almost nothing, however, has been published about nutritional requirements.

The Egyptian and Israeli scientists agreed that the most important route to follow would relate to quantifying protein and energy needs of mullet to ensure optimal growth. These are recognized as the major nutritional needs of fish for maintenance and growth. This methodology, derived from terrestrial animal husbandry, has already been applied to a number of marine cultured species, including gilthead seabream, European sea bass, and white grouper (Lupatsch et al 1998, 2001, 2003).

The Eilat and Alexandria laboratories pursed somewhat parallel lines of research, adding measured amounts of feed on a time-controlled basis, keeping careful track of environmental changes (e.g. temperature, salinity etc.) trading their data along the way.

Objectives
The agreed-upon goals of both laboratories included:

(a) Development of an optimal growth curve for mullet growth in tanks (Israel) and earthen ponds (Egypt) which can be used to predict growth of the fish along the whole range of sizes from juvenile to market size (400g).
(b) Determination of digestible protein and energy requirements for maintenance and growth of mullets at different sizes from juvenile to market size. Use of four protein levels for growth trials in Egypt.
(c) Gross body composition changes across the weight range from juvenile to market size.
(d) Determination of metabolic body weights for protein and energy from the starvation treatments in the short-term growth trials (primarily in Israel).
(e) Determination of the efficiency of utilization of protein and energy from data of the short term feeding trials, involving formulas developed at both laboratories.
(f) Development of a database for daily protein and energy necessary for different sizes of mullets to achieve their growth potential at specific water temperatures.

Methods- NCM

a- Fish were grown out in experimental tanks to market size on a surplus of feed (fed to satiation) in experimental 700 L tanks with constant sea water exchange and aeration..

b- Daily feed intake of fish was monitored during the growout of fish to market size.

c- Mathematical expressions were developed to describe weight gain and feed intake of fish grown to market size.

d- Periodic sampling was carried out at different sizes of fish during the development of the optimal growth curve for analysis of body composition changes that are occurring.

e- Laboratory analysis of feeds and mullet for proximate composition: protein, lipid, moisture, and ash using standard methods AOAC were carried out on samples. Energy content of feeds and mullet was determined using a Parr bomb calorimeter with benezoic acid as the standard.

f- Development of mathematical expressions defining metabolic body weight for protein and energy from the cumulative data of the starvation trials were made.

g- Short term feeding trials (30 – 45 days) with increasing levels of feed from starvation to excess to determine protein and energy retention as a function of protein and energy intake were carried out.

h- Mathematical expressions to describe the relationship between nutrient intake (protein or energy) and their retention and the efficiency with which the fish utilizes these nutrients were developed.

i- Calculation of daily protein and energy needs for different size of mullets to achieve their growth potential was made from the mathematical expressions developed.

j- Use was made of the mullet's daily protein and energy requirements to show how we set the feed formula criteria to meet these needs for fish of different sizes.

Results

a- The following equation describes the formal approach used in calculations of protein and energy requirements of mullets for maintenance and growth:

$$\textbf{Energy or protein requirement} = a \times BW\,(kg)^{b} + c \times \textbf{growth}$$

Where $BW\,(kg)^{b}$ = metabolic body weight

a = maintenance requirement

c = cost in units of energy or protein to deposit energy or protein as growth.

b- The equation developed for defining daily weight gain at specific fish sizes (Figure 13) is:

$$\textbf{Weight gain (g / day)} = 0.00226 \times BW\,(g)^{0.557} \exp^{(0.140 \times T)} \qquad r^2 = 0.89$$

for W = Weight (g) of fish for weights between 1 and 400 g.

T = Temperature between 20 - 27^{0}C.

c- The equation describing feed intake at certain fish sizes and water temperatures (Fig. 13) is:

$$\textbf{Feed intake (g / day)} = 0.0029 \times \textbf{Weight (g)}^{0.653} \times \exp^{0.135 \times \text{Temp}} \qquad r^2 = 0.93$$

d- Changes in body composition as a function of fish size are defined by the following three equations and the changes are illustrated in Fig. 14:

$$\textbf{Energy (kJ / g)} = 5.25 \times BW\,(g)^{0.131} \qquad r^2 = 0.91$$

$$\textbf{Lipid (\%)} = 6.12 \times BW\,(g)^{0.196} \qquad r^2 = 0.89$$

Moisture (%) = **$75.96 \times BW\ (g)^{-0.038}$** **$r^2 = 0.92$**

e- Metabolic body weights for protein and energy are defined from the short term growth trials using the starvation data:

Energy loss (kJ fish^{-1} day^{-1}) = $46.2\ (\pm 1.84) \times BW(kg)^{0.80}$ **$r^2 = 0.97$**

Protein loss (g fish^{-1} day^{-1}) = $0.38 \times BW(kg)^{0.70}$ **$r^2 = 0.96$**

The exponents of $(kg)^{0.80}$ and $(kg)^{0.70}$ can thus be described for mullet as the metabolic body weights for energy and protein, respectively (Fig. 15).

f- Figure (16) which illustrates the relationship between digestible protein intake ($x=g/kg^{0.70}$) and protein gain ($y=g/kg^{0.70}$) in mullets at two different temperatures and indicates linear relationships defined by the equations:

Trial at 23^{0}C $y = -0.20 + 0.42\ x$ **$r^2 = 0.96$**

Trial at 26.5^{0}C $y = -0.31 + 0.41\ x$ **$r^2 = 0.95$**

What is clear from these expressions and the Figure is that at 0 protein gain the maintenance requirement for protein increases with water temperature, being 0.48g $(kg)^{-0.70}$ at 23^{0}C and 0.75g $(kg)^{-0.70}$ at 26.5^{0}C. But, as the slopes of both expressions, which refer to the efficiency of use of protein for growth, are the same (0.42) we know that efficiency is not affected by water temperature.

g- The relationship between digestible energy intake ($x=g/kg^{0.80}$) and energy gain ($y=g/kg^{0.80}$) at the same temperatures (Figure not shown) are also linear and described by the equations:

Trial at 23^{0}C: $y = -27.3 + 0.54\ x$ **$r^2 = 0.97$**

Trial at 26.5^{0}C $y = -39.0 + 0.55\ x$ **$r^2 = 0.97$**

As with protein, energy needed for maintenance (0 growth) also increases with temperature being 50.7 kJ $(kg)^{-0.80}$ at 23^{0}C and 70.9 kJ $(kg)^{-0.80}$ at 26.5^{0}C. Slopes of both expressions are parallel and equal to 0.55, the efficiency of energy use or growth in mullets so as in protein they are not effected by temperature.

h- Using the various mathematical expressions developed in this study, we calculated the required feed intake and it's digestible protein and energy content for mullets of four sizes that is needed for them to obtain their predicted weight gain at 23°C. The following table provides this information:

Body weight, per fish	5g	50g	250g	750g
Predicted wt. gain, g day^{-1}	0.14	0.50	1.23	2.26
Feed intake, g day^{-1}	0.21	0.93	2.67	5.47
DE need, kJ fish^{-1}day^{-1}	2.38	12.61	40.95	91.90
DP need, g fish^{-1} day^{-1}	0.066	0.257	0.670	1.298

i- The following table shows how we use the information provided in h- above to set criteria for feeds to meet the fish's protein and energy needs at these weights:

Body weight, per fish	**5g**	**50g**	**250g**	**750g**

Using a predetermined DE content of feed of 12 or 15 MJ per kg								
DE content of feed	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*	*12 MJ*	*15 MJ*
Required feed intake, g $fish^{-1}$ day^{-1}	0.20	0.16	1.05	0.84	3.41	2.73	7.66	6.13
Resulting DP content of feed, g kg^{-1}	330	412	245	306	196	245	169	212
Predicted FCR	1.43	1.14	2.10	1.68	2.77	2.22	3.39	2.71
DP/DE ratio, g MJ^{-1}	27.5	27.5	20.4	20.4	16.3	16.3	14.1	14.1
Using a predetermined DP content of feed of 250 or 350g per kg								
DP content of feed	*250g*	*350g*	*250g*	*350g*	*250g*	*350g*	*250g*	*350g*
Required feed intake, g $fish^{-1}$ day^{-1}	0.26	0.19	1.03	0.73	2.68	1.91	5.19	3.71
Resulting DE content of feed, MJ kg^{-1}	9.10	12.73	12.24	17.27	15.27	21.43	17.70	24.77
Predicted FCR	1.86	1.36	2.06	1.46	2.18	1.55	2.29	1.64
DP/DE ratio, g MJ^{-1}	27.5	27.5	20.4	20.4	16.3	16.3	14.1	14.1

Discussion and Conclusions

The mathematical expressions developed in the course of this study allow us to accurately formulate feeds for the rearing of grey mullet. Feeds that provide sufficient nutrients (protein and energy the major nutrient needs of fish) for both maintenance and predicted growth while having a minimal effect on environmental enrichment. Such feeds are more efficient hence, less costly and are more environmentally friendly, very desirable characteristics for fish farming enterprises.

Methods - NIOF

a- Average growth performance of grey mullet M. cephalus, reared in earthen ponds was determined for fish fed at four dietary protein levels (30, 40, 50 and 60%).
b- Digestibility coefficients of the four dietary protein levels were obtained using the stripping method to collect feces.
c- Gross body composition of grey mullet flesh (initial and final body weight) was determined for the reared fish.
d - Nutrient retention efficiencies of protein lipid, and energy were obtained using the formula:

NRE = [Nutrient gain in carcass/(Nutrient intake x Digestibility of nutrient)].

e- Economic evaluation was applied to the experimental diets.

Actual experimental procedures

(a) Three ponds with water volume of about $90m^3$ and a fourth pond with about $290m^3$were used.
(b) Stocking density was one fish/m^3 in all ponds.
(c)The experimental period lasted for three months.
(d) The amount of daily ration (3% from live body weight) was divided into two equal meals at 10 a.m. and 2 p.m. daily .
(e) Hand feeding was applied to ensure that the feeds are available to all the fish in the ponds for consumption .
(f) The chemical analyses were carried out to estimate moisture, crude protein, ether extract, crude fiber and ash content according to the methods of AOAC (1984), and

nitrogen-free extract was calculated by difference. Gross energy were measured using Oxygen Bomb Calorimeter.
(g) Digestibility trial was conducted for ten days. 0.5% chromic oxide was added to the diets .The feces were collected by stripping.
(h) Water samples were taken every two weeks for analysis of dissolved oxygen, ammonia, and salinity, using the American Public Health Association (APHA, 1992) methodology. The pH values were measured by digital pH meter (model 68 of "Systems and Engineered Designs").

Calculations:
*** Specific growth rate (%/day) =**
[(Ln Final body weight – Ln Initial body weight)/Period (day)] x 100

***Feed conversion ratio (g./g.)=Feed (g.)/Gain (g.)**

Digestibility coefficient,% = 100-100[(Ii / If) x (Nf / Ni)],
Where I=concentration of chromic oxide,I = ingest, N=concentration of nutrient (%,demand f = feces.

Nutrient retention efficiency = NRE=[Nutrient gain carcass/(Nutrient intake X Digestibility of nutrient)].

Results
a- Dietary protein level requirements were decreased to 30% for grey mullet *M. cephalus* reared in earthen ponds as compared with 60% in tanks.

b- Digestibility coefficient of protein was higher for grey mullet, *M. cephalus* fed at 30% protein level compared to those fed at 40, 50 and 60%.

c- Nutrient retention efficiency of protein was higher for grey mullet *M. cephalus* fed at 30 % protein than 40, 50 and 60 %.

d-The net revenue percentage of grey mullet *M. cephalus* fed at 30% protein level has increased by about 76%, higher than those 40, 50 and 60% level .

e-The artificial feed could be considered as supplementary feed beside the natural feed.

Conclusions:

The effectiveness of diets formulated on the basis of digestible energy and nutrients can be evaluated by measuring weight gain ,feed efficiency (gain ,g./feed g.) or feed conversion (feed, g./gain ,g.) and body composition of fish receiving the diets under particular culture regimes.

The efficiency of each feedstuff in the diets can be evaluated using the following scheme:-
(a) Analyzing feed ingredients for their chemical composition.
(b) Measuring digestibility of feed ingredients.

(c) Formulating and reformulating balanced diets in combination with several ingredients or substitution with other ingredients.
(d) Observing the levels of productivity supported by these diets.
(e) Measuring the feed intake and weight gain and calculating the feed efficiency.
(f) Calculating energy and nutrient retention efficiencies by analyzing fish carcasses. NRE can be calculated using the formulae: **NRE = [Nutrient gain in carcass / (Nutrient intake x digestibility of nutrient)].**

IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

With the obvious exception of political unrest, there can be no subjects of greater importance to Middle East cultural and economic well-being than food resource expansion and water conservation.

Prior to the advent of MERC a quarter of a century ago, technology transfer in the Middle East was hopeless. Although the Israelis had perhaps the best technology in the region, external contact was zero. The dramatically expanding influence of MERC itself, aided by Egyptian leadership, has already and will undoubtedly continue to, develop relationships, as already demonstrated, with Lebanon, Jordan, Tunisia, Morocco, and the Palestinians. The author's personal contacts with Jordanians and Palestinians brought assurances of desire for collaboration nearly two decades ago. Several projects between them and Israelis have already matured.

At one time, the Saudi Government actually signed an agreement to collaborate with the Egyptians on a cooperative Egypt/Israel mariculture project; this was unfortunately curtailed. A very high level American consultant team, which has recently been contracted by the Saudis to review their Red Sea facilities, has been advised to explore the possibilities of enlarging their mariculture program, in which grey mullet might be featured.

As referred to above, both laboratories intend carrying the project further. The Egyptian government is already providing assistance and the Israeli Ministry of Agriculture is already sponsoring preliminary studies designed to expand monoculture of mullets. It is difficult to state how much future expansion will occur but it is fairly obvious that mullet production will expand in both countries. Of two projects submitted to MERC by Egypt and Israel which involve grey mullet, one is in the last stages of contract negotiation and the other is in the review process. Both studies are expected to advance mullet culture significantly in the region in the years to come.

By their own admission, all of the project scientists have learned much about the mullet; their level of knowledge is world class. Clearly, were it not for the maddeningly frustrating political tensions, mullet culture would become a win-win proposition.

PROJECT ACTIVITIES/OUTPUTS

The project scientists all met together at the newly developing Cyprus Research Foundation at Larnaca, Cyprus. The Cypriots, with assistance from MIT, are in the

process of forming a Research Enterprise with a formidable facility and an ambitious program plan. Their mariculture activity is already impressive.

The Foundation's Board of Trustees actively seeks a strong and productive relationship with our MERC scientists, as a means of furthering their own marine-oriented aspirations. Accordingly, they assumed responsibility for all costs associated with the meetings except transportation. This was particularly appealing to the author who was, and is, paying all of his own expenses on the project. These meetings took place in March 2004, 2005, and 2006.

In addition to the intensive work sessions at the annual meetings, the project scientists maintained electronic mail contact through out the project. This facilitated moving the project research forward and allowed the development of two additional grant proposals which are in various stages of negotiation or review for eventual funding. Both proposals are natural outcomes of the grey mullet work done under this project and should help in the expansion of regional mullet culture and the development of regional land-based polyculture systems that are environmentally friend.

The project produced the following publications:

- Aizen, J., Meiri, I., Levani-Sivan, B. and Rosenfeld, H. 2005. Enhancing spawning in Grey mullet by removal of dopaminergic inhibition. *General and Comparative Endocrinology*, 143: 212-221.
- El-Garabawy, M.M. and Assem, S.S. 2006. Spawning induction in the Mediterranean grey mullet *Mugil cephalus* and larval developmental stages. *African Journal of Biotechnology,* vol. 5(19): 1836-1845.
- El-Garabawy, M.M, Assem, S.S. and ismail, R.F. 2006. Steroid hormone in serum of female *Mugil cephalus* in relation to steroidogenic tissue (ultrastructure) in Lake Quaron. *Egypt. J. Aquat. Bil. & Fish.* vol. 30.
- El-Garabawy, M.M, Assem, S.S. and Fahmy, A.F. 2007. Steroid hormone in serum of male *Mugil cephalus* in relation to steroidogenic tissue (ultrastructure) in Lake Quaron. In press.
- Abdel Rahman, S.H. and Helal, A.M. 2007. Evaluation of various feeding regimes for optimal growth and survival of early stages of grey mullet, *Mugil cephalus. Egypt. J. Aquat. Bil. & Fish.* vol. 30.

A number of students, and technicians took part in this project in Israel and Egypt. Some of the participants were doing research dealing with grey mullets towards meeting their master's thesis requirement, while others were undergoing training in aquaculture research methodologies.

Israeli participants: M.Sc students: a) Y. Aizen – 'Spawning induction of grey mullet (*Mugil cephalus)'* b) R. Solomonovich – 'Moleculat characterization of sex reversal in grey mullet (*Mugil cephalus*).

Reseach associates & technicians: a) Dr. Iris Meiri, b) Dr. I. Lupatsch, c)G. Heinisch, d) E. Toledano, e) S. Lutzky, f) O. Nixon-Stupler, g) I. Geva, h) B. Ginsberg, i) Y. Chernov and j) R. Kastel.

Egyptian participants: M.Sc students: a) Ranai Fahmy Ismael (Masters thesis- Alexandria University, b) Hasan Ez El Din Mohammed (Masters thesis – Asyout University).

Reseach associates & technicians: a) Dr. Saffa Mohammed Abdel Kawy, b) Dr. Samira Shehata Asem, c) Suzan Mohammed Fadda, d) Mohammed Abdel Hamid El Okby, e) Mohammed Ata Allah, f) Abdallah Saad Mahmoud and g) Mahmoud Gaber Mohammed.

PROJECT PRODUCTIVITY

Did the project accomplish all of its goals? Candor requires the response: Technically – very much; socially – only partly.

On the plus side, the Egyptians have completed an excellent building program and have made wonderful progress in their R&D start up. The Israelis have made outstanding inroads in mullet culture and with the Egyptians, have solved many of the reproduction, larval rearing and nutrition problems.

Mid-East politics continues to dominate an otherwise marvelously effective program. The scientists learned how to culture mullet. They will continue to learn more about this invaluable food resource as time goes on. The technology is fostered by both governments and business interests in Israel. Many stomachs will be satiated by mullet and money will be made.

While one's instincts may argue that the U.S. Government might, itself, do more to ease both official and interpersonal contacts, political considerations are beyond the ken of this program and its principal investigators, including this author. At the minimum, however, it must be pointed out that travel by Egyptians to Israel and Israeli to Egypt was permitted by neither of their governments, for ost of the period of the project.

Regardless of the reasons, honesty compels the admission that interpersonal contact during the course of this project was disappointing and could have been helpful. The three annual meetings were among the most productive and impressive within this author's memory. More personal travel by the Principal Investigator (this author) might have been useful, but he had personally spent far beyond his means on the project, as it was, and simply could not afford further costs.

FUTURE WORK

One of the most promising achievements of this project is its stimulation of future work. The project has occasioned interest, not only with its own technical community, but broadly through the concerned societies in both countries. Work is already underway in both Egypt and Israel in all three of the major research subdivisions: reproduction, larval rearing and nutrition. The scientists believe in common that their goals are much closer than when the project began, and can be achieved in the

immediate future. The ultimate result will be possibly the most completely desirable food source in the region.

The Egyptians have already demonstrated an insatiable market for mullets as a food resource, and Israeli kibbutzim have begun to consider monoculture of mullets to expand annual production. The development and expansion of by-products of mullet culture (Batarekh/Karasumi) are also been investigated.

The mullet project, however, is only one of a catalog of excellent projects of MERC, possibly the most imaginative, productive, and socially enhancing programs that this country ever formulated for the Middle East. Properly developed, MERC can provide outstanding leadership for American government and business interests in that troublesome but vitally important region. In this regard, however, USAID has failed to capitalize its potential.

The fact is that MERC's Achilles heel is its internal communications failure. Each of the Principal Investigators has developed, individually, both technical, administrative, and diplomatic techniques ranging from successful to highly successful. Each year, we try to persuade USAID to call together all of its imaginative PIs to trade ideas, techniques, problems, and opportunities. And (at risk of sounding like a broken record) this author must admit total failure.

Sixty-five years of working in-or-with Government has failed to provide this P.I. with any degree of rationale as to why these potentially invaluable advisors cannot be convened at least twice per decade, to further advance a cooperative food-oriented program in the Middle East on which the U.S. Government spends one twentieth of one percent of what it spends on military assistance.

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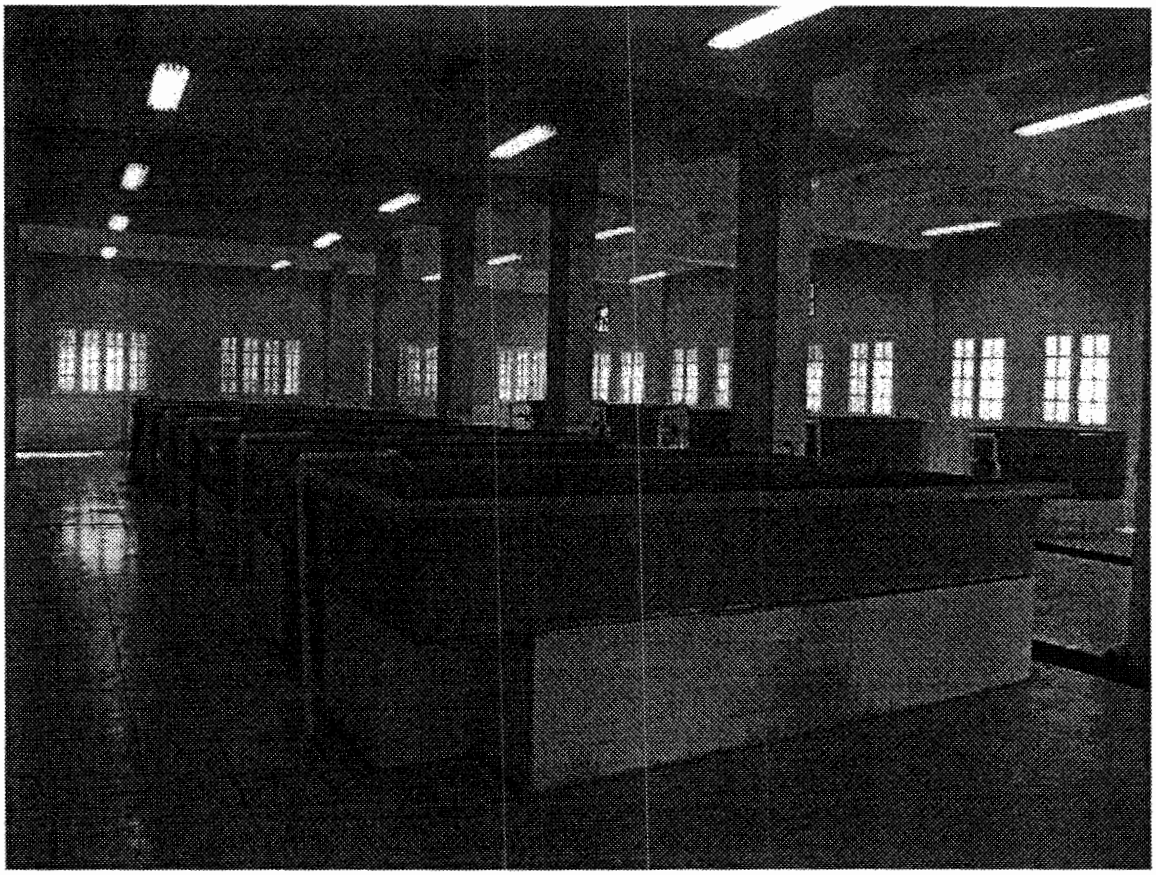
SECOND APPENDIX: FISH HATCHERY AND MACHINERY DESIGNED AND CONSTRUCTED AT FAYOUM UNDER USAID GRANT.

Fayoum Hatchery, Round Tanks with Running Water System for Fish Larvae Rearing

BEST AVAILABLE COPY



Fayoum Hatchery, Rectangular Tanks for Nursing of Fish Larvae

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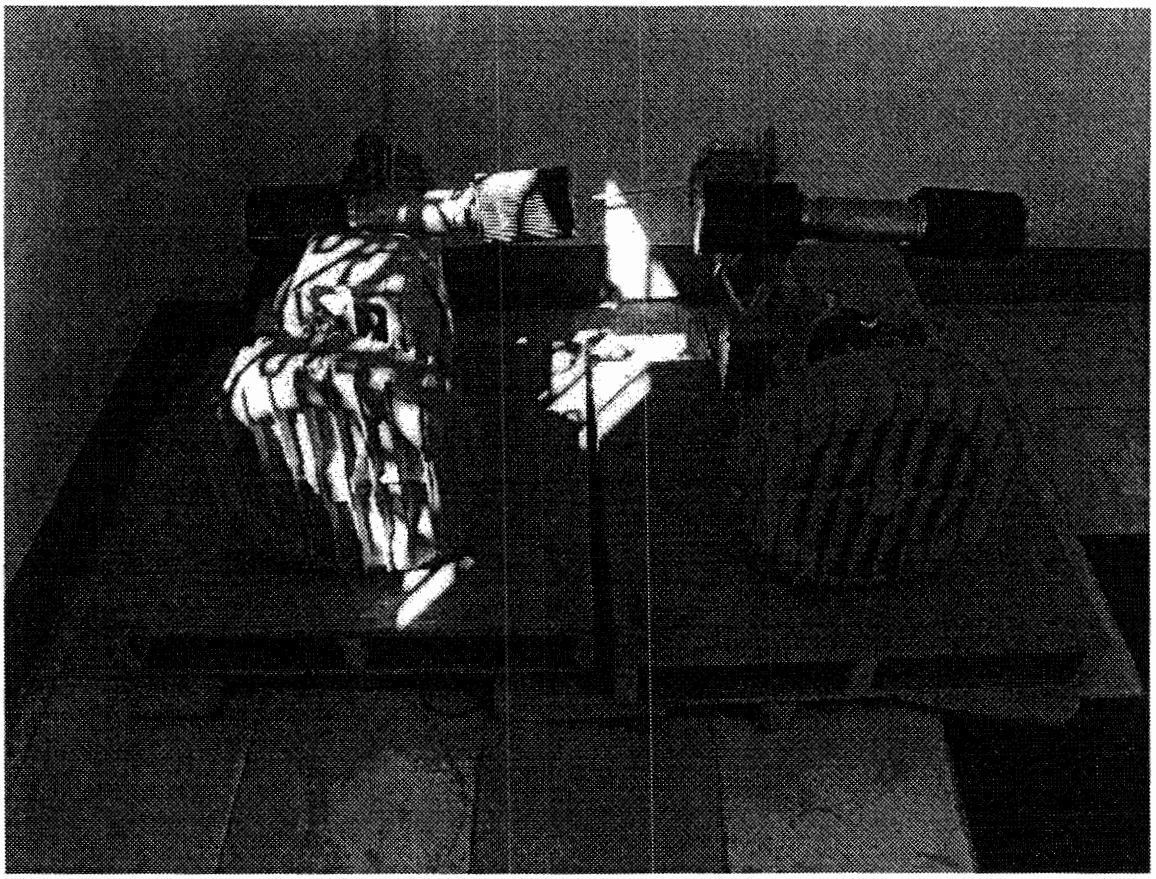

Fayoum hatchery, 5 hp Blowers for Air Supply to the Hatchery

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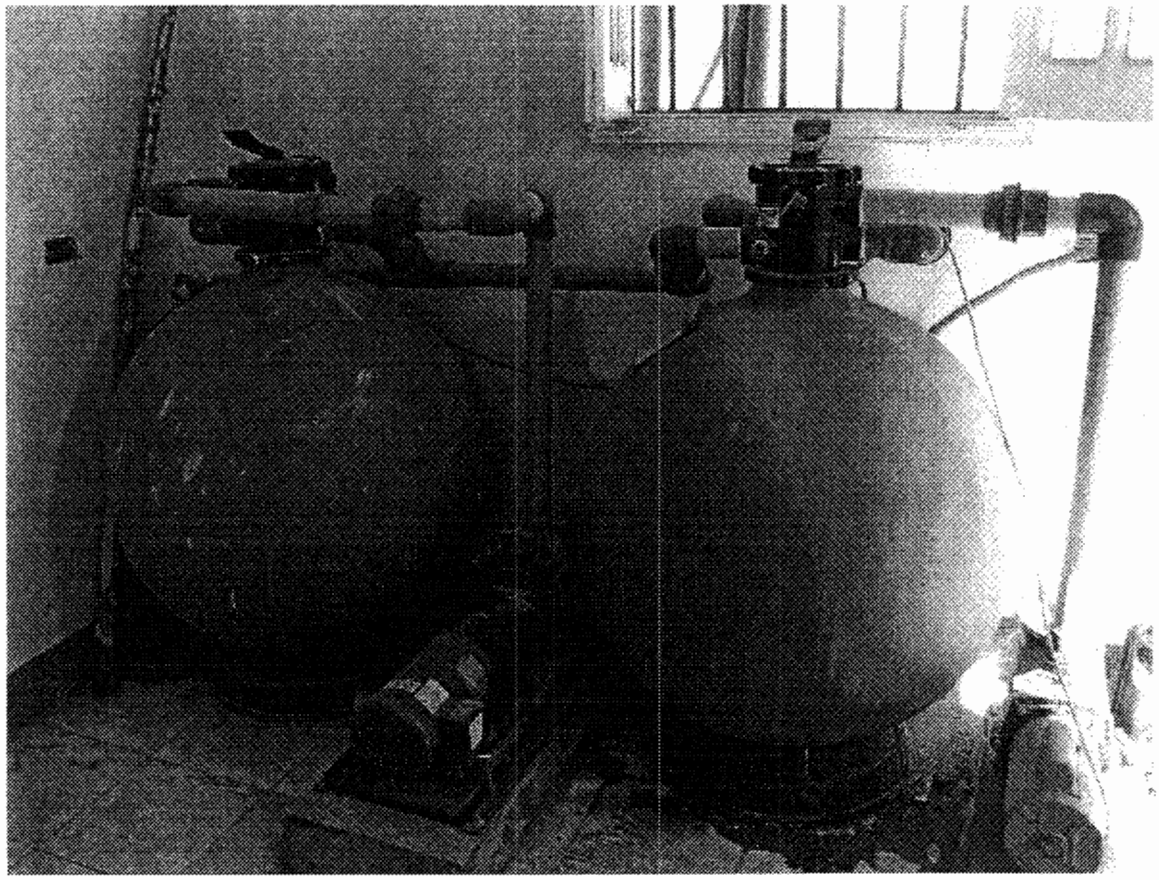

Sand Filters and Pumps to provide Hatchery with clean Seawater

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Fayoum Hatchery, UV unit for Sterilization of Hatchery Seawater

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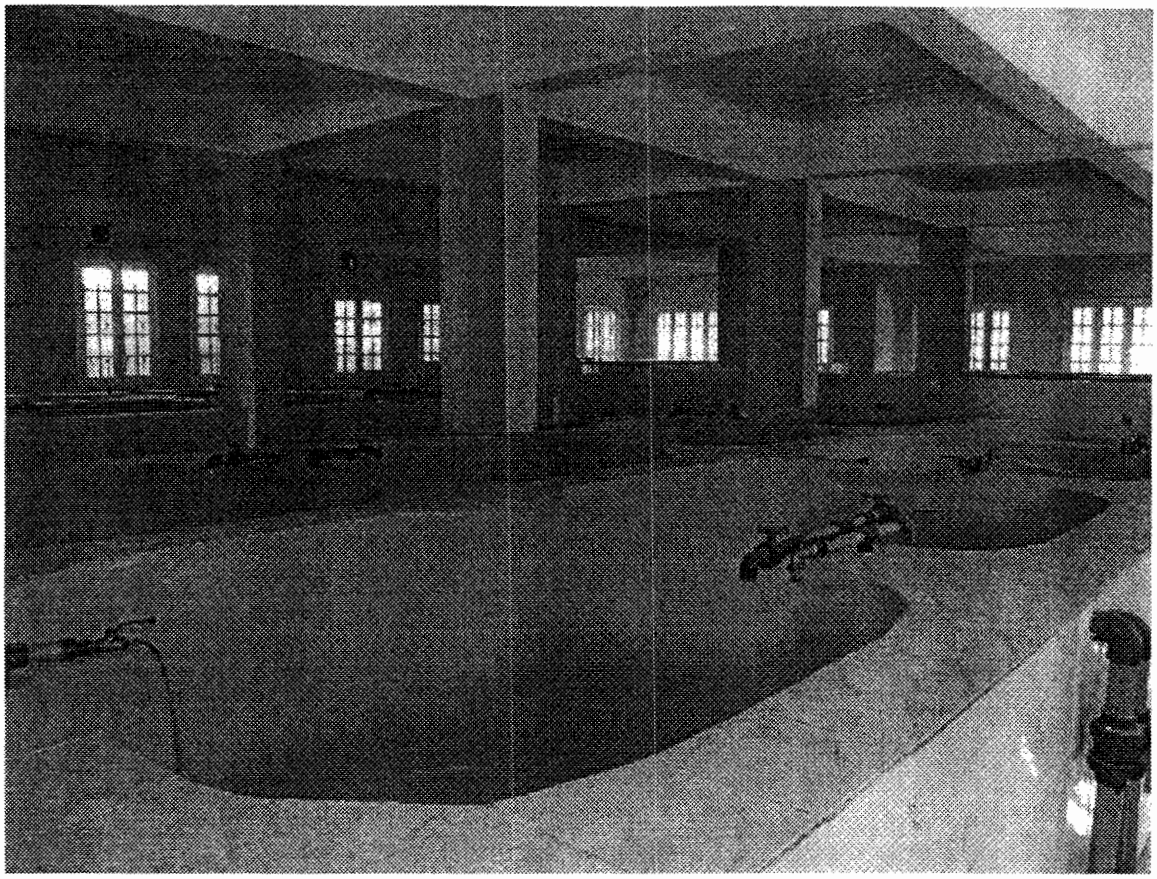

Fayoum Hatchery, Painted Concrete Tanks for Rotifer Culture

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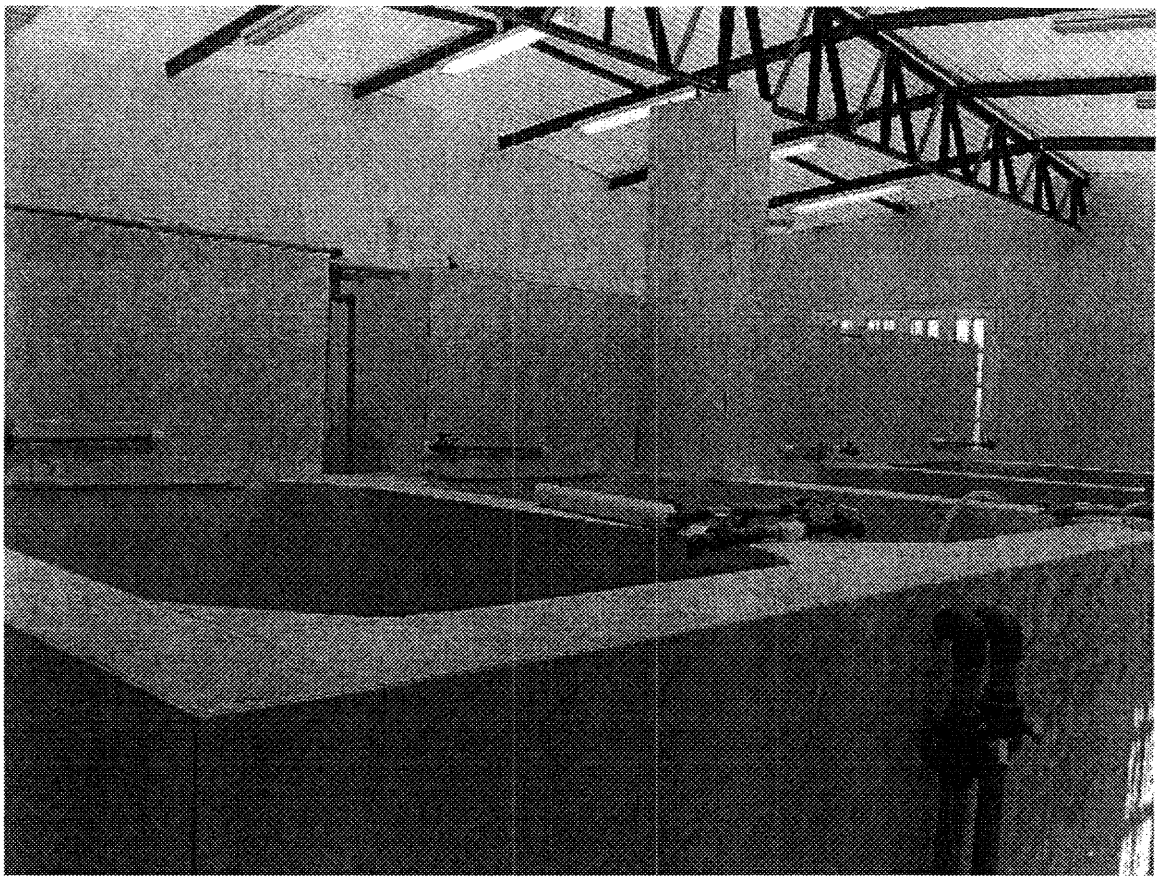

Fayoum Hatchery, Green House for Mass Culture of Micro-Algae

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