

PD-ABN-826

93311

Cooperative Marine Technology Program
for the Middle East (Phase IV)

Third Annual Report

Compiled and Edited by

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Submitted to U.S. Agency for International Development

(Contract No. HNE-0158-G-00-2062-00)

1 December 1995

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the Middle East - Phase IV**

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**Cooperative Marine Technology Program for the Middle East
(Phase IV)**

Contract No. HNE-0158-G-00-2062-00

Third Annual Report

1.- Foreword

This is the Third Annual Report of Phase IV of the Cooperative Marine Technology Program for the Middle East. The research activities which were carried out between September 1994 and September 1995 in the following three projects are reported herein:

1. *Trophodynamics of the Southeastern Mediterranean with Special Reference to Commercially Important Fishes.*
2. *Investigations of Lake Ecosystems in Egypt and Israel with Implications for Fisheries and Water Quality Management.*
3. *Seafood Safety and Fish Decontamination- Technical and Health Implications..*

Also included in this report is a section on Program Management.

As the Program Principal Investigator and Chief Scientist, I wish to express on behalf of the Projects' Principal Investigators and the National Coordinators/ Managers of the Program my sincere thanks to the U.S. Agency for International Development for the financial support of this cooperative research Program.

1 December 1995

Sayed Z. El-Sayed
Project Principal Investigator &
Chief Scientist

Third Annual Report (2) Executive Summary

Project I. Trophodynamics of the Southern Mediterranean with Special Reference to Commercially Important Fishes.

During the reported period, the efforts of the Israeli investigators were directed toward carrying out multidisciplinary surveys of the Israeli continental shelf; tracking data from the surveys conducted during the previous period; and at optimization of design and further surveys.

With the replacement of problem-ridden, old BioSonics Echosounder with a reconditioned Model 102 Dual- Beam Echosounder, two surveys were successfully conducted on 14-18 May and 17-20 September 1995. On both cruises acoustic surveys of pelagic fish stock and concurrent sampling statistics to measure environmental parameters were made on board the R/V *Shikmona* by the Israeli scientific team in collaboration with Mr. Gert van Dijken, of Texas A&M University. The acoustic surveys consisted of 19 transects and 29 concurrent sampling stations. At these stations, samples to study the concentrations of zooplankton, phytoplankton standing crop, and inorganic nutrients were collected. Also at these stations, salinity and temperature were measured.

Concurrent with the acoustic surveys, trawling surveys of the demersal fish stocks were undertaken on commercial trawlers by personnel from the Fisheries Department of the Ministry of Agriculture. In May 1995, the trawling survey was conducted by three fishing vessels, and in September, 1995 six vessels were employed in the survey.

The treatment of the data from the surveys carried out between 27 February and 2 March 1994 and between 14 and 18 August, 1994 revealed that during the latter survey, the biomass of the red mullet was estimated at 470 tons, whereas the biomass of pelagic fish was estimated to be 6,080 tons.

The Texas A&M effort continued to concentrate on estimating primary production off the Israeli coast. During the survey cruises multiple primary productivity experiments were successfully completed. Additionally, water samples were collected for pigment analysis, flow cytometry, bacterial counts and fluorescence profiles to study the distribution of the phytoplankton population.

Project II.

Investigations on Lake Ecosystems in Egypt and Israel with Implications for Fisheries and Water Quality Management.

1. Lake Kinneret (Israel)

With the acquisition of the reconditioned Model 102 Dual-Beam Echosounder in May 1995, the Israeli investigators (joined by three Egyptian scientists), embarked on an intercalibration survey between the BioSonics Echosounder and the HADAS system. The results of the survey showed that the two systems gave compatible results in terms of number of fish per unit area.

The acoustic surveys showed that the lavnun population continued to be at a level higher than considered desirable from water quality considerations. Based on the results obtained, a lavnun reduction program was instituted in the winter of 1994/95. It was hoped that the removal of some of the small fish would improve conditions for the rest of the fish to allow them to reach a size which would permit the regular fishery to resume. Unfortunately, budget constraints will undoubtedly hamper the fish removal program, which will impact negatively on the water quality of Lake Kinneret.

Also during the period covered by the report, an investigation of the "Effects of Cyanobacteria on zooplankton grazing and nutrient excretion was carried out in Lake Kinneret. The results to date suggest that the presence of cyanobacteria, especially in high abundance, could be detrimental to zooplankton, and could thereby affect the entire Lake Kinneret food web.

2. Lake Qarun (Egypt)

The Research activities carried out in 1994/95 focused on:

- (a) Environmental Studies of Lake Qarun
- (b) Fisheries and Fish Production

(a) Environmental Studies:

Monthly surveys of water quality of Lake Qarun and the two main drains (El-Bats and El-Wadi) were continued. The monitoring program included the following parameters: depth, air-water temperature, pH, Secchi disc depth, DO, COD, alkalinity, organic nitrogen, nutrient chemistry, hardness (Ca and Mg), cations (Na and K) and TDS. Pollutants by

heavy metals as well as pesticides were also studied. Additionally, monitoring the standing crops of phytoplankton, zooplankton and benthic organisms was conducted on a monthly basis.

(b) Fisheries and Fish Production

The yield per recruit model of Beverton and Holt (1957) to estimate the effect of fishing and natural mortalities and age at first capture, was applied to the yield of the most economically important fish species in Lake Qarun.

Research activities in 1994/95 were noted for the extensive contacts and frequent site visitations between the Egyptians and Israeli scientists who are involved in the Lake Management Project.

Project III. Seafood Safety and Fish Decontamination- Technical and Health Implications.

The Research activities undertaken in 1994/1995 entailed surveys of the safety of fish marketed in Egypt and Israel; experiments on the rate of depuration of toxic chemicals, and legislation dealing with seafood safety in Egypt.

Microbiological and parasitological surveys of fish catches from different locations in Egypt have been conducted by a research team from Cairo University under the direction of Dr. Mohyi S. Easa. These surveys included locations at (a) the High Dam Reservoir and the Nile at Aswan, (b) the Nile at Giza, (c) Lake Manzala, and (d) El-Max and Abukir at Alexandria. We have reported earlier on surveys conducted in the Aswan region.

The selected sites from Aswan in the south, to Lake Manzala and Alexandria in the north of Egypt, represented stark differences in the degree of water pollution, and the quality of the fish harvested from these waters. In the extreme south, the level of pollution was minimal and the quality of sampled fish were relatively high. In contrast, with increasing population density and waste discharges in the north, the polluted waters of Lake Manzala and the Alexandria region contained diseased fishes, infested with parasites and bacterial pathogens. Clearly, the prevalence of parasite encysted metacercarial in fish samples increased northward from Aswan (2.5 percent infection) to Cairo (17 percent infection), to Lake Manzala (100 percent infection). Similarly, bacterial counts of fecal coliform increased from low levels in Aswan to very high levels in Lake Manzala and Alexandria.

The public health significance of these results indicate that fish harvested from the polluted regions is unsafe if consumed raw. More significantly, the health risk is quite high for persons involved in fish handling, e.g. fishing, marketing, and food preparation.

Microbiological surveys of fish marketed in Israel gave the same results. In Israel, fishponds are generally supplemented with animal manure, and in some farms, with sewage. Bacterial and viral pathogens introduced in the fishponds may pose a health hazard for fish handlers, and consumers.

The rates of elimination of bacterial pathogens from fish were investigated by Dr. Mohamad El-Samra and his research team, at the laboratories of the National Institute of Oceanography and Fisheries, in Suez, Egypt. Research findings indicate that it will take several days to achieve 95% depuration of bacterial pathogens from fish. These are ongoing investigations. Research results will be useful in assessing the time required for fish depuration practices.

Investigations on the survival of bacteria and viral pathogens in natural water at the Hebrew University, Israel, provided very interesting results. This included a variety of pathogens, e.g. Hepatitis A. Virus (HAV), Poliovirus 1, F+ Bacteriophages, and E. coli. Different microorganisms exhibited different die-off rates. However, the startling results were that the virus can survive for several months, especially at low temperatures in the winter.

Studies on the uptake, accumulation, and elimination of pesticides by fish are being conducted by Dr. Mohamed Sherief, Ain Shams University, Cairo. Also the results of research done on an organophosphorus pesticide, commonly used in Egypt, are presented. These studies were conducted with the Nile Tilapia. Research findings provide valuable information on pesticide depuration rates, which will be used in the development of advisories for seafood safety.

A section on the legislation pertaining to seafood safety is also included. This relates to fresh and processed fish marketed in Egypt. A discussion on the limitations of some of these regulations is also included.

The plans for the fourth and final year of this project include providing a comprehensive account of (a) the microbiological and parasitological conditions of fish marketed in Egypt and Israel, (b) surveys of chemical contaminants in fish, (c) depuration rates of chemicals and pathogens from fish and their health and technical implications for fish farming practices, and (d) guidelines for improving seafood safety management, which includes procedures for assessing health risk, protection of consumers, and specific elaboration of pertinent legislation in Israel and Egypt.

Program Management

The Cooperative Marine Technology Program for the Middle East is coordinated by a Steering Committee composed of: Dr. H.K. Badawi (Egypt) assisted by Mr. A.I. El-Ibiary; Dr. Y. Cohen (Israel); Dr. R.B. Abel and Dr. S.Z. El-Sayed (U.S.A)

Dr. El-Sayed (Principal Investigator and Chief Scientist) continues to maintain the overall responsibility for the scientific effort of the Program, Dr. Abel (Program Manager) is responsible for maintaining the close interaction and the cooperation between the Programs' National Coordinators and the projects' Principal Investigators. He also shares the responsibility of planning the Steering Committee meetings and workshops,

Meetings and Workshops

The Steering Committee met in Haifa (Israel) and Cairo (Egypt) during September 20-28, 1994. Topics of discussion included: logistics and finance problems, advance planning, and ship acquisition.

On April 4-6, 1995 an historic 'first' took place when the Israeli, Egyptian and Jordanian teams met with the Steering Committee at the Marine Science Station in Aqaba, Jordan. The objective of the meeting was to discuss plans to re-submit a proposal dealing with the Gulf of Aqaba.

The meeting of the Steering Committee was followed by the Second Annual Workshop of Phase IV of the Cooperative Marine Technology Program for the Middle East.

Future Plans

- A one year no-cost extension of Phase IV was requested, and later approved by AID. Phase IV will thus be extended till 29 September 1996.
- The Third Annual Workshop of Phase IV will be held in Egypt between 8-11 January 1996.

(3) Program Management

As mentioned in the First and Second Annual Reports, the Cooperative Marine Technology Program for the Middle East is coordinated by a Steering Committee composed of: Dr. H.K. Badawi (Egyptian Coordinator) assisted by Mr. A.I. El-Ibiary; Dr. Y. Cohen (Israeli Coordinator); Dr. R.B. Abel (Manager) and Dr. S.Z. El-Sayed (Principal Investigator and Chief Scientist).

Dr. El-Sayed continues to maintain the overall responsibility for the scientific effort of the Program, as he has done from its inception. Assisted by Dr. Abel, he also compiles, edits, and submits the technical, semi-annual, and annual reports. Collaborative management is provided by Dr. Abel who is responsible for maintaining the interaction and cooperation between the Program's National Coordinators. He also shares the responsibility of planning the Steering Committee meetings and workshops.

Meetings and Workshops

A. The Steering Committee met in Haifa and Cairo during September 20-28, 1994. Principal subjects of discussion concerned:

1. The need for accelerating progress in the Trophodynamics Project- mainly due to logistics problems, these include : the BioSonics echosounder failure and acquisition of a ship for the Egyptian investigators.
2. Communications between the Egyptians and the various Israeli units.

B. On April 4-6, 1995 an historic 'first' took place when the Israeli, Egyptian and Jordanian teams met at the Marine Science Station in Aqaba, Jordan. The main objective of the meeting was to discuss progress made on the Gulf of Aqaba proposal.

1. Concerning the Aqaba proposal
 - a.) The Israeli contribution was nearly complete, except for some data to be furnished by the InterUniversities Institute.
 - b.) The Jordanians wished to review the text and revise it to suit their current needs.

2. Aspects of the Program document needing special emphasis included capacity building, education and training, and recruitment of other institutions.

Phase IV Workshop

The Steering Committee meeting was followed by the Phase IV Workshop. It was convened and chaired by Dr. El-Sayed with Dr. Abel acting as rapporteur. El-Sayed and Abel summarized the purpose of the Workshop, and gave a brief account of the proceedings of the Steering Committee meeting.

Seafood Safety and Fish Decontamination Project

In Dr. Mancy's absence, P.I., Dr. Mohamed Sherif made a presentation of the Sea Food Safety Project and he stated the project's goals to be:

1. Assessment of public health and safety of the production, marketing, and consumption of seafood in Egypt and Israel.
2. Generation of critical information to policy and decision makers and non-governmental organizations (NGO's); and
3. Developing better awareness of health risks of seafood contaminants and the improvement of seafood production and marketing.

Dr. Sherif listed sources of fish contamination, and related health aspects, and provided examples of economic loss. He tabulated the types of information needed for development of a public health advisory. Dr. Sherif was followed by Dr. M.I. El-Samra who was concerned with heavy metals in Tilapia and full-scale depuration studies. Sampling sites included Cairo, Port Said, and Aswan. Dr. Samra described the uptake of lead, zinc, cadmium, and copper. The results found in gills, muscles, and liver were compared

Dr. Mohey El-Said described microbiological and parasitological aspects of infections of Tilapia, mullet, and rabbit fish. His results were also tabulated and graphed.

Dr. Badri Fattal had outlined his project plan for fish decontamination at a previous meeting. This year his team has tested the survival of Hepatitis A. Virus in the environment as it pertains to food safety. His team has also screened fish samples for infectious agents and chemical contaminants in skin, liver, spleen, and muscle of Tilapia and mullet.

Investigations of Lake Ecosystems Project

1. ISRAEL

Dr. Moshe Gophen introduced the Lakes Management project, by discussing the twin objectives of water quality and fish concentration. His group's findings have led to shifts in the Israeli Government's fishing regulations. He brought the workshop participants up to date on the delicate interrelationships among phytoplankton, zooplankton and sardines. He also presented statistics on predator-prey relationships.

Dr. Paul Walline brought the participants up-to-date on his acoustic fish estimation research in Lake Kinneret. He graphically depicted the abundance from 1987 through 1993 and in a 3-dimensional analysis abundance per square kilometer. Of particular interest was his description of sardines as a counterproductive stock and the Government's effort to remove them. He further demonstrated the efficacy of sonic method by comparing the data with those obtained by purse-seining.

2. EGYPT

Dr. Ezzat Ibrahim described his parallel research in Lake Qarun, depicting 3-dimensionally, annual fish production from 1970 through 1993. He showed that although the salinity in Lake Qarun had risen dramatically from 33‰ to 38‰ since 1993, fish production has actually increased during that same period. He does not believe there is any casual relationship between fish production and salinity increase. His team conducted extensive studies of fish parasites, and using mathematical models displayed data depicting yield per recruit as a function of fishing mortality at different levels of natural mortality.

Dr. Fahmy El-Gammal discussed the catch per effort in Lake Qarun, estimating the maximum sustainable yield at 706 tons. He suggested a reduction of fishing effort from 384 to 165 fishing boats to maintain that figure. The solution is evidently to establish a hatchery, stocked annually with 60 million mullet fry.

Trophodynamics of the Southeastern Mediterranean with Special Reference to Commercially Important Fishes

1. ISRAEL

Dr. Yigal Kalikhman related the loss to the project caused by the BioSonics Echosounder failure. He described fish surveys out to 200 meters. He then compared field data with his mathematical models, and compared nutrient data with phytoplankton growth. [Dr. Cohen interjected by stating that the need for special care, in this instance, owing to the apparent phosphate-limiting along the coast except near, and in, Haifa harbor where industrial effluent furnishes abundant phosphate].

2. USA

Dr. El-Sayed gave an overview of Texas A&M research effort in the study of the phytoplankton standing crop and primary production off the Israeli coast.

3. EGYPT

In the absence of Dr. Ali Beltagy, P.I., Dr. M. Zaki described the results of two cruises made in September and October, 1994, in which 34 stations in 7 sectors were occupied, and in which 205 km of echograms were completed. She displayed current vectors and explained the water mix off the Nile delta. She discussed the distribution and abundance of the same chemical parameters which were measured as by Kalikhman.

Following the presentations, Dr. El-Sayed asked each project P.I. to discuss his/her plans through the end of his/her project. He urged immediate and intensive collaborative activity in order to have better material to present to the AID evaluation team. Following discussion of time needs, Abel stated that we would ask AID for a one year no-cost extension for all the projects' activities. Abel also agreed to explore the matters of:

- a) The \$15,000 promised to Israel for the extra cost of the BioSonics equipment;
- b) Delayed funding to the Egyptians from the University of Michigan and
- c) Delays in equipment procurements for the Egyptians.

In turning to the future, Abel and El-Sayed explained the futility of attempting to submit any more Program packages to AID. From now on, only individual projects will be designed and submitted. The Israelis and Egyptians agreed on the following priorities:

- 1) Coastal Process

- 2) Lakes Management
- 3) Mariculture of the Gray Mullet

Logistics

1. The major logistics problem continued to relate to the poor performance of the BioSonics gear, however, most of the problems were finally overcome, and the Israelis conducted successful cruises both during the Spring of 1995 and a final cruise in September 1995. Both cruises enjoyed Egyptian participation.
2. Largely through excellent cooperation from NOAA, Dr. Abel was finally able to identify an excellent research vessel available for transfer to the Egyptians. The *Deere Island* had the right dimensions and was well equipped. Unfortunately, however, the transfer never took place.

Management Issues

1. Two issues continue to bedevil the program. The first relates to Egyptian communications, which in spite of El-Sayed's efforts to force feed the systems, continues to lag.
2. The second concerns the Egyptians' lack of sea going capacity. The marine program, by definition, requires work at sea and yet, after 8 years of constant search and labor to provide that capacity (including personnel and material) have proved somewhat discouraging.
3. Reduced operational funding forced AID to cancel the Program's evaluation, scheduled for last Spring/Summer. This is unfortunate, because:
 - a) Each evaluation team recruited by AID has conducted its examination of the Program in a fair, and business-like and sagacious manner.
 - b) Such examinations have consequently contributed materially to Program conduct, both technically and operationally.
 - c) In turn, the analysis reports have enabled the Program's responsible coordinators to improve certain conditions that they may not have identified themselves.

It is hoped that the evaluation process can be continued in the future.

4. Interactions of all kinds need to be intensified. During the past year, Drs. Shazly, Zaki, and Hamza spent various periods of time in Israeli Kinneret and Elat Laboratories respectively.
5. In June, Dr. Able participated in a conference in New York: "Business and Economic Cooperation in the Middle East after the year 2,000." He described the technological and social benefits developing from Phase IV. It was apparent from the participants' reactions that the MERC program, and in particular, the marine program, are most welcome in the Middle East

but not in many sectors of American society. AID, MERC, and its component projects need more and better PR.

Future Plans

1. A one-year no-cost extension of Phase IV has been granted by AID. While the Israelis have exhausted their funds, more work remains, which they will undertake. The Egyptians must accelerate their operations considerably, especially in the Trophodynamics Project, to attain their objectives within this year.

2. Under Abel's coordination, with AID's blessing, the MERC project directors will convene in Washington, DC on December 12, to acquaint each other with their respective projects and to discuss common opportunities with particular reference to the rapidly shifting dynamics in the Middle East.

(4.) Trophodynamics of the Southeastern Mediterranean
With Special Reference to
Commercially Important Fishes

**(4.a) Trophodynamics of the Southeastern Mediterranean
With Special Reference to
Commercially Important Fishes
(ISRAEL)**

Third Annual Report

**Principal Investigators: Yigal Kalikhman and Paul Walline
Associate Investigators: Yosef Yacobi, Nurit Kress and Moshe Tom**

**Israel Oceanographic and Limnological Research
ISRAEL**

Introduction

During the reported period, our efforts were aimed at carrying out multidisciplinary surveys of the Israeli continental shelf, treating data from the surveys conducted during the previous period, and at optimization of design of further surveys.

Multidisciplinary surveys

An attempt was made to carry out a survey in January 1995, however the BioSonics ES-1000 Dual-Beam Echo Sounder failed at the beginning of the study, and we had to cancel the cruise. Due to multiple failures, the echo sounder was sent to the manufacturer for repair or replacement. It was replaced with a reconditioned Model 102 Dual-Beam Echo Sounder. After the sounder arrived, two surveys were conducted: on 14-18 May and 17-20 September 1995.

On both cruises acoustic surveys of pelagic fish stock and concurrent sampling stations to measure environmental parameters were conducted from the Research Vessel "Shikmona" by the Israeli scientific team with the participation of Gert van Dijken (Texas A&M University, USA). The acoustic surveys consisted of 19 transects and 29 concurrent sampling stations. On these stations samples to determine the concentrations of zooplankton, chlorophyll and nutrients were taken, and water salinity and temperature were measured.

The distance between acoustical transects, chosen on the basis of the analysis previously conducted (Kalikhman *et al.*, 1986; Kalikhman, 1989; Annual Technical Reports on "Trophodynamics of the Southeastern Mediterranean", 1992-93 and 1993-94), ensured a relative error in the field stock assessment within 0.1. The analysis carried out during the reported period (see the following section of the present report) indicated that the chosen distance between acoustical transects also ensured an adequate reconstruction of a fish distribution field (the correlation between the reconstructed field and that really existing in the sea, although unknown, $r > 0.85$).

Trawling surveys of the demersal fish stock were carried out on commercial trawlers by experts from the Fisheries Department of the Ministry of Agriculture of Israel simultaneously with the acoustic survey. In May the trawling survey was carried out on three fishing vessels, and in September on six vessels.

Treatment of data from previous surveys

Data from the surveys carried out on 27 February - 2 March and 14-18 August 1994 were treated. On 27 February - 2 March, the water temperature in the study region was very uniform: the surface temperature ranged from 17.0° to 17.7°, and the temperature at the bottom - 16.9° to 17.5°. The primary production (calculated from chlorophyll and Secchi disk measurements) was highest in the inshore areas between Caesarea and Hadera and in Haifa Bay (Fig. 1). Pelagic fish densities were low. Comparatively dense concentrations were observed only in the areas near Haifa, Atlit and Netanya (Fig. 2). The biomass of pelagic fish was estimated to be only 570 tons. The heaviest catches of the commercially important demersal fish "red mullet" were obtained as a result of trawlings conducted between 200 and 400 m isobaths (Fig. 2). The biomass of red mullet was evaluated as 320 tons.

On 14-18 August the surface water temperature ranged from 28.0° to 29.5°, while the water temperature at the bottom - from 18.0° to 30.0° (Fig. 3). The heaviest catches of red mullet were obtained in the areas with the high water temperature at the bottom (Fig. 3). The biomass of red mullet was evaluated as 470 tons. Pelagic fish densities were comparatively high and independent on the water temperature. Dense concentrations were observed in the north and in the area near Haifa (Fig. 4). The biomass of pelagic fish was estimated to be 6080 tons.

Optimization of survey design

In order to determine characteristics of an acoustic survey design required to obtain a realistic image of an actual fish distribution field, mathematical experiments were conducted with simulated patchy fields and acoustic surveys.

A formula for choosing the distance between acoustic survey transects (D), ensuring a specified precision of fish distribution maps, was obtained earlier on the basis of mathematical simulation of isotropic patchy distribution fields and acoustic surveys (Annual Technical Report on "Trophodynamics of Southeastern Mediterranean", 1993-94). To specify this relationship, during the reported year we carried out a series of mathematical experiments with simulated anisotropic patchy fields and acoustic surveys.

Anisotropic fields were generated as a set of anisotropic patches with the same spatial orientation (Fig. 5). For each simulated anisotropic field, the axis of the autocorrelation ellipse in

the direction of patch elongation or in the perpendicular direction was determined as an average of the autocorrelation radii along the transects taken in this direction (Fig. 5). The simulations conducted (examples are given in Fig. 5) indicate that the surveys in the direction of elongation of the patches give results considerably better than those in the perpendicular direction.

The distribution fields were rotated to a certain angle (an example of rotation for 30° is given in Fig. 6) to examine the efficiency of surveys which, for some reason, cannot be conducted in the optimal direction. The autocorrelation radius (R) for a field in an arbitrary direction was defined as half a length of the projection of the autocorrelation ellipse on the axis in this direction (Fig. 6). The results of the experiments indicate that the increase of the angle between the survey direction and that of the patch elongation results in lower correlation (r) between the reconstructed field and that originally generated (Fig. 6). This is explained by the fact that the D/R ratio in the direction of the major axis of autocorrelation ellipse is smaller than that in any other direction.

In general, surveys of isotropic and anisotropic fields give similar results (Fig. 7). The existence of a distinct relationship fitting the generalized data set (r versus D/R ratio) confirms the possibility of using the autocorrelation radius as a parameter of a field in choosing the distance between acoustic survey transects. From Fig. 7 it can be seen that with the D/R ratio increasing to 1.0-1.5, the correlation changes only slightly and remains comparatively high, while with further increase, the correlation falls considerably. For this reason, we suggest choosing the distance between transects to be less than $(1.0-1.5)R$. Thus, when designing a survey, if *a priori* information on the autocorrelation radius for a field is available, the distance between transects can be chosen, ensuring the required match between the field reconstructed on the basis of the data from the survey, and the field really existing in the sea.

The autocorrelation radius for the fish distribution fields in the Israeli fisheries zone was estimated earlier as $R=6.0$ km (Annual Technical Report on "Trophodynamics of the Southeastern Mediterranean", 1993-94). According to the results of mathematical simulation given above, the distance between acoustical transects should not exceed 6.0-9.0 km. As the extension of the Israeli fisheries zone is about 170 km, 19 acoustical transects are required. The grid of transects, chosen on the basis of the preliminary analysis and used in our surveys, contains 19 acoustical transects. Thus, the conclusion can be made that this grid of transects ensures the adequate reconstruction of the patchy fish distribution fields which characterize the shelf of the SE Mediterranean.

References

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Kalikhman I.L. (1989). Correcting distance between acoustic survey tracks. In: *"Progress in Fisheries Acoustics", an Underwater Acoustic Group Conference held in MAFF Fisheries Laboratory, Lowestoft, England, 21/22 March 1989*. Proceedings of Fisheries Acoustics, v. 11, pt. 3, 212-215.

"Trophodynamics of the Southeastern Mediterranean", Annual Technical Report, 1992-93.

"Trophodynamics of the Southeastern Mediterranean", Annual Technical Report, 1993-94.

Legends to Figures

Fig. 1. 27 February - 2 March 1994. Distribution of primary production calculated from chlorophyll and Secchi disk measurements.

Fig. 2. 27 February - 2 March 1994. Distributions and stocks of pelagic fish (left) and the demersal fish "red mullet" (right).

Fig. 3. 14-18 August 1994. Distribution of the water temperature at the bottom (left), and distribution and stock of the demersal fish "red mullet" (right).

Fig. 4. 14-18 August 1994. Distribution and stock of pelagic fish.

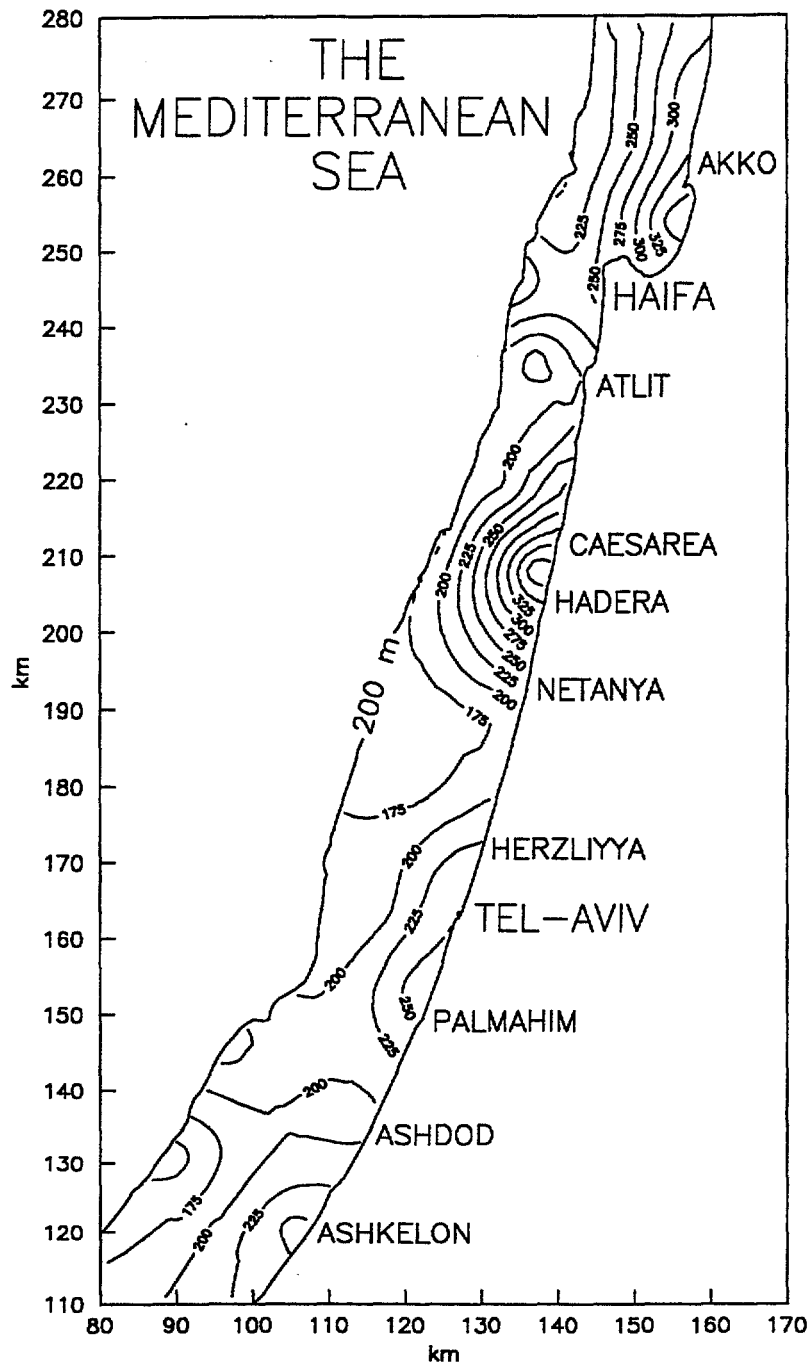
Fig. 5. First panel - simulated patchy anisotropic fields (straight lines indicate the transects used to estimate the autocorrelation functions). Second panel - the autocorrelation ellipses for the fields. Third and fourth panels - the paths of the simulated surveys (dotted lines) conducted in the directions of the major or minor axes of the autocorrelation ellipses, and the distribution fields as reconstructed.

Fig. 6. First panel - simulated patchy anisotropic fields (the straight lines indicate the transects used to estimate the autocorrelation functions). Second panel - the autocorrelation ellipses for the fields (the autocorrelation radii in two arbitrarily chosen perpendicular directions are indicated). Third and fourth panels - the paths of the simulated surveys (dotted lines) conducted

in the directions chosen, and the distribution fields as reconstructed.

Fig. 7. Correlation between the reconstructed field and that originally generated with different D/R ratios. Symbols correspond to the results of the simulated surveys of the fields given in Figs. 6 and 7 of Annual Technical Report on "Trophodynamics of Southeastern Mediterranean", 1993-94, and in Figs. 5 and 6 of the present report: squares - Fig. 6 of the Report on the previous year study; circles - Fig. 7 of the Report on the previous year study; triangles - Fig. 5, left; asterisks - Fig. 5, right; circles with four radial beams - Fig. 6, left; circles with eight radial beams - Fig. 6, right (filled symbols correspond to surveys in the direction of abscissa axis, empty ones - to surveys in the direction of ordinata axis). The solid line represents the non-linear regression ensuring the minimal sum of squared residuals. The dotted lines are the borders of the confidence interval with the probability $p = 0.99$.

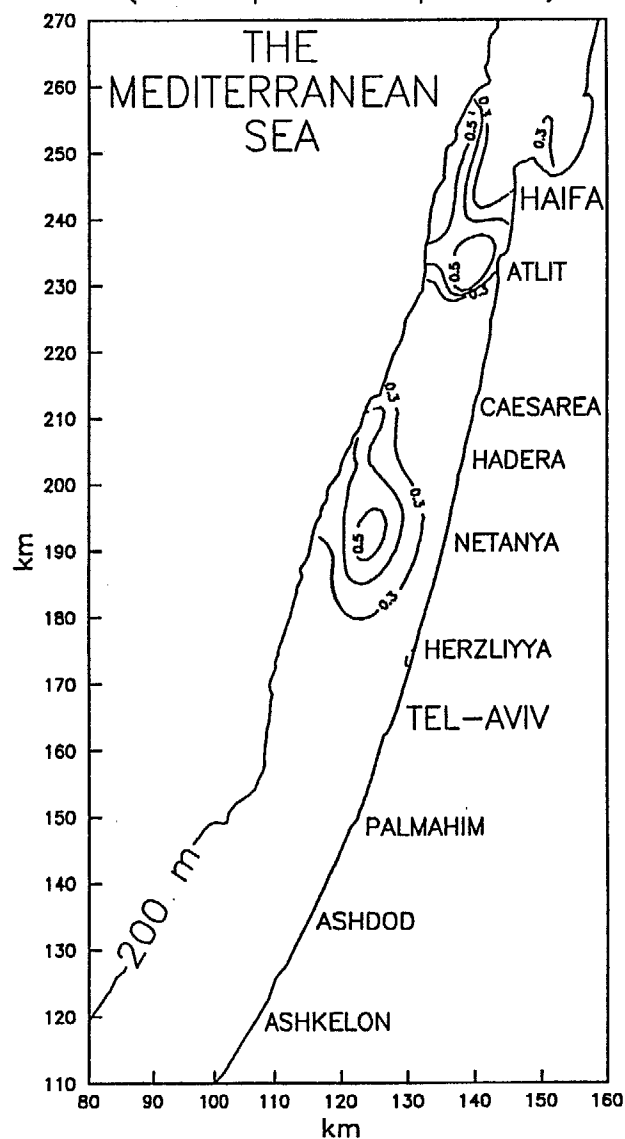
PRIMARY PRODUCTION



27.2-2.3.1994
 Area 3100 sq km
 Total 677000
 Average 218

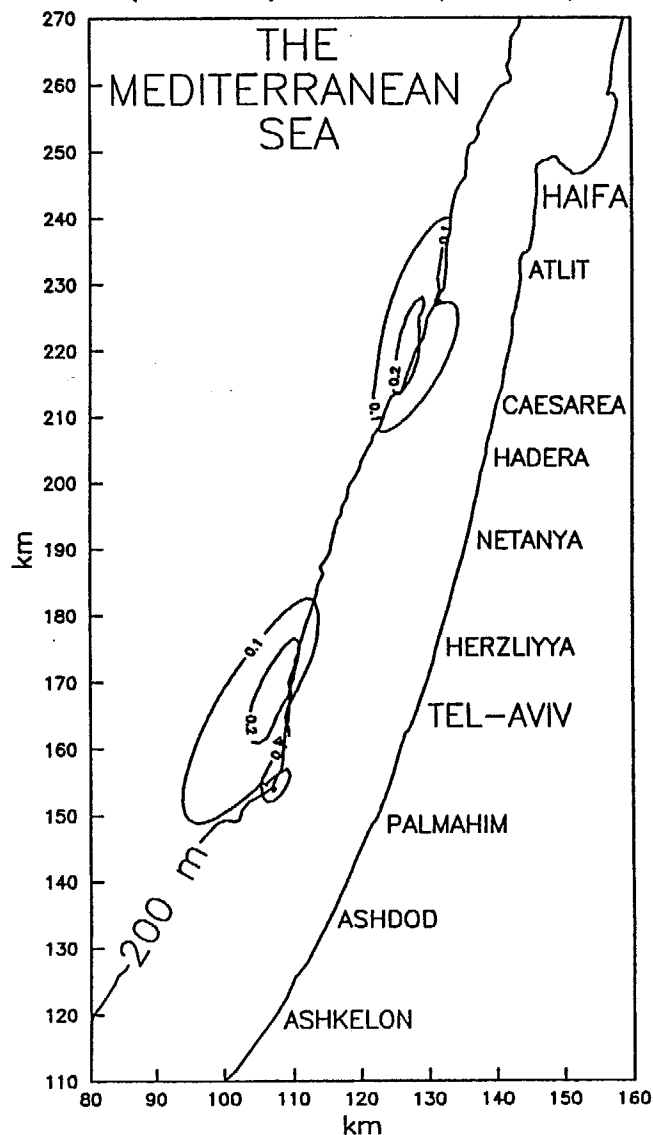
Fig. 1

PELAGIC FISH
(ton per sq km)



B=570 tons

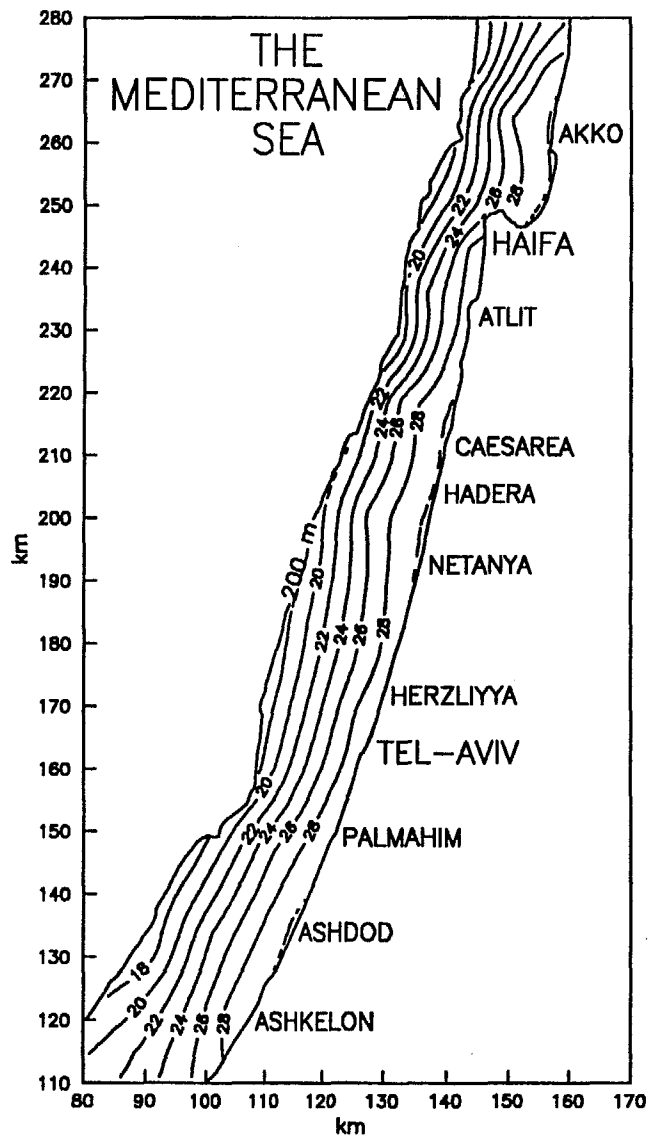
RED MULLET
(ton per sq km)



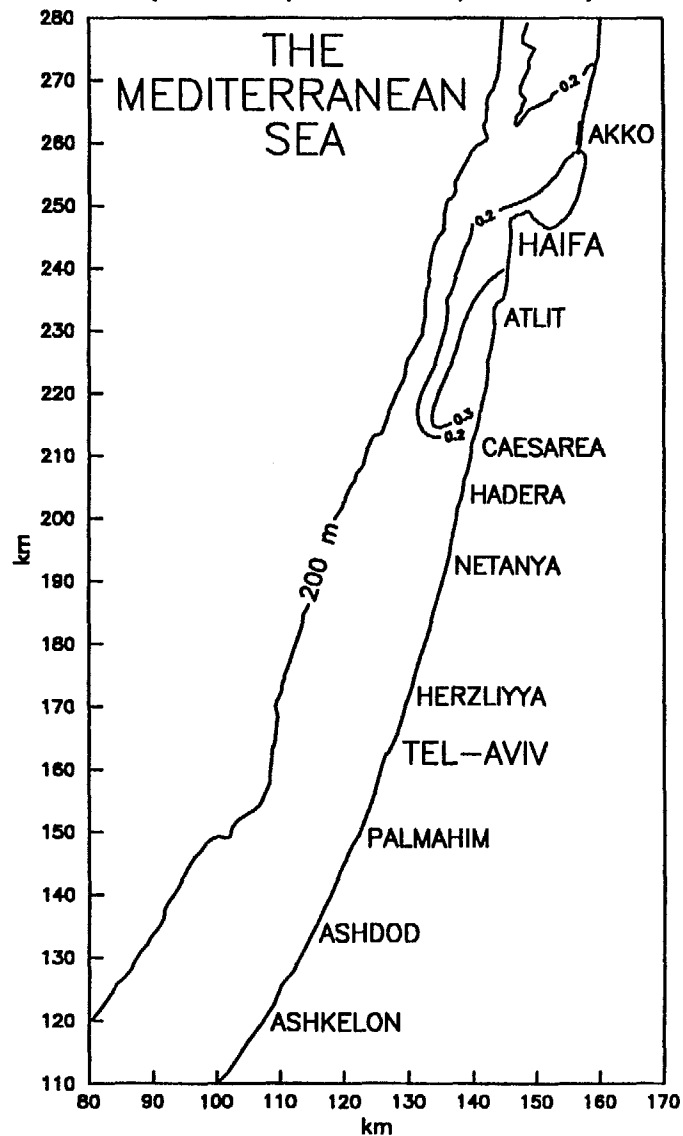
B=320 tons

Fig. 2

TEMPERATURE AT THE BOTTOM



RED MULLET (ton per sq km)



B=470 tons

Fig. 3

PELAGIC FISH (ton per sq km)

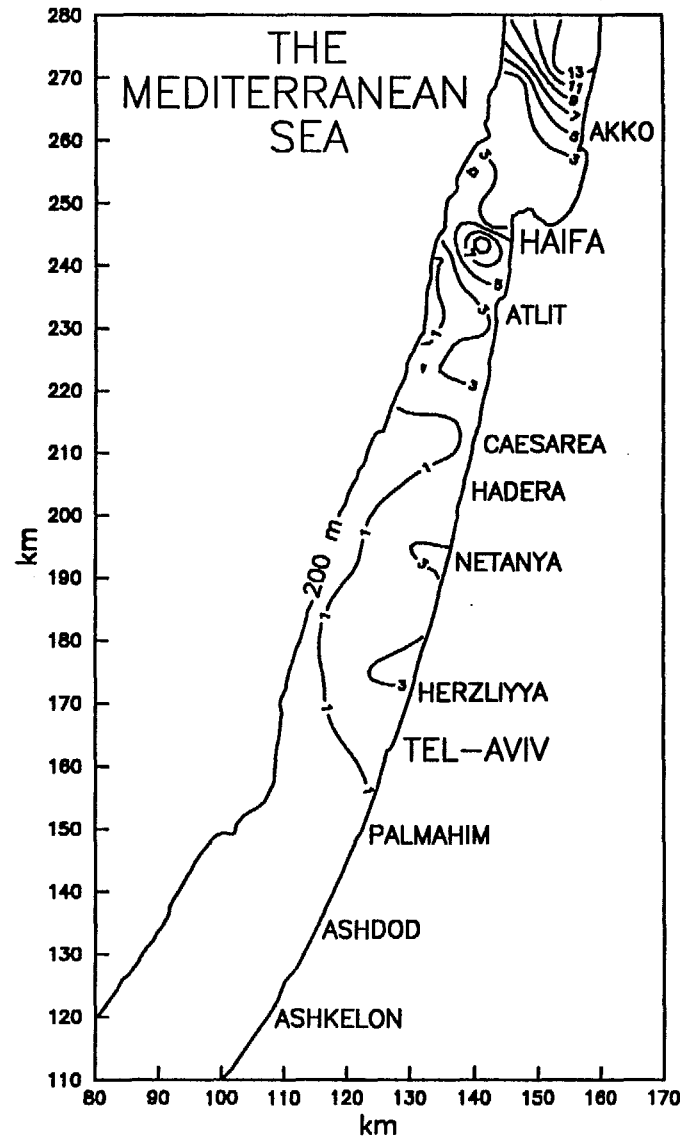
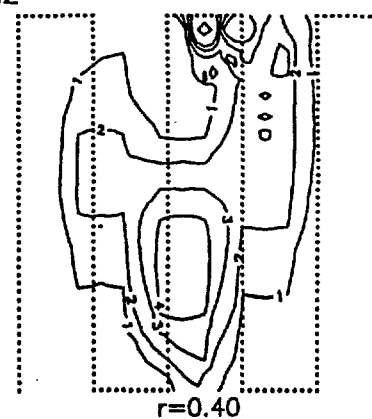
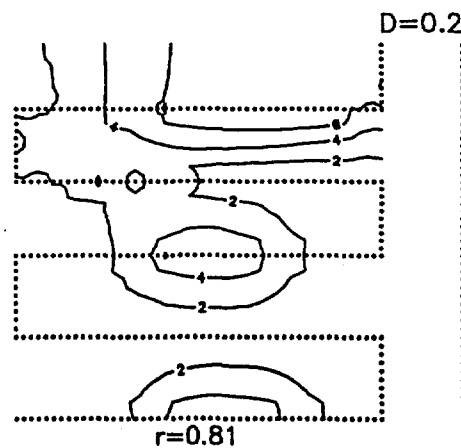
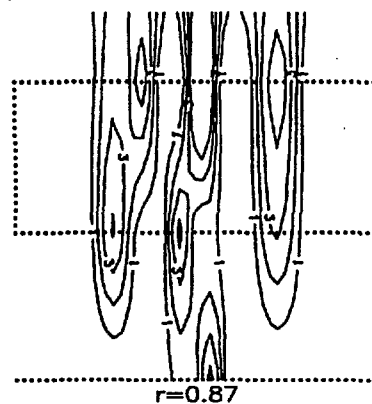
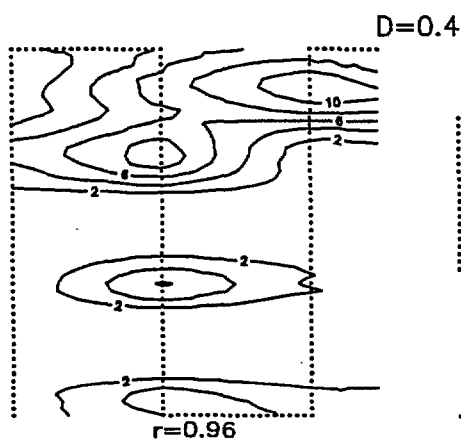
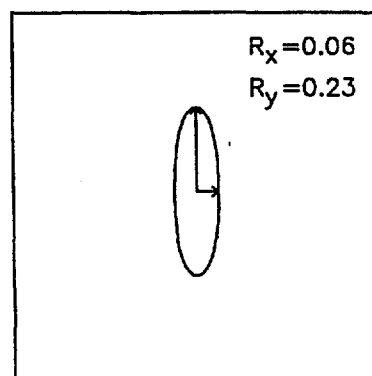
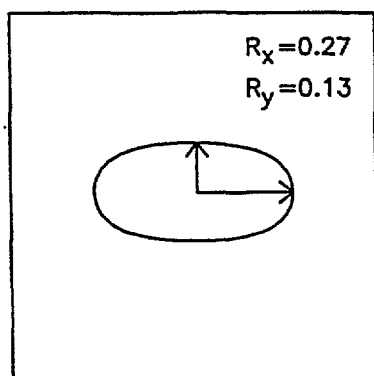
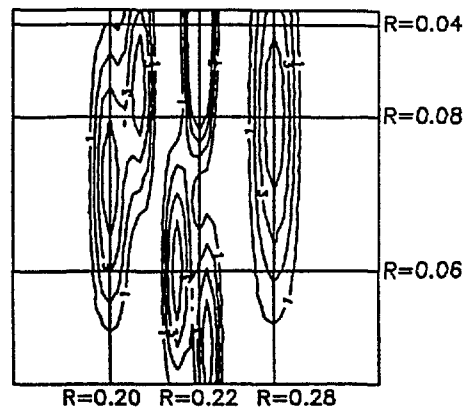
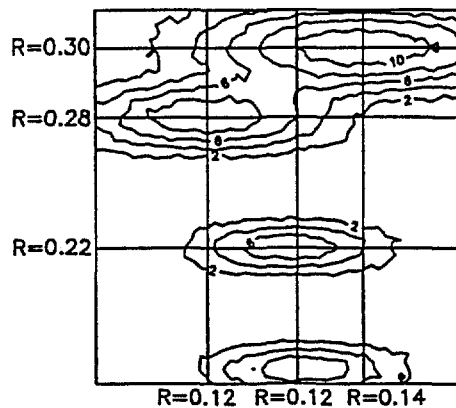
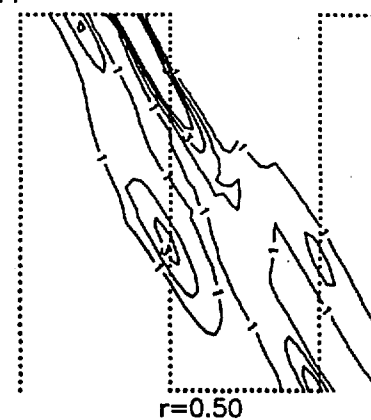
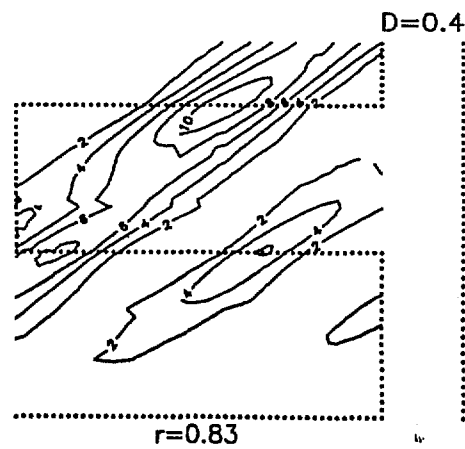
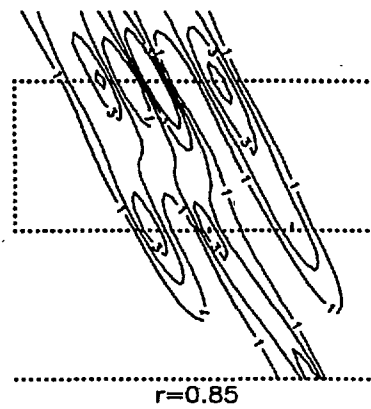
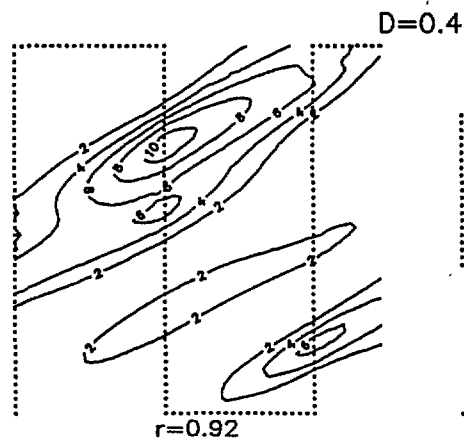
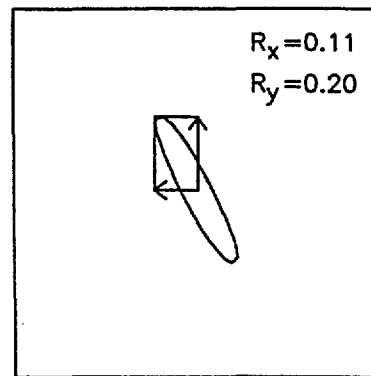
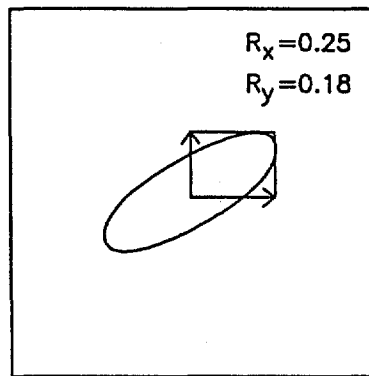
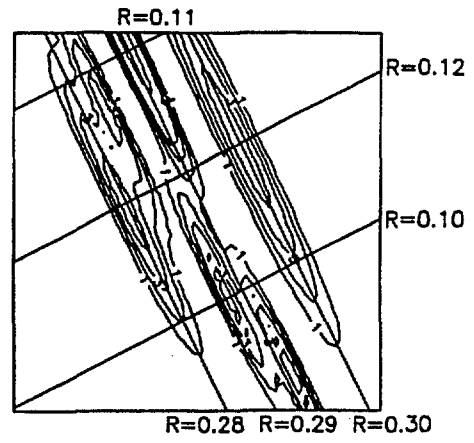
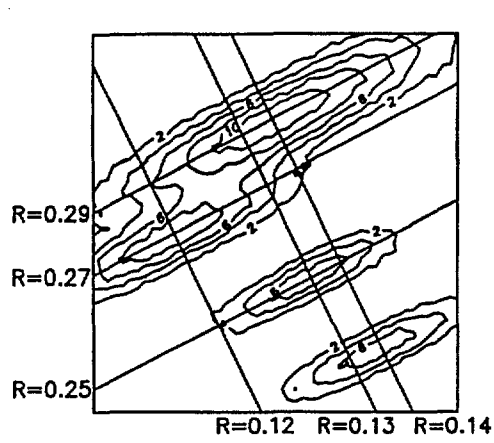


Fig. 4





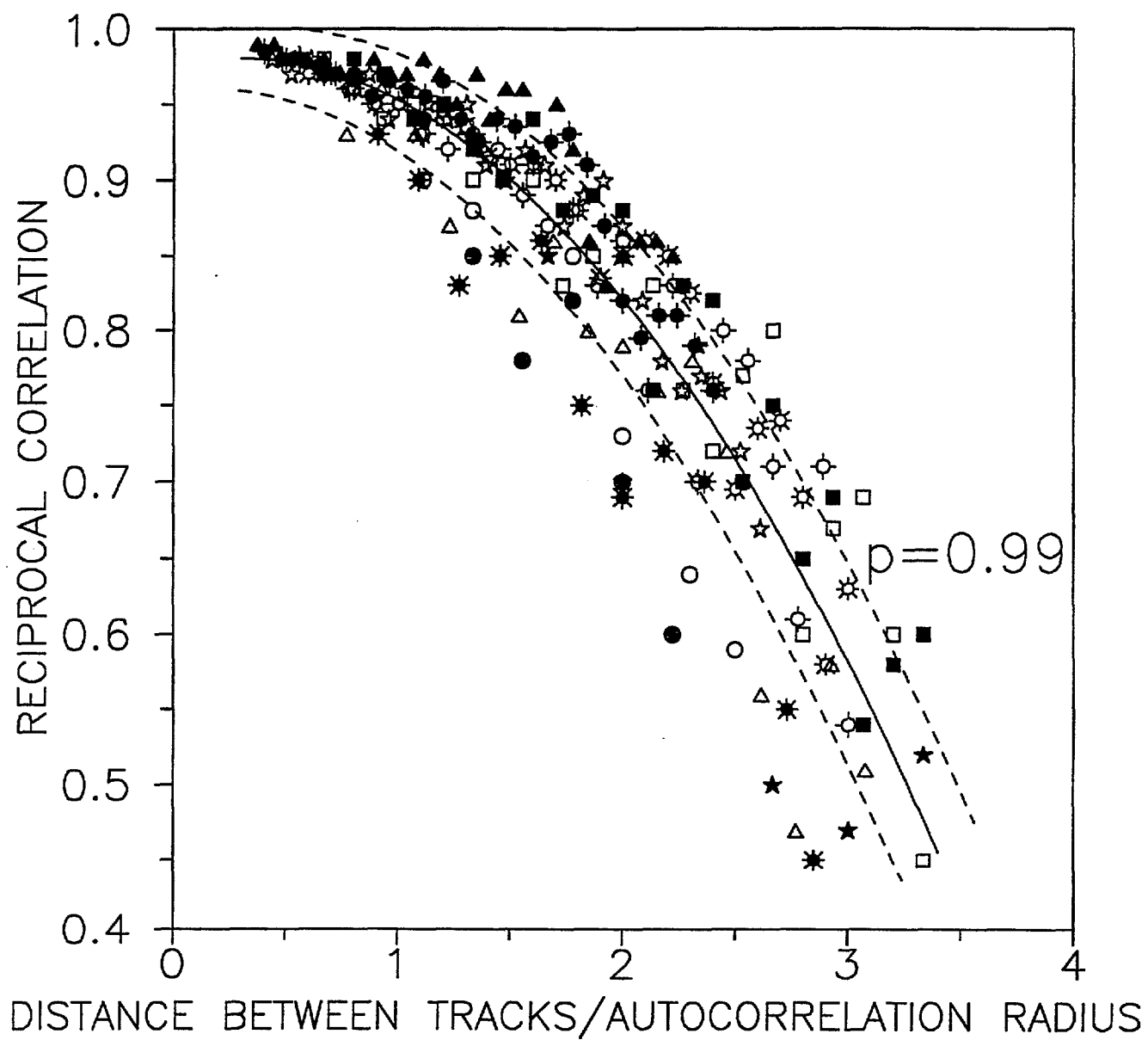


Fig. 7

**(4.b) Trophodynamics of the Southeastern Mediterranean
With Special Reference to
Commercially Important Fishes
(U.S.A)**

Third Annual Report

**Principal Investigator: Dr. Sayed Z. El-Sayed
Associate Investigator: Gert van Dijken**

**Department of Oceanography
Texas A&M University
College Station, Texas**

Introduction:

The focus of our research effort continues to concentrate on estimating primary production in the study area, and in particular to assess the contributions of the different sizes of the phytoplankton to the standing crop and primary production. It is well known that study of the size contribution of the phytoplankton is of paramount importance in the study of the marine food web. To this end, fractionated primary production experiments were carried out, as well as size fractionated pigment analyses, flow cytometry, bacterial counts, and fluorescence profiles were made. During this period 3 multi-disciplinary cruises were undertaken, in January, May and September, 1995. The results of the primary production experiments are summarized below.

Cruise IV (January)

This cruise was canceled shortly after we left the harbor due to equipment failure of the echosounder. Therefore, no primary productivity experiments were performed. However, two nutrient-enrichment experiments were carried out at the Israeli Kinneret Limnological Laboratory in order to study the effects of phosphate, iron and oxine (a chelating agent, which makes trace metals unavailable to phytoplankton) on carbon incorporation. The results were described in more detail in the semi-annual technical report (1 June, 1995). It was found that phosphate was the most important limiting nutrient of the two analyzed. The responses were similar for the different size-classes of phytoplankton.

Cruise V (14-18 May)

During this cruise, four primary productivity experiments were carried out. Methods used were similar to those previously described in the Second Annual Report (7 November, 1994). The primary production in the region was estimated by carrying out two different kinds of experiments: primary productivity 'stations' and 'transects'. At the stations, one specific spot is occupied where samples are taken from 6 different depths, and incubated for a certain amount of time at their *in situ* light level. When doing a primary productivity transect, three different stations are sampled within a short time at two different depths. The sampled stations are representative of the bathymetry range of the study region; i.e. stations with a bottom depth of 15, 50 and 200 meters, respectively. The major advantage of the transects is that a much greater

spatial coverage is rendered, which is important in order to get an accurate estimate of the total annual primary production in the study area. The multidisciplinary cruises are relatively short, while only one primary productivity experiment can be carried out per day. Therefore, if primary production would be estimated at just one station per day, only four different spots would be occupied during one cruise. By doing transects the whole continental shelf is sampled from shallow to deep in one day (three different spots). A tradeoff is that samples can be taken from only two depths instead of six to seven. However, for intercomparison of the results, care is taken that samples are taken from the same optical depths, i.e. from the surface and at 13% of the surface irradiance (I_0). These data are necessary for the modeling of primary production from chlorophyll *a* measurements, fluorescence profiles and light penetration in the water column. Furthermore, previously analyzed satellite images of ocean color will aid in the understanding of the spatial variability of algal biomass in the study region.

At the first station (May 15, #9a, bottom depth=250 m) total integrated production over the day was 0.19 g C m^{-2} . The phytoplankton smaller than 20mm was responsible for 92% of the total production, and those smaller than 2 mm for around 45% of the total. Maximum production rates were measured close to the surface at 6 m and exceeded $0.7 \text{ mg C m}^{-3} \text{ h}^{-1}$.

Total integrated production on the second station (May 18, #11-2, bottom depth=200 m) was $0.19 \text{ g C m}^{-2} \text{ day}^{-1}$. At this station the netplankton (>20mm) had a relatively large contribution to total production (27%). Maximum production rates were around $0.4 \text{ mg C m}^{-3} \text{ h}^{-1}$.

During the first transect (May 16) stations 21, 22 and 23 were occupied. As expected photoinhibition occurred at the surface; at every station the production at depth was higher than at the surface. The production of the shallow station at the surface was three times higher than at the deeper stations. At 13% of I_0 this was a factor 10. Production rates at the two deeper stations were very similar.

The second transect experiment (May 17) was carried out with samples from stations 28-2, 29A and 29-2. Again, production rates at 13% light level were higher than at the surface, although in general production rates were lower than at the other transect. Production rates at the shallow station were 3 times higher at 13% of I_0 than at the other two stations. At the surface, it was around two times.

Cruise VI (17-20 September)

During this cruise 2 primary productivity stations (bottom depths 200 m.) were occupied and 1 transect was carried out. Production rates were lower than during the May cruise. At station 8 (Sep 18) total production amounted to $0.09 \text{ g C. m}^{-2} \cdot \text{day}^{-1}$, and at station 20A (Sep 19) $0.11 \text{ g C. m}^{-2} \cdot \text{day}^{-1}$. The contribution of the phytoplankton smaller than 20 mm to the total production was 95%, more or less equally divided between the nano- and pico-plankters.

During the transect (Sep 20), stations 29 (200 m.), 30 (50 m.) and 31 (15 m.) were occupied. In contrast to the May cruise, the production rates at the shallow and intermediate stations at the 13% light-level were not significantly higher than at the surface, which is probably due to the lower light intensity during this time of the year. Production rates at the shallow station were 10 times higher as compared to the intermediate station. The production rates at the deep station compared very well to that of rates measured at station 8 and 20A.

Table 1: Primary production estimates at stations occupied during the May and September cruises.

Stations	May 15	May 18	Sep 18	Sep 19
Total production (g C. $\text{m}^{-2} \cdot \text{day}^{-1}$)	0.194	0.188	0.092	0.112
Σ net	8%	27%	5%	6%
Σ nano	47%	28%	61%	43%
Σ pico	45%	46%	35%	51%
P_{max} (mg C. $\text{m}^{-3} \cdot \text{h}^{-1}$)	0.76	0.41	0.35	0.22

Table 2: Production rates measured during the transects (mg C. $\text{m}^{-3} \cdot \text{hour}^{-1}$).

Transect	16 May	17 May	20 Sep
<i>shallow station</i>			
Σ surface	1.56	0.76	3.23
Σ 13% of I_0	6.90	1.44	3.37
<i>intermediate station</i>			
Σ surface	0.48	0.33	0.31
Σ 13% of I_0	0.66	0.45	0.21
<i>deep station</i>			
Σ surface	0.53	0.40	0.16
Σ 13% of I_0	0.68	0.49	0.26

**(4.c) Trophodynamics of the Southeastern Mediterranean
With Special Reference to
Commercially Important Fishes*
(Egypt)**

Third Annual Report

Principal Investigator: Dr. Ali I. Beltagi

**National Institute of Oceanography and Fisheries
Alexandria, Egypt**

[* Editors note: The Technical Report was not received at the time when the Third Annual Report was being compiled]

**(5.) Investigations of Lake Ecosystems in Israel, with
Implications for Fisheries and Water Quality
Management**

**(5.a) Investigations of Lake Ecosystems in Israel, with
Implications for Fisheries and Water Quality
Management
(Lake Kinneret)**

Third Annual Report

**Principal Investigators: M. Gophen, P. Walline, D. Hambright
Collaborating Investigators: I. Kalikhman, I. Ostrovsky, T. Zohay, B. Azoulay,
and J. Easton**

**Israel Oceanographic and Limnological Research
Kinneret Limnological Laboratory**

Hydroacoustic surveys of fish populations

Paul Walline, Iygal Kalichman and Ilya Ostrovsky.

During the last year we continued to have severe problems with the BioSonics ES1000 echosounder. It was returned to the company for repair or replacement after it failed (and burned up for the 3rd time) in January 1995. It was replaced with a reconditioned Model 102 Dual-Beam Echo Sounder, which has worked without problems since May. Although the analysis system (software and ESP card) remained the same, it was necessary to check calibration and again compare results with the Hadas system used in the past in order to continue the 5-yr time series of hydroacoustic surveys started in 1988.

In May an intercalibration survey was made with the participation of 3 visiting scientists from the Egyptian National Institute of Fisheries and Oceanography. Results (Fig. 1) showed that when fish are favorably distributed, the two systems (Hadas and BioSonics) give comparable results in terms of number of fish per unit area.

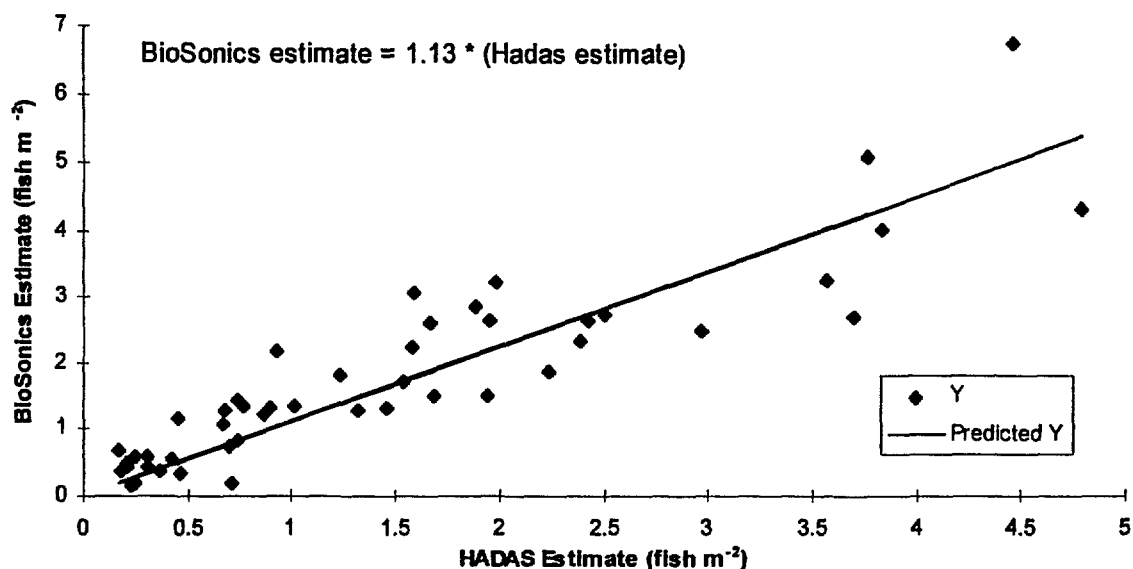


Figure 1. Comparison of estimates of fish density made with the Hadas and Biosonics systems. Fourteen transects (subdivided to make 52 short sections of 3-4 min. each) were made on the night of 24 May '95. The Hadas and Biosonics systems were operated simultaneously from opposite sides of the RV Hermona. Conditions were ideal for operating the Hadas system since fish were concentrated in mid-water. The BioSonics estimate is for fish > -60 dB, approximately the same size as the smallest fish observable with the Hadas system as operated.

It would be useful to be able to convert the data obtained with the Hadas system from numbers of fish per unit area to fish biomass per unit area. For this purpose a conversion factor is necessary which relates the length (and therefore weight) of fish to their acoustic size. In order to determine this factor for lavnun, we have measured thousands of fish from 6 stations sampled repeatedly (6-10 times) during the course of the study. Peaks in the length-frequency (Fig. 2) can be related to peaks in the acoustic size (target strength, TS) frequency.

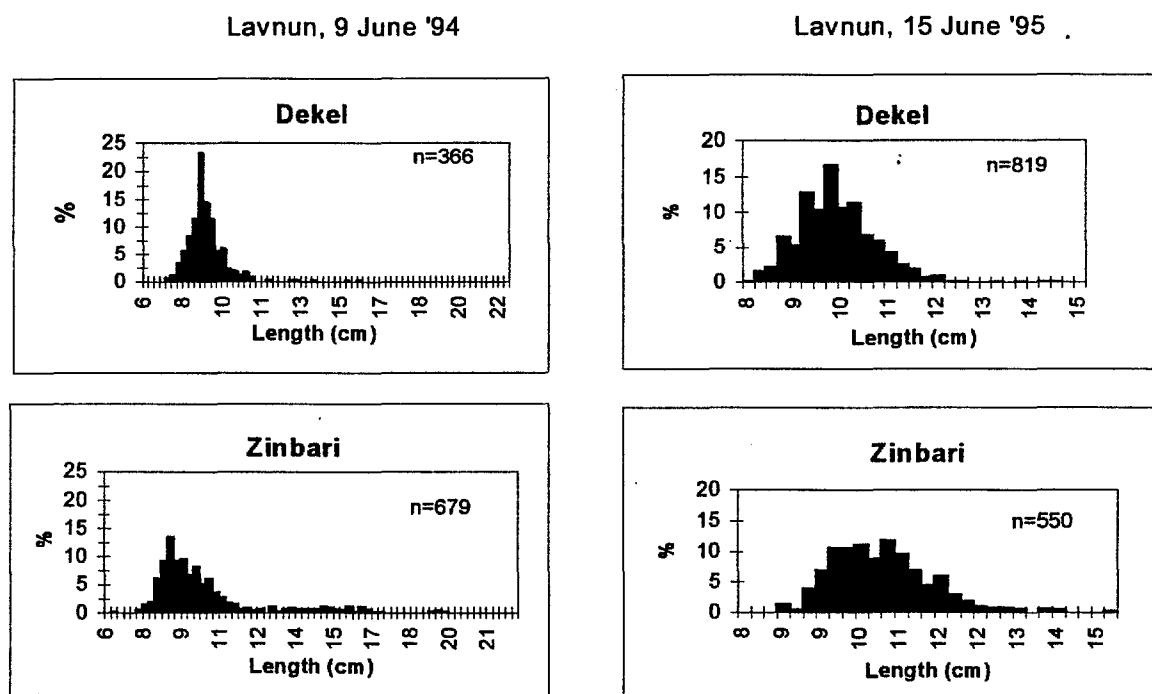


Figure 2. Length-frequency (in %) for two stations before and after the lavnun reduction program in which 450 tons of lavnun were removed in the winter of '94-'95. There appears to be a slight increase in the size of the fish over this time period.

The lavnun population continues to be at a level greater than considered desirable from water quality considerations (Fig. 3), and as can be seen in Figure 2, they remain small. Following recommendations from KLL based on results obtained in this study, a lavnun reduction program was instituted in the winter of 1994-95. It was hoped that removal of some of the small fish would improve conditions for the remainder and allow them to reach a size which would permit the regular fishery to resume. A total of around 500 tons were removed and taken to a disposal site. Budget considerations did not allow removal of a greater quantity, so the total removal was only about half of that removed by the fishery in a "normal" year. In part because so few fish

were removed, there seems to have almost no effect on the fish population. Numbers did decline between May 1994 and May 1995 (Fig. 3), but only slightly. There also seems to have been a slight increase in average size (Fig. 2). However, it is clear that even if these differences could be attributed to the reduction program, to attain the aims of the program, it would be necessary to remove much greater quantities of fish. We have suggested the removal of at least as many fish as were removed by the fishery in the years prior to 1982. Unfortunately, budget restraints make it likely that the removal program will not be continued in winter 1995-96. The fishing cooperatives are suggesting that if no subsidies are forthcoming for the current fishing season, they may be forced to leave the fishery permanently. This would mean that fishing pressure would be reduced almost to zero, with probable negative effects on the water quality of L. Kinneret. We hope that arguments based on the research we have completed during the current project will have an impact on the responsible authorities, and that some way of continuing to remove lavnun will be found.

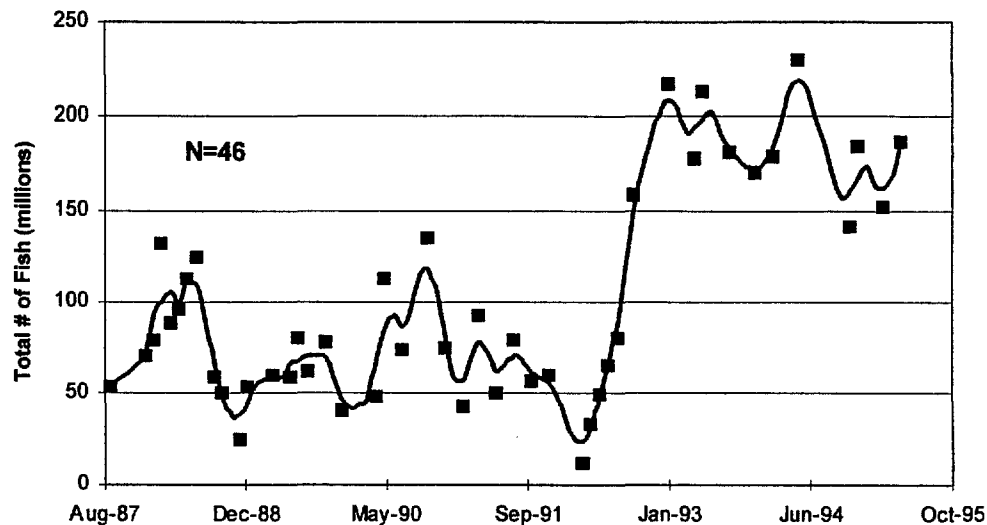


Figure 3. Abundance of fish (mostly lavnun, *Acanthobrama terraesanctae*) in Lake Kinneret, Israel by sampling date. The line is a running average.

EFFECTS OF CYANOBACTERIA ON ZOOPLANKTON GRAZING AND NUTRIENT EXCRETION

D. Hambright, T. Zohary, B. Azoulay, J. Easton, and M. Gophen

Purpose. To determine the effects of cyanobacteria (*Microcystis aeruginosa* and *Aphanizomenon ovalisporum*) on grazing and nutrient excretion by Lake Kinneret zooplankton.

As reported previously, (e.g., Hambright et al. 1993, 1994, KLL and AID Annual Reports), we have been regularly examining zooplankton grazing on various planktonic groups experimentally since 1991 as part of the AID Food Web Studies. Hence, during the recent occurrences of *Aphanizomenon* (October 1994 and July 1995) and *Microcystis* (winter-spring 1995), we examined the potential effects of these cyanobacteria on grazing and nutrient excretion by natural Kinneret zooplankton.

In winter-spring 1995, *Microcystis aeruginosa* bloomed, constituting up to 50% of the phytoplankton biomass. Zooplankton species composition and abundance was similar to previous winter-spring periods, with cladocerans (*Ceriodaphnia*, *Bosmina*, and *Diaphanosoma*) and cyclopoid copepods dominating the assemblage; the calanoid copepod *Eudiaptomus dreishii* were also conspicuous members of the zooplankton. Plankton assemblages in the lake differed somewhat between the two *Aphanizomenon* periods. In 1994, *Aphanizomenon* bloomed during late summer-early fall, dominating the phytoplankton (up to 3000 filaments per mL), though some *Peridiniopsis* was present. Zooplankton biomass was dominated by cladocerans (61%) and cyclopoid copepods (38%). A new rotifer (*Platytias patulus*) was also very abundant, numerically constituting 17% of the zooplankton, but due to its low per capita biomass, ca. 2% of the total biomass. In 1995, *Aphanizomenon* appeared earlier in summer but remained at lower abundances (500–1000 filaments per mL). Unlike during the 1994 bloom period of *Aphanizomenon*, nanoplanktonic phytoplankton species (especially *Oocystis* sp.) were very abundant during the 1995 *Aphanizomenon* period. Zooplankton during the 1995 *Aphanizomenon* period were similar to the previous summer of 1994 except that *Platytias* were rare.

Four sets of experiments were conducted during the cyanobacteria periods. The experimental protocol has been detailed previously (e.g., see 1993 and 1994 KLL and AID Annual Reports). The basic approach of the experiment was to measure changes in phytoplankton abundances in experimental mesocosms containing different levels of zooplankton biomass during a 24-hr period. Generally we measured changes in phytoplankton by measuring changes in size-fractionated chlorophyll (< 20 and ≥ 20 µm) which was later converted to phytoplankton biomass.

Generally, grazing by Lake Kinneret zooplankton compares favorably to published grazing studies of other zooplankton, although the rates measured were low relative to published rates determined for individual taxa. Because grazing rates typically increase with increasing

zooplankton size, we suspect the lower rates measured in our study are a result of using a composite zooplankton assemblage, including a predominance of small-sized species and life-history stages. Clearance rates (measures of the mass-specific volume of water filtered per unit time) and ingestion rates followed expected patterns (see dashed lines Fig. 1). Clearance rates declined with increasing phytoplankton biomass. Ingestion rates increased initially with increasing phytoplankton biomass, but leveled off after 50 μg phytoplankton / mg zooplankton / d. The presence of both *Microcystis* and *Aphanizomenon* reduced grazing efficiency, but not consistently. The effect of *Microcystis* was seen on small zooplankton ($<300\mu\text{m}$). *Aphanizomenon* reduced grazing rates only at high densities; during the heavy bloom period of 1994 and when *Aphanizomenon* abundance was artificially supplemented from cultures in 1995. Extrapolation of grazing rates measured experimentally to the standing stock estimates of zooplankton and phytoplankton in the lake, show that 0.8-41% of the nanophytoplankton biomass could be consumed on a daily basis (Table 1). The highest proportion of predicted biomass consumed was during the *Peridinium* period when total nanophytoplankton biomass represented $<5\%$ of the total biomass. During the summer and fall periods, in which nanophytoplankton dominate the phytoplankton ($>50\%$), 0.8-8% of the nanophytoplankton biomass would be predicted to be consumed on a daily basis.

Patterns in nutrient excretion rates were related to grazing rates (Fig. 2), but only below ca. 50 μg phytoplankton / mg zooplankton / d. At higher grazing rates, nutrient excretion rates did not increase above ca. 11 and 2 μg nutrient / mg zooplankton / d, for nitrogen and phosphorus, respectively. The presence of cyanobacteria seemed to have no effect on nutrient excretion rates independently of the effects on grazing. Extrapolation of nutrient excretion rate estimates to the lake indicate that zooplankton may excrete up to 7,000 tons of nitrogen and 1,800 tons of phosphorus annually (Table 2), constituting a 2- and 20-fold turnover of the standing stock inventories of these nutrients. Although this extrapolation is tenuous, it does suggest, as do many published studies, that nutrient excretion by zooplankton may play a decisive role in nutrient availability to phytoplankton in Lake Kinneret.

We are presently continuing the analyses of these experiments, including further direct algal counts of selected species and experiments to clarify which phytoplankton were and were not grazed. Regardless, however, these results to date suggest that the presence of cyanobacteria, especially at high abundances, could be detrimental to zooplankton, and could thereby affect the entire Lake Kinneret food web.

Acknowledgements

We wish to thank T. Fishbein and M. Sela for phytoplankton counts, O. Hadas for supplying cultured *Aphanizomenon*, and U. Pollinger for data on nanophytoplankton biomass.

Literature Cited

Smith, S.V., S. Serruya, Y. Geifman, and T. Berman. 1989. Internal sources and sinks of water, P, N, Ca, and Cl in Lake Kinneret, Israel. *Limnology and Oceanography* 34: 1202-1213.

Grazing

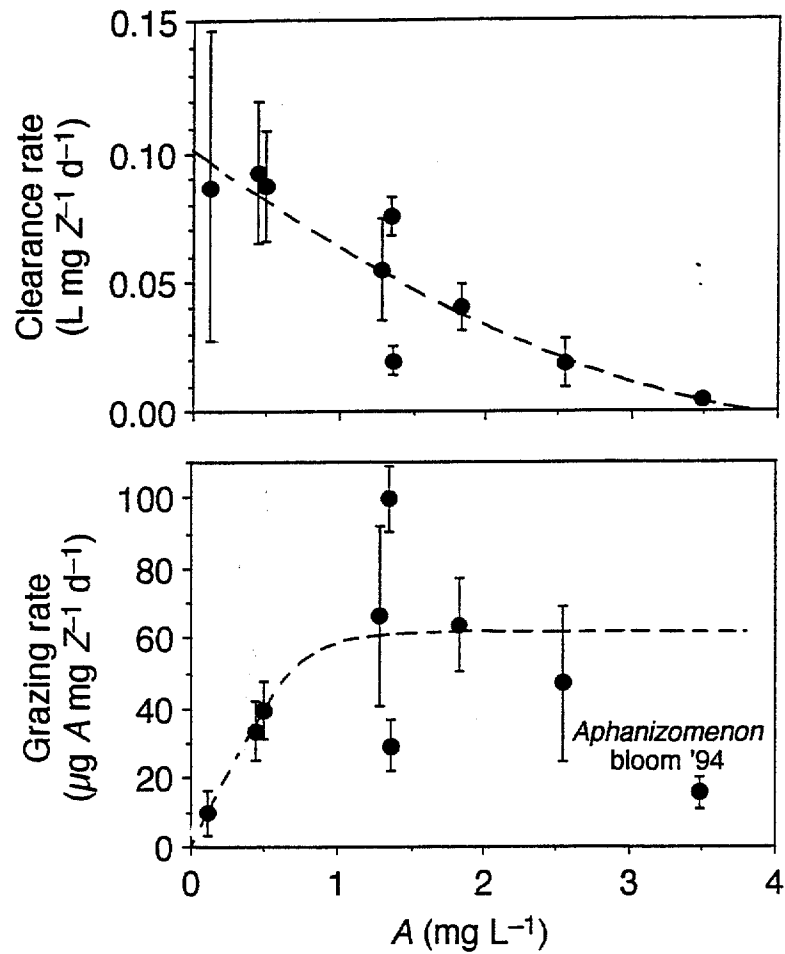


Figure 1. Clearance rates ($\text{L/mg zooplankton/day}$) and grazing rates ($\mu\text{g phytoplankton/mg zooplankton/day}$) of Lake Kinneret zooplankton plotted as functions of phytoplankton biomass ($\text{mg phytoplankton/L}$).

Nutrient Excretion

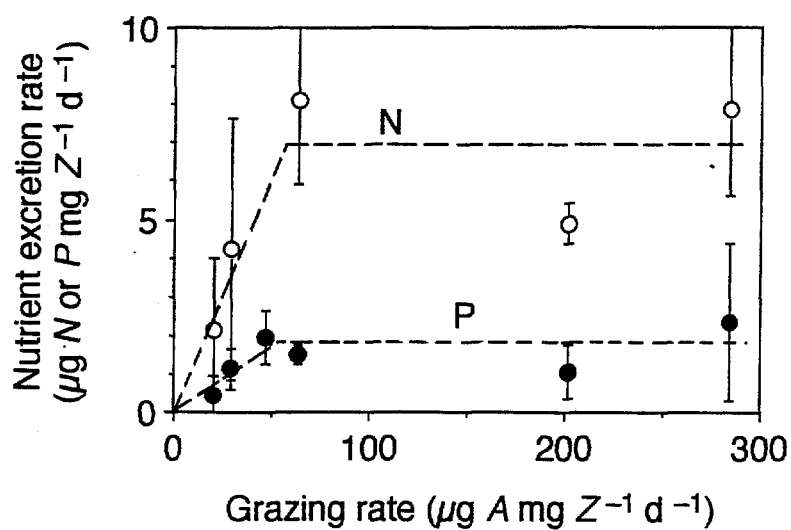


Figure 2. Excretion rates ($\mu\text{g P or N/mg zooplankton/day}$) of phosphorus and nitrogen by Lake Kinneret zooplankton plotted as functions of zooplankton grazing rates ($\mu\text{g phytoplankton/mg zooplankton/day}$).

Table 1. Experimentally determined zooplankton grazing rates on nanophytoplankton expressed as μg phytoplankton (A) consumed per mg zooplankton (Z) per day and as a percentage of the nanoplankton standing stock present in the lake at the time of each experiment.

Experiment	Zooplankton grazing rates		Comments
	$\mu\text{g A mg Z}^{-1} \text{ d}^{-1}$	% nano biomass	
<i>Peridinium</i> Season			
Z-5	39	41	calanoid- <i>Asplanchna</i>
Z-6	54	35	<i>Ceriodaphnia</i>
Z-9S	29	8	<i>Microcystis</i> , small zooplankton
Z-9L	66	19	<i>Microcystis</i> , large zooplankton
Nanoplankton Season			
Z-3	99	8	typical summer spp.
Z-7	20	0.8	<i>Aphanizomenon</i> bloom 1994
Z-10	64	5.5	<i>Aphanizomenon</i> 1995
Z-11	47	4.1	<i>Aphanizomenon</i> + culture

Table 2. Lake Kinneret inventories and annual budget of phosphorus and nitrogen (from Smith et al. 1989) and estimated daily and annual zooplankton excretion of phosphorus and nitrogen.

	Phosphorus (t)	Nitrogen (t)
Standing stock	92	3682
Inflow	120	1585
Outflow	11	407
Sedimentation	109	185
Denitrification	-	1120
Excretion		
Daily	5	19
Annual	1852	7020

Feeding Mechanism of Daphnia: High-Speed Camera Analysis

M. Gophen and K.D. Hambright

Introduction: Due to the past difficulties with the High-Speed-Video-Camera (technical problems and lost during shipment) we only have completed our preliminary work. Moreover, in addition we have analysed High-Speed Cinematography work (Gophen et al. unpublished data) using the AID funded computer software for Video and motion analyses and these results are presented here.

Purpose: To study the feeding mechanism of *Daphnia* as part of algal consumption analysis and phytoplankton biomass control by zooplankton to improve water quality.

Methods: Single *Daphnia*, was placed in filtered lake water, glued to the tip of a plastic fibre. The fibre was fixed on a holder of a fine tipped micromanipulator. A fine (~100 micron diameter of the internal space) glass tube was located close to the *Daphnia* and very small pulses (controlled by threaded injector) of India Ink were injected. The *Daphnia's* activity (body parts motions, especially the thoracic appendages) were taped by High - Speed - Camera through microscopical lenses. The tapes were analysed by slow motion and motion-time-analyser.

Results and Discussion: Number of milliseconds per stroke of appendages at different levels of Ink quantities are given below (number of observations are given in parenthesis) (SD's were <30%):

	<u>No Ink</u>	<u>Low Ink</u>	<u>High Ink</u>
Young <i>D. magna</i>	275 (41)	297 (53)	367 (8)
Adult <i>D. magna</i>			447 (6)

Increasing Ink quantity lowered number of strokes (filters activity).

Calculated number of strokes per second are given below:

Young *D. magna* (body length 1.9 mm): 2.7-3.6

Adult *D. magna* (body length 3.7 mm): 2.2

Older and larger daphnids are less active (lower number of strokes, i.e. filter movements, per second) than young specimens. Nevertheless it is possible that larger daphnids have a higher rate of filtration because of their larger filtering surface of the "combs".

Two internal microflows inside the carapace space of the *Daphnia* were indicated: 1) Lateral Subcarapace Flow (LSF), and 2) Median Filter Flow (MFF).

The LSF microflow starts from the water inlet zone above appendage 2 (P2) and continues along the internal surface of the carapace until being pushed away unfiltered to the exit zone at the distal body area. The MFF microflow starts also from the inlet zone and continues downward between the two filter rows (P3 and P4). The flow is then split between the filters and tangentially passes through the combs

towards the water outlet zone. Approximately 50% of incoming water flows through as MFF and 50% as LSF. This mechanism ensures a smooth and non-pulsated outflow of the water from the *Daphnia*'s body. It was indicated by volumetric image analysis that the filtering rate in our mesurments was 1.02 ml per hour.

We continue our analyses and further results will be included in the final report.

**(5.b) Investigations of Lake Ecosystems in Egypt, with
Implications for Fisheries and Water Quality
Management
(Lake Garun)**

Third Annual Report

Principal Investigator: E.A. Ibrahim

**National Institute of Oceanography and Fisheries
Inland Waters Aquaculture Branch
Cairo, Egypt**

Lake Ecosystem Studies Lake Qarun, Egypt

The present report summarizes the research activities carried out in Lake Qarun during the period between October, 1994 and September, 1995.

The major accomplishments of the different sub projects are summarized below:

I. Environmental Studies

a. Monitoring Water Quality

During the period covered by this report, detailed surveys of water quality of Lake Qarun and the two main drains (El-Bats and El-Wadi) were continued. The surveys were conducted on a monthly basis and consisted of nine stations in the Lake and two stations in the drains.

The monitoring program included the following parameters: depth, air-water temperature, pH, Secchi disc depth, DO, COD, alkalinity (CO_3^{2-} & HCO_3^-), organic nitrogen (DON & PON), nutrient chemistry, hardness (Ca & Mg), cations (Na & K) and TDS. The results are presented in Figs. 1,2&3.

Three hydro-stations representing the eastern, middle and western regions of the Lake were selected. At each station the following observations were made:

- Photosynthetic Active Radiation (PAR) from the surface down to the bottom.
 - Water quality parameters at different depths in the euphotic and aphotic zones. The water quality parameters studied were: temperature, depth, pH, inorganic nutrients, chl. *a* and *in situ* primary productivity.
- The results are given in Table 1.

The diurnal variations of temperature, DO and pH were recorded for 24 hours during August 1995 using YSI Environmental Monitoring System. The results are shown in Fig.4.

b. Pollutants

• Heavy Metals

During the summer of 1995, detailed study of heavy metals was performed on water and sediment samples collected from Lake Qarun and the drains. The metallic ions were measured by a new AAS (Perkin Elmer model 3110 equipped with Graphite Furnace HG 600) The results are given in Tables 2&3.

• Pesticides

Residues of 19 widely used organochlorine pesticides in the Fayoum Governorate (project site) were investigated in the sediment samples collected from Lake Qarun and drains the during summer 1995. The results are given in Table 4.

c. Biological Parameters

• Phytoplankton

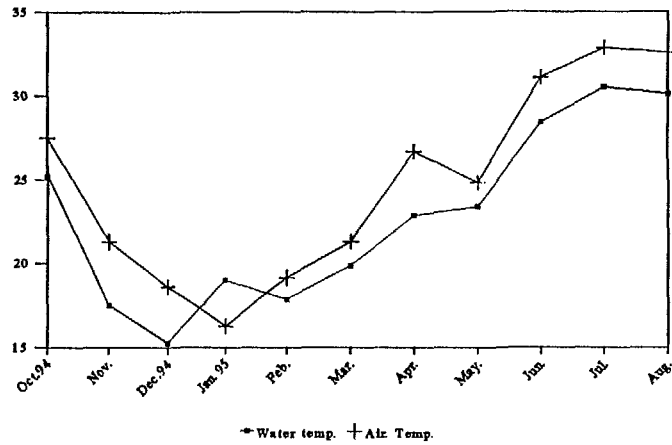
Monitoring of phytoplankton standing crop and species composition was continued at 9 stations in the lake. chlorophyll *a* and phaeophytin values of net (>20 μm) nano (< 20 μm) and picoplankton (< 2 μm) were studied. The monthly values of the pigments concentration as well as the composition of the phytoplankton are shown in Figs. 5&6.

• Zooplankton and Bottom Fauna

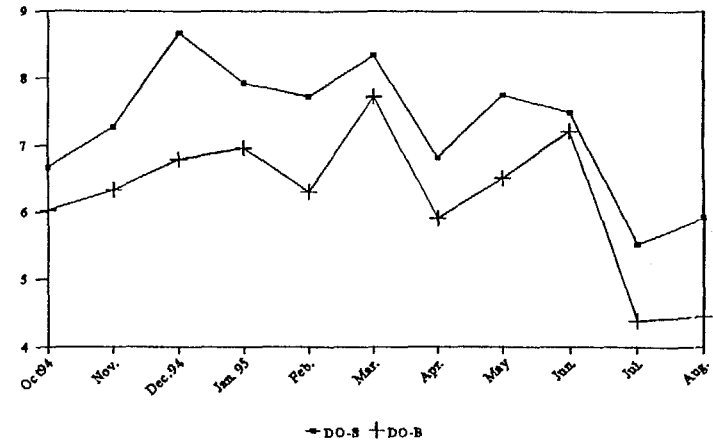
The standing crops of zooplankton (in terms of number of organisms L^{-1}) and benthic organisms (i.e. number of organisms m^{-2}) were investigated monthly in the Lake. The results are shown in Fig.7.

Fig. (1):

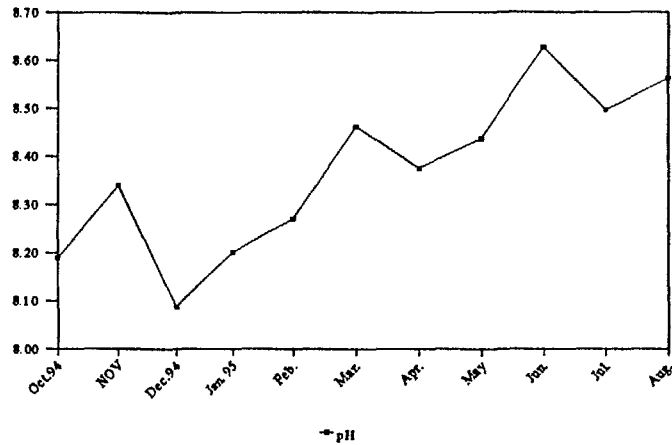
Air & Water Temperature (°C)
1995



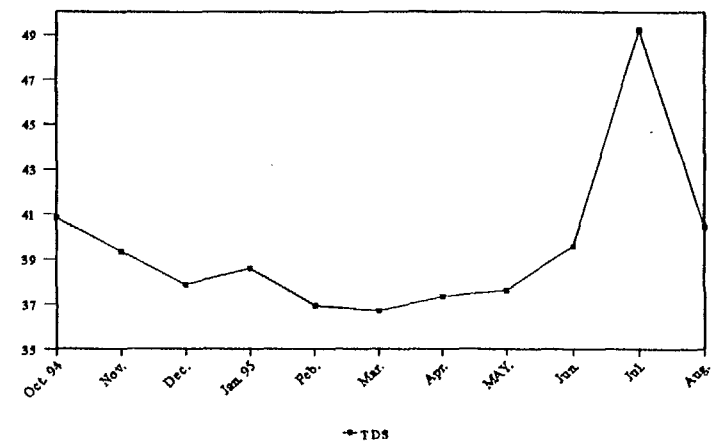
Dissolved Oxygen (mg/L)
1995



pH value
1995

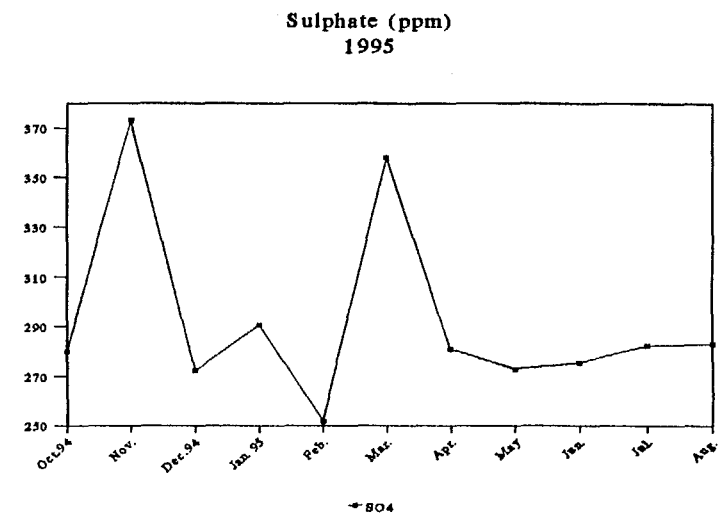
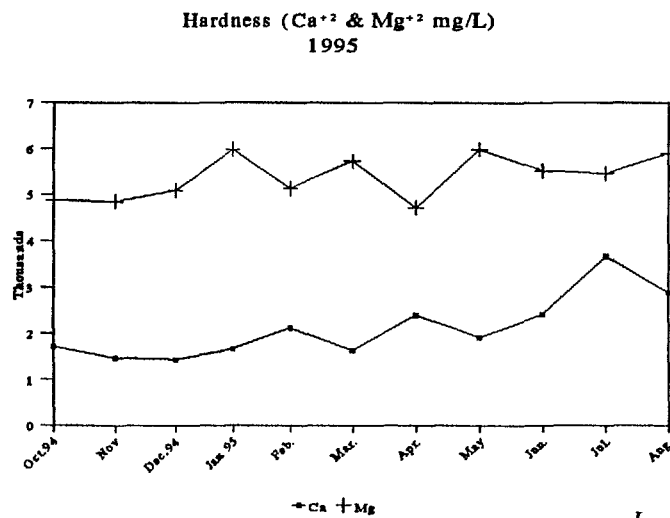
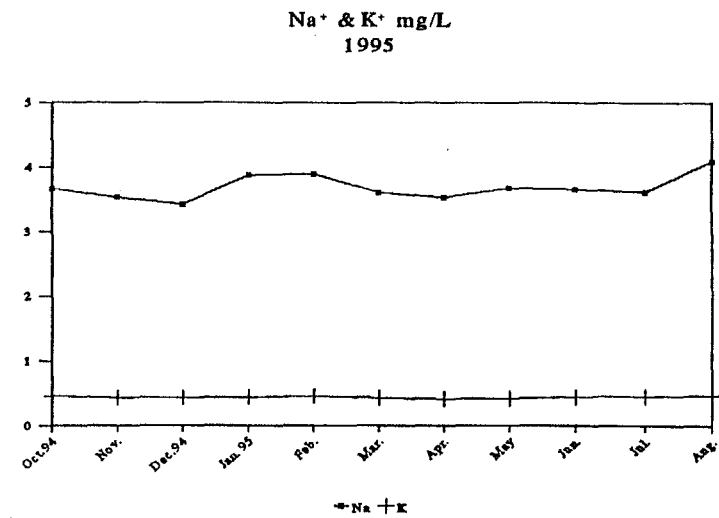
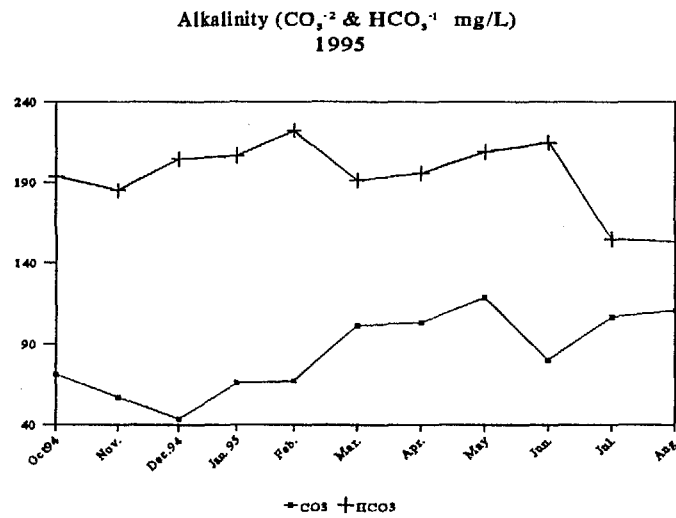


TDS (mg/L)
1995



Lake Qarun

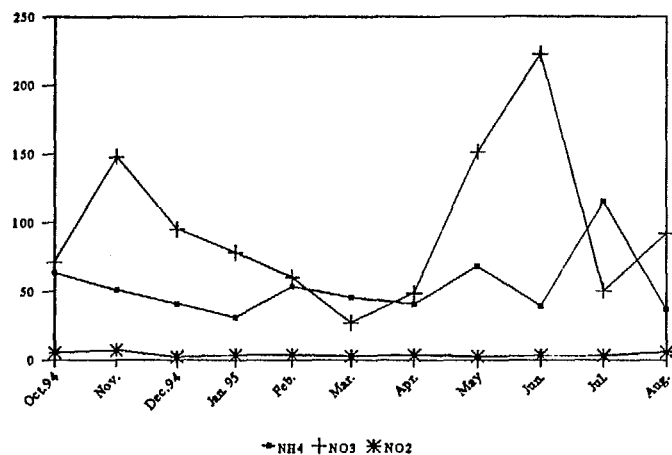
Fig. (2):



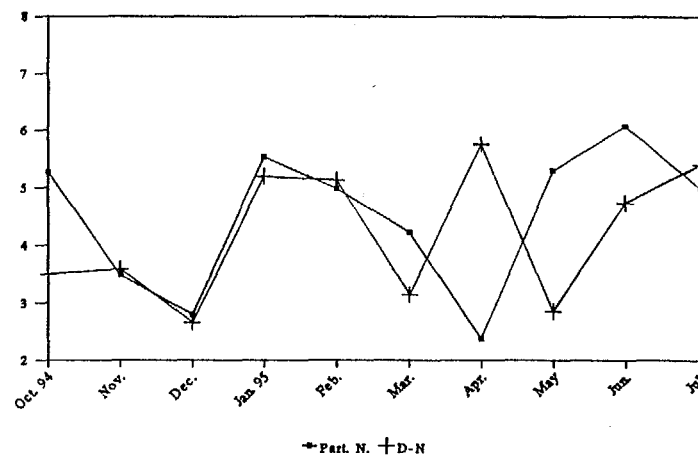
Lake Qarun

Fig. (3): Nutrients

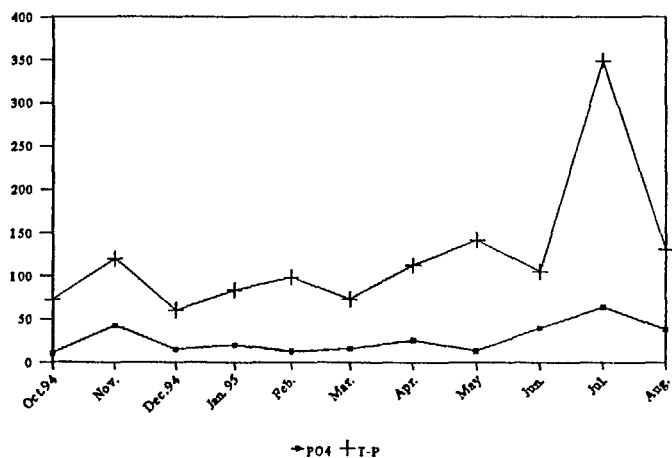
Nitrogen (NO_3 , NO_2 , & NH_4 $\mu\text{g/L}$)
1995



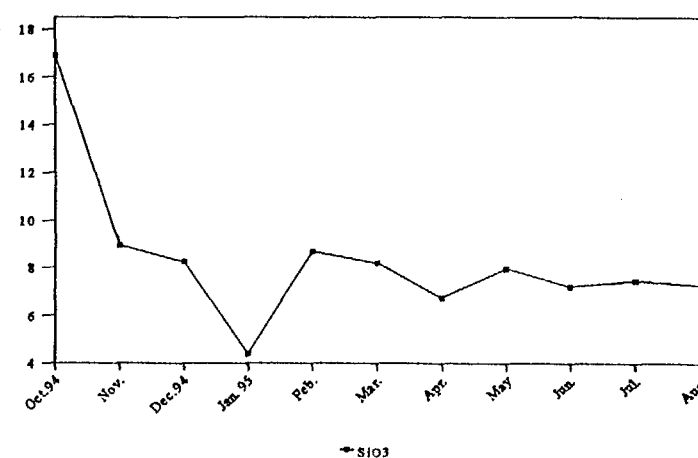
Particulate & Dissolved Nitrogen (mg/L)
1995



Phosphorus ($\mu\text{g/L}$)
1995



Silica (SiO_2 mg/L)
1995



Lake Qarun

Table (1): Annual Average of Depth, PAR, PAR%, Productivity, *Chloro. a* & Nutrients At the three Hydro-Stations

DEPTH Cm	PAR	PAR%	PROD. mg C m ⁻³ h ⁻¹	<i>CHL. a</i> µg L ⁻¹	NO3 µg L ⁻¹	NO2 µg L ⁻¹	NH4 µg L ⁻¹	PO4 µg L ⁻¹	pH	Temp. °C	Cond. µmols Cm ⁻¹	ORP
East												
0.00	223.53	100	172.34	29.23	42.00	2.69	52.04	32.61	8.45	21.97	42.78	-74.00
35.71	200.84	90	205.51	33.13	106.41	2.94	56.35	31.26	8.50	21.70	43.12	-76.20
60.71	112.66	50	139.54	28.13	61.08	3.99	54.90	25.84	8.52	21.50	42.62	-76.60
123.57	22.36	10	283.62	34.27	27.39	3.86	39.42	31.31	8.51	21.56	42.96	-76.60
230.00	2.24	1	154.19	20.81	73.56	3.25	55.00	31.32	8.51	21.44	43.24	-75.60
Middle												
0.00	171.93	100	114.01	15.64	38.58	3.55	52.90	27.78	8.39	23.16	46.42	-72.00
54.38	154.53	90	165.82	15.64	154.56	4.28	83.28	21.70	8.43	22.96	46.82	-72.50
82.50	86.14	50	217.42	19.28	90.82	3.96	59.15	28.12	8.45	22.84	46.68	-74.83
198.75	17.24	10	156.93	15.84	56.22	3.01	69.09	28.58	8.46	22.23	46.68	-74.33
304.38	1.70	1	106.21	13.61	75.44	3.52	56.20	26.06	8.48	22.01	47.13	-74.33
West												
0.00	215.28	100	86.52	18.57	30.64	2.49	45.96	24.74	8.43	24.38	46.48	-72.5
55.00	189.70	90	136.64	17.47	43.77	2.99	46.14	22.55	8.44	23.56	46.47	-73.33
82.50	107.71	50	167.34	13.69	45.16	2.73	40.80	25.42	8.43	23.38	46.48	-74.17
167.50	21.53	10	163.67	12.86	52.21	2.63	39.64	26.10	8.42	23.10	46.60	-73.5
294.63	2.15	1	75.12	19.10	61.17	3.17	42.22	29.11	8.43	19.32	46.47	-74.50

Fig.(4): Dirunal changes in Dissolved Oxygen (ppm), Temperature (°C) & pH

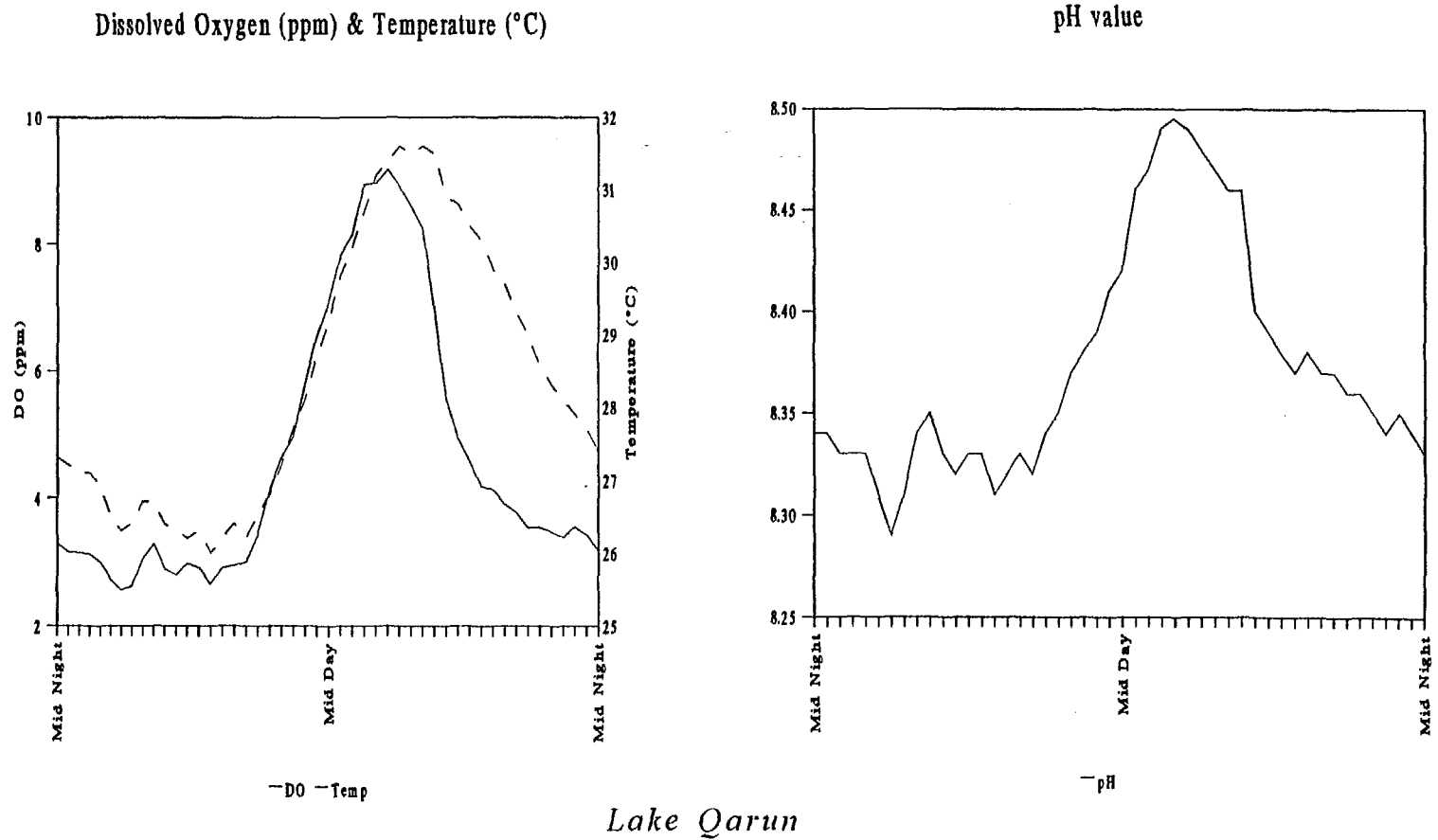


Table (2): Heavy metals in Lake Qarun water during summer
(µg/L)

Water									
Site	Ni	Co	Cr	Cu	Pb	Fe	Zn	Mn	Hg
1	430.00	141.33	326.33	136.00	390.00	3951.50	188.00	5465.50	250
2	340.67	131.00	175.33	105.00	330.00	1125.50	750.67	1832.00	300
3	446.33	143.33	390.00	99.50	293.33	2016.50	111.00	741.00	450
4	415.67	210.67	191.00	95.00	436.67	1236.00	243.67	132.00	450
5	631.00	218.33	269.00	71.50	356.67	1606.50	454.67	197.00	640
6	485.50	207.00	272.67	72.50	346.67	2838.00	1820.00	325.00	*
7	343.00	216.33	290.00	84.80	366.67	2084.00	873.50	3207.50	620
8	510.00	147.67	370.00	60.50	393.33	2778.00	1011.00	246.50	*
9	674.50	195.33	346.33	80.00	383.33	1505.50	232.50	283.00	680
El-Bats Drain	444.67	148.67	399.67	49.50	140.00	16412.50	186.00	4041.50	*
El-Wadi Drain	403.00	157.67	364.33	30.00	170.00	10994.50	217.00	4275.50	*

Table (3): Heavy metals in Lake Qarun Sediment during Summer
($\mu\text{g/Kg}$)

Site	Ni	Co	Cr	Cu	Pb	Fe	Zn	Mn
1	751.00	317.00	1093.33	536.50	453.33	27391.50	851.33	6084.50
2	647.67	233.67	960.67	512.50	406.67	27343.50	805.00	6368.50
3	834.33	237.00	796.67	419.50	420.00	27027.00	714.00	6295.00
4	818.50	191.00	852.33	448.00	466.67	26485.00	684.67	6063.00
5	697.33	143.33	745.33	271.00	440.00	25076.50	772.00	4269.00
6	1031.67	187.00	1023.00	458.00	473.33	26999.00	2444.33	5010.00
7	1018.33	185.00	973.00	529.00	593.33	27383.00	854.00	4785.00
8	1287.50	255.33	1444.67	44697.50	2683.33	26989.00	5972.50	4927.00
9	585.33	217.33	618.33	338.00	473.33	25435.00	428.33	2897.50
El-Bats	587.67	263.00	975.67	294.00	396.67	26527.50	581.00	6764.00
El-Wadi	799.00	335.00	1226.00	370.00	466.67	27695.50	822.67	5562.00

**Table (4): Pesticide in Lake Qarun sediment.
($\mu\text{g/Kg}$)**

	June 1995					July 1995				
	West	Middle	East	El-Bats	El-	West	Middle	East	El-Bats	El-Wadi
BHC-ALPHA	0.48	0.42	0.12	0.50	0.48	0.16	0.00	0.15	0.13	0.10
QUINTOZENE	0.20	0.19	0.12	0.31	0.19	0.08	5.72	11.10	14.00	0.00
LIDAN GAMMA-BHC	0.00	0.00	0.80	0.00	0.00	0.31	0.11	100.32	0.13	0.11
HEPTACHLOR	0.15	0.21	0.00	0.21	0.13	0.18	0.00	0.00	0.18	0.64
ALDRINE	0.00	0.53	0.20	0.00	0.10	0.00	0.15	13.20	0.13	13.70
BHC-BETA	1.36	0.95	9.22	1.05	0.68	0.00	0.00	12.90	0.24	6.87
BHC-DELTA	2.55	3.87	64.30	3.64	5.34	6.36	0.00	0.00	3.89	0.00
HEPTACHLOR	0.00	0.00	0.00	0.00	0.00	0.86	0.00	0.00	0.00	0.00
ALPHA-ENDOSULFAN	1.63	1.31	0.87	1.38	1.91	0.00	0.00	145.80	1.13	174.00
BETA-CHLORDAN	0.38	0.63	11.90	0.42	0.52	0.00	0.00	19.00	0.80	27.00
ALPHA-CHLORDAN	0.40	0.18	0.95	0.21	0.49	0.45	0.12	0.83	0.79	0.67
4,4 DDE	0.29	0.25	0.00	0.41	0.26	0.00	0.00	3.30	0.00	6.84
DIELDRIN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ENDRIN	0.00	1.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ORTHO-PARA DDT	0.34	4.30	0.96	0.05	0.48	0.99	0.37	0.00	0.37	0.00
4,4 DDD	0.25	6.00	8.52	0.00	0.47	0.00	0.00	78.70	0.00	95.80
4,4 DDT	1.12	1.52	0.05	50.50	3.86	0.99	0.00	0.56	0.69	0.00
METHOXYCHLOR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.32
	0.25	47.50	42.60	50.50	3.86	0.22	5.41	25.60	19.50	98.21

Fig. (5): Class composition of phytoplankton
No. of cells $\times 10^3 \text{ L}^{-1}$ (1995)

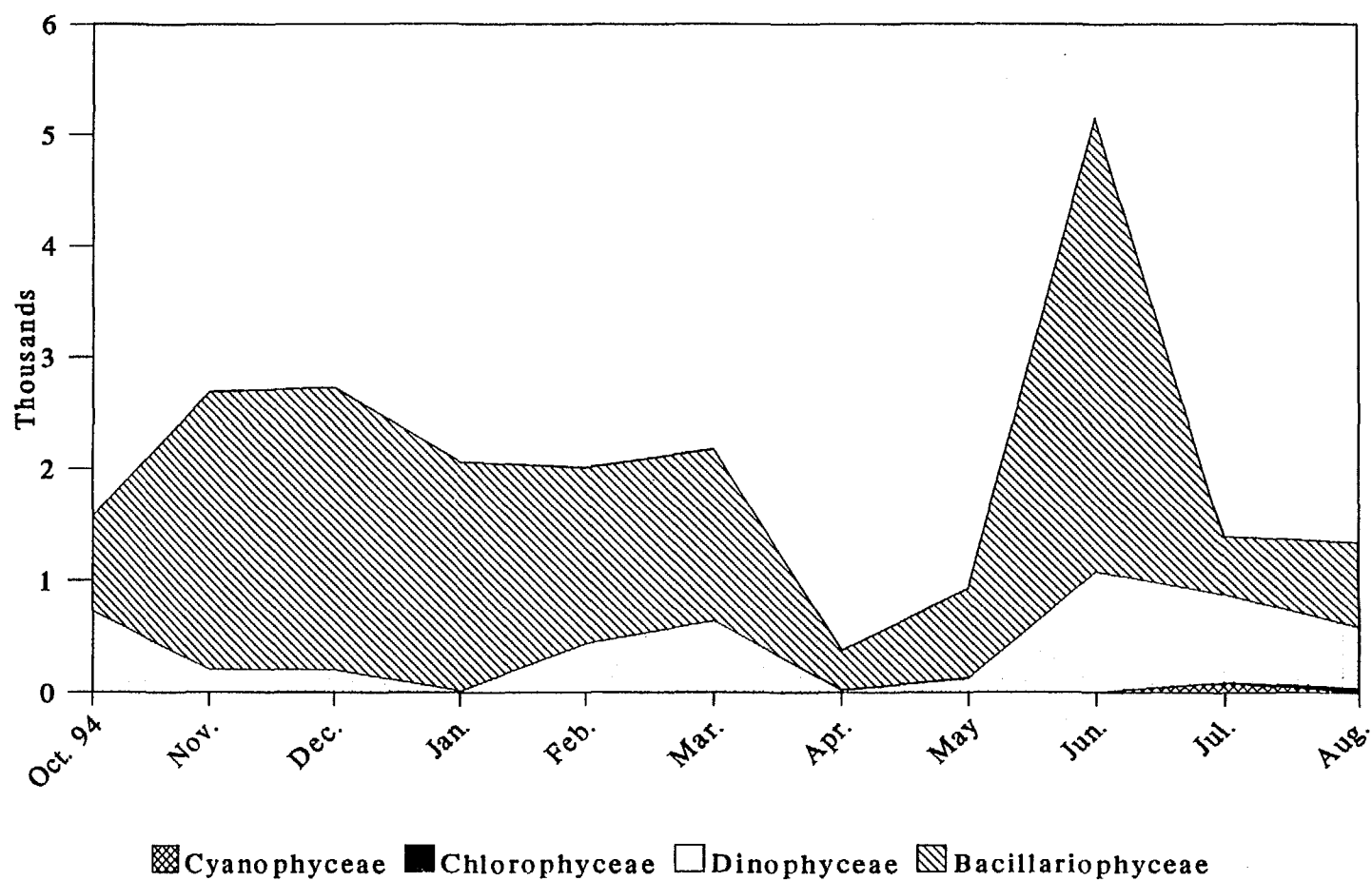
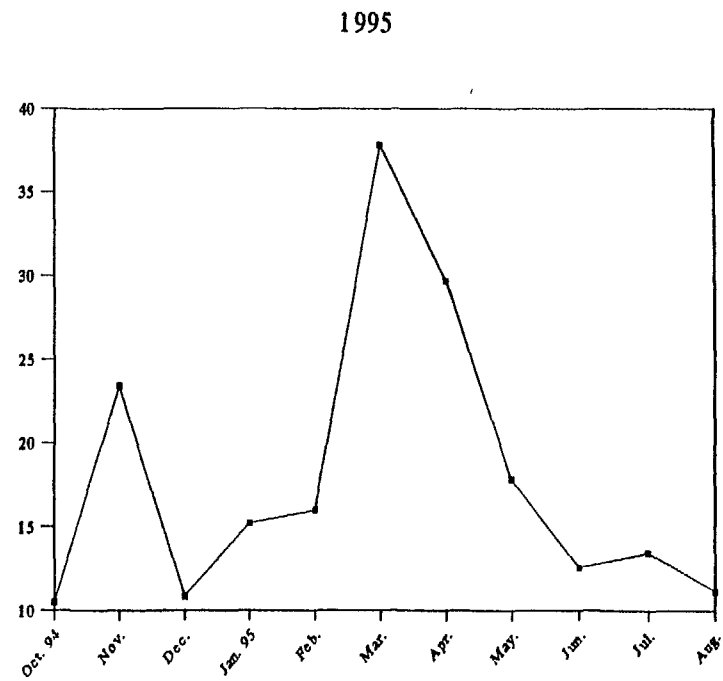
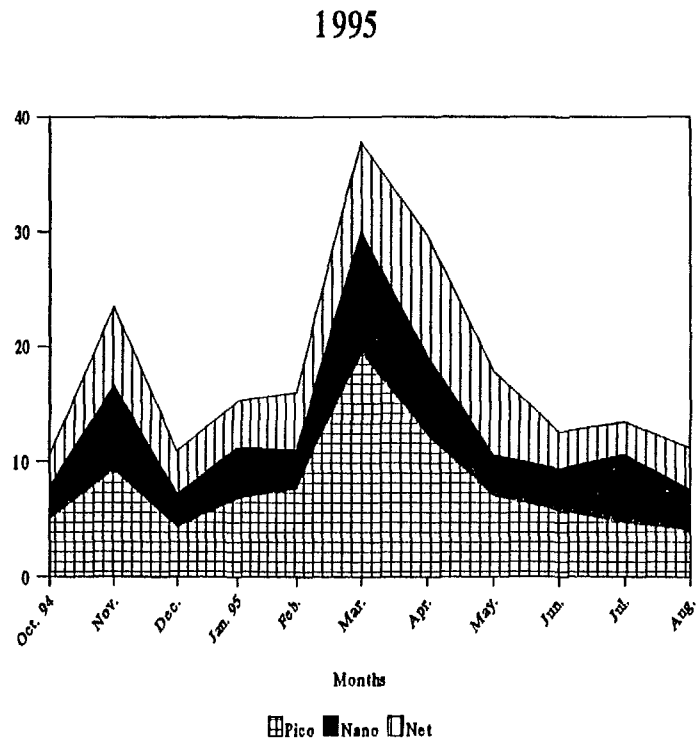


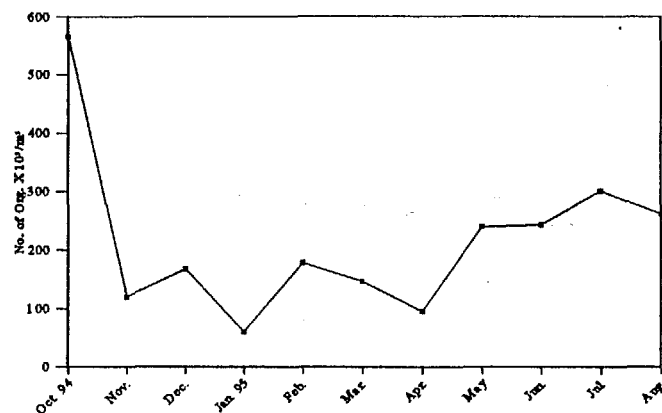
Fig. (6): Chlorophyll a ($\mu\text{g L}^{-1}$)



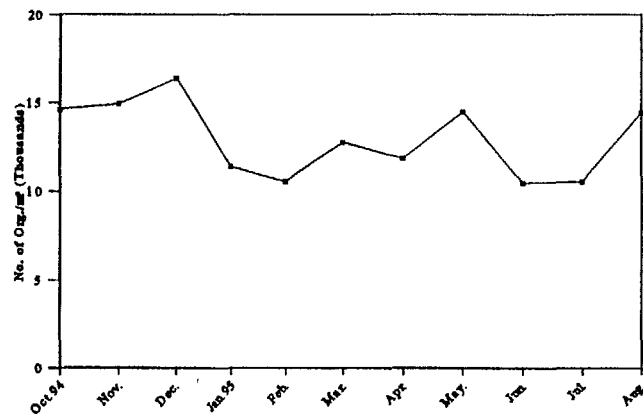
Lake Qarun

Fig. (7): Zooplankton & Bottom Fauna.

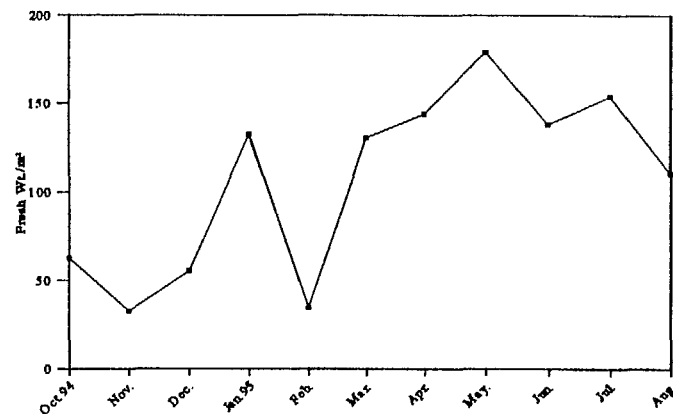
Total Zooplankton in Lake Qarun
1995



Standing crop of Benthic animals
1995



Biomass of Benthic animals
1995



Lake Qarun

- Feeding Behavior of Mullet Fry in Lake Qarun

In late summer 1995, one phase of the research focused on the feeding selectivity of mullet fry and their impacts on zooplankton community in Lake Qarun. Preliminary feeding experiments of mullet fry was conducted in 30 L glass aquaria under laboratory conditions, and 20 L transparent bottles *in situ* (received as a gift from the Kinneret Limnological Laboratory). The standard length (ST) of the tested mullet fry ranged from 46 to 64 mm & their weight varied from 1.68 to 3.86 g. Three sets of either glass aquaria or plastic bottles were used in these experiments. The first set served as control (i.e. zooplankton without fry); the second and third sets were stocked with 4 and 9 each. Feeding procedure consisted of adding freshly caught zooplankton collected from Lake Qarun using a plankton net (55 µm mesh diameter).

Fish feeding selectivity was investigated by monitoring the zooplankton densities in aquaria and plastic bottles before feeding and after 24 hours of feeding. Another experiment was conducted in the glass aquaria where feeding behavior of mullet fry was followed before feeding and after 30, 60 minutes and 24h of feeding. The results of these experiments are presented in Table 5

- Total Bacteria Counts (TBCs)

TBCs of the Lake Qarun the and the drains' water were investigated (at 22° and 37° C) during summer 1995. The data are given in Table 6.

II Fisheries and Fish Production

The yield per recruit model of Beverton and Holt (1957) to estimate the effect of fishing mortality coefficient "F", age at first capture " t_c " and natural mortality coefficient "M", was applied on the yield of the most commercially important fish species in the lake. The objective was to develop a management policy for the rational exploitation of the fish stocks.

Applying the Beverton/Holt model for *Solea aegyptiaca* showed that the maximum yield per recruit is 51.99 gm, which is achieved at a fishing mortality coefficient of 0.50. The results indicated that the present level of fishing mortality, natural mortality (1.34) is higher by about 168% than the corresponding maximum yield per recruit.

As for *Liza saliens*, the yield per recruit at the present level of fishing mortality, natural mortality and age at the first capture was 45.62 gm, while the maximum yield per recruit would correspond to a fishing mortality of 0.70.

Regarding *Oreochromis aureus*, the calculated maximum yield per recruit was 118.44 gm at a fishing mortality level of 0.30, whereas yield per recruit at present levels of natural mortality, fishing mortality and age at first capture was 89.19 gm. Accordingly, the present level of fishing mortality leads to maximum yield per recruit by 216.6%.

The estimated maximum yield per recruit for *Oreochromis niloticus* was 79.15 gm which corresponds to a fishing mortality of 0.30. Consequently, the present level of fishing mortality (1.04) is higher than the maximum yield per recruit by about 248%.

Estimated values of yield per recruit for *Tilapia zillii* showed that the present level of fishing mortality (0.63) is higher than that corresponding to the maximum yield per recruit (0.20) by about 213.5%.

To evaluate the effect of changing age at first capture (t_c) on the yield per recruit for the different fish species, the yield per recruit was calculated at different level of t_c . Results are illustrated in Figs. 8, 10, 12, 14, & 16 for *S. aegyptiaca*, *L. saliens*, *O. aureus*, *O. niloticus* and *T. zillii*. respectively. It is evident from these figures that as the age at first capture increases the yield per recruit increases, and vice versa.

**Table (5): Feeding behavior of Mullet fry in Lake Qarun
24 h experiments**

Species	Plastic bottles						Glass Aquaria					
	0 time			After 24 hrs			0 time			After 24 hrs.		
	Without fish	With 4 fry	With 9 fry	Without fish	With 4 fry	With 9 fry	Without fish	With 4 fry	With 9 fry	Without fish	With 4 fry	With 9 fry
<i>Brachiomus plictilis</i>	990	1020	930	1100	270	88	975	950	1175	855	363	38
<i>Syncheata pectinata</i>	30	60	60	38	15	-ve	13	38	75	15	-ve	-ve
<i>Napulis larvae</i>	330	410	360	200	45	13	225	188	213	240	75	27
<i>Cal-copepodid</i>	-ve	-ve	30	13	15	-ve	13	25	25	15	-ve	13
<i>Polychaeta larvae</i>	30	30	60	13	-ve	-ve	50	25	25	15	13	-ve
Total	1380	1520	1440	1364	345	101	1276	1226	1513	1140	451	78

**Feeding behavior of Mullet fry
Short to Long term Experiment in glass Aquaria**

	0 time		30 minutes		60 minutes		24 hrs	
	Without Fry	With 9 fry	Without Fry	With 9 fry	Without Fry	With 9 fry	Without Fry	With 9 fry
<i>Brachiomus plictilis</i>	1525	1590	1375	900	1338	615	1763	275
<i>Syncheata pectinata</i>	138	90	88	60	100	-ve	250	13
<i>Napulis larvae</i>	575	360	570	195	300	75	138	-ve
<i>Cal-copepodid</i>	-ve	30	-ve	-ve	-ve	-ve	-ve	-ve
<i>Polychaeta larvae</i>	25	60	-ve	30	13	-ve	-ve	-ve
Total	2263	2130	2033	1185	1751	690	2151	288

Table (6): Total bacterial counts (TBCs) in surface Lake
Qarun waters
(TBCs X 10¹² ml⁻¹)

Site	TBCs at °C	TBCs at °C
1	11	1.6
2	9.8	9.5
3	6.1	6
4	6	5.9
5	9.6	8.2
6	5	4.8
7	4.3	4
8	2	1.6
9	1.5	1
El-Bats Drain	14	11.8
El-Wadi Drain	13.1	11.2

* Each number is the mean value of three plates

The effect of natural mortality coefficient yield per recruit was estimated at different values of natural mortality coefficient (shown in Figs. 9, 11, 13, 15 & 17). The results indicated that the decrease in the natural mortality was accompanied by an increase in the yield per recruit. On the other hand, the increase in the natural mortality coefficient resulted in a decrease in yield per recruit.

Scientific Cooperation

This year was marked by extensive contacts and frequent site visitations between the Egyptian and the Israeli scientists. These activities can be summarized as follows:

The P.I. and Co-P.I. of Lake Qarun Project (Drs. Ezzat A. Ibrahim and Fahmy I. El-Gamal) visited the Kinneret Limnological Laboratory during the period of 15-19 October, 1994. There they met with Dr. Paul Walline and discussed the research progress and prepared a draft proposal for a new project dealing with food production in Wadi El-Raiyan Lakes (Egypt) and Kinneret Lake (Israel). Also during the visit, Dr. Paul Walline agreed to invite three Egyptian Ph.D. students for training purposes at the Kinneret Limnological Laboratory.

In May 1995, the three doctoral students visited the Kinneret Limnological Laboratory during the period from 22 May to 1st June. They undertook intensive training under the supervision of Drs. Moshe Gophen and Paul Walline, in the following subjects:

- measurements of phyto-pigments by HPLC technique (Mr. Adel Hassan Konsowa).
- acoustic surveys using single and dual beam systems (Mr. Osama Kalefa).
- Effect of planktivorous fish on zooplankton communities (Mr. Gamal El-Shabrawy).

The Israeli P.I. and the Co-P.I. (Prof. Drs. Moshe Gophen and Paul Walline) visited Egypt from 25 to 29 June 1995. They visited the project site at Lake Qarun and held discussions regarding the exchange of scientific information as well as conducting mutual experiments on the feeding behavior of mullet fry in freshwater environment (Lake Kinneret) and saline water (Lake Qarun). They were kind enough to send 24 transparent plastic bottles as a gift to the Egyptian investigators. The bottles are essential for conducting the *in situ* and feeding behavior experiments.

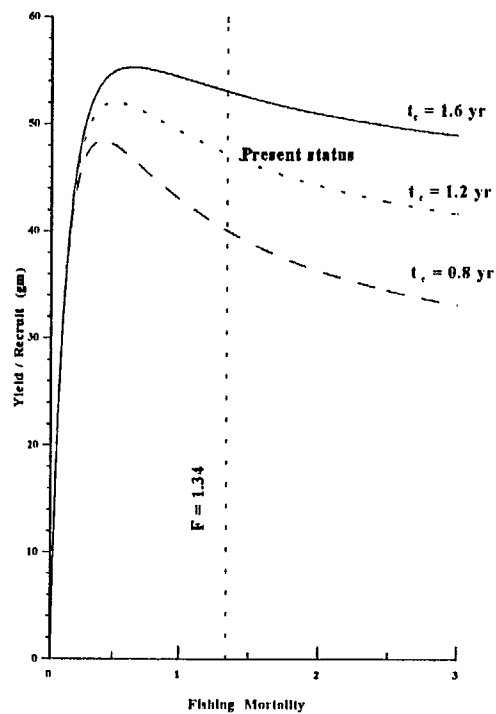


Fig. (8): Yield per recruit (gm) of *Solea aegyptiaca* as a function of fishing mortality (F) and different ages at first capture (t_c).

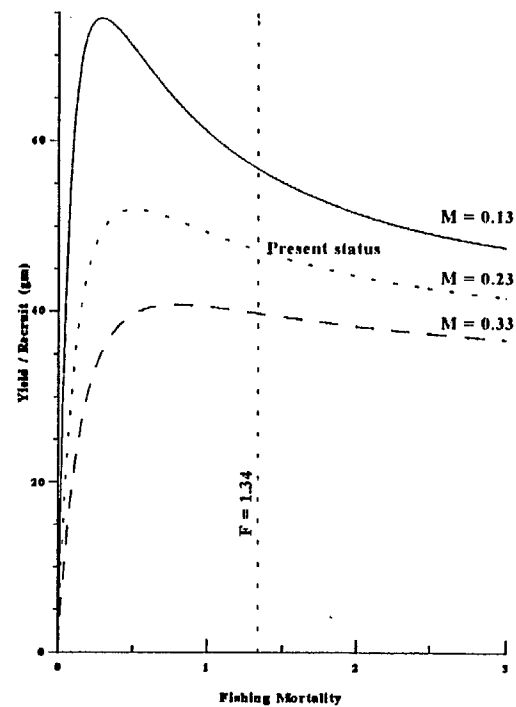


Fig. (9) : Yield per recruit (gm) of *Solea aegyptiaca* as a function of fishing mortality (F) and different levels of natural mortality (M).

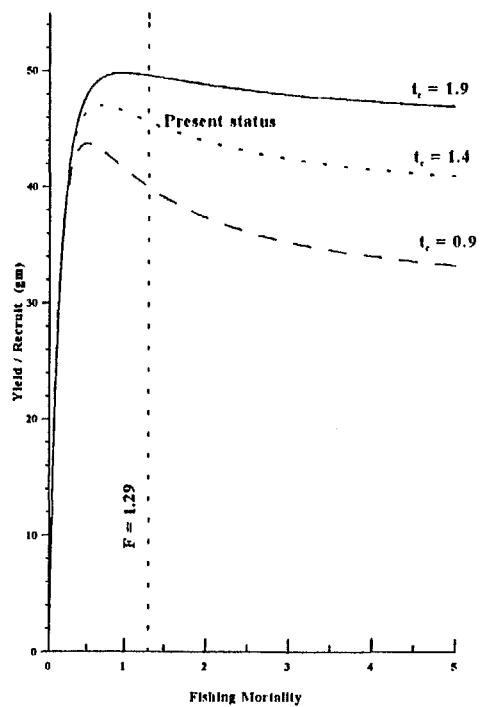


Fig.(10): Yield per recruit (gm) of *Liza saliens* as a function of fishing mortality (F) and different ages at first capture (t_c).

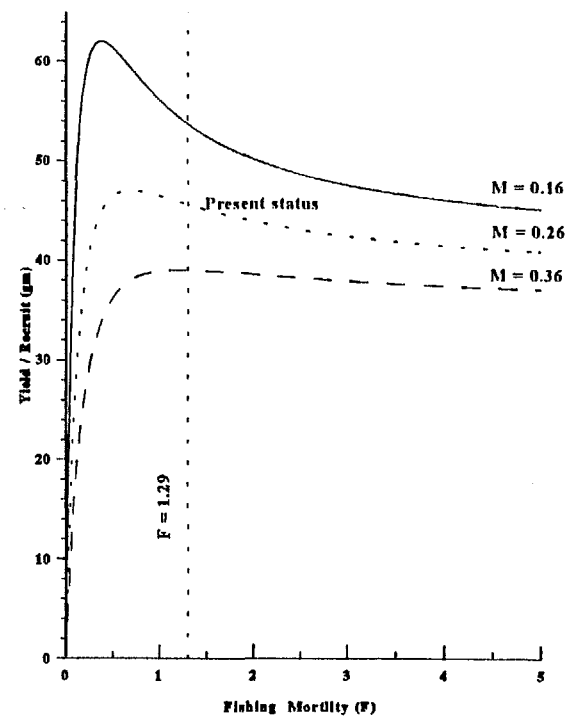


Fig.(11): Yield per recruit (gm) of *Liza saliens* as a function of fishing mortality (F) and different levels of natural mortality (M).

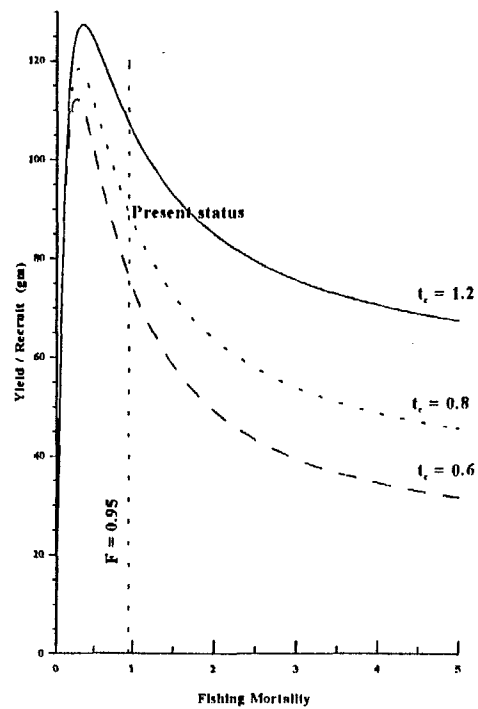


Fig.(12):Yield per recruit (gm) of *Oreochromis aureus* as a function of fishing mortality (F) and different ages at first capture (t_c).

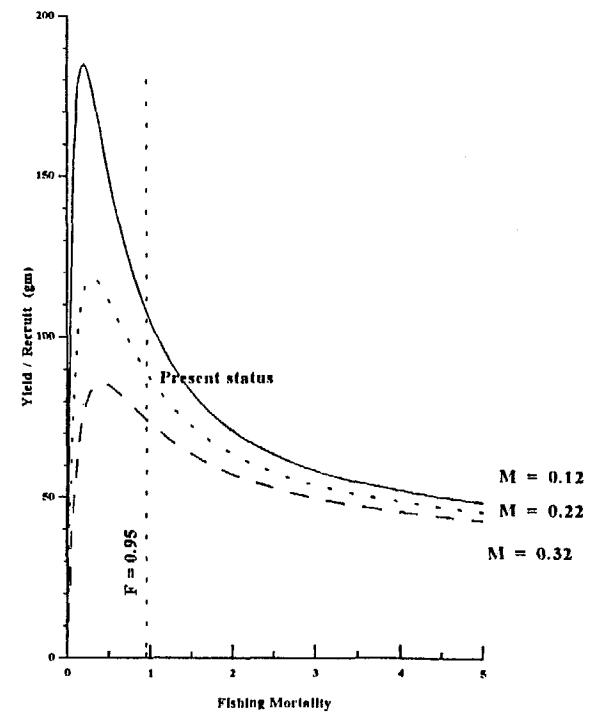


Fig.(13): Yield per recruit (gm) of *Oreochromis aureus* as a function of fishing mortality (F) and different levels of natural mortality (M).

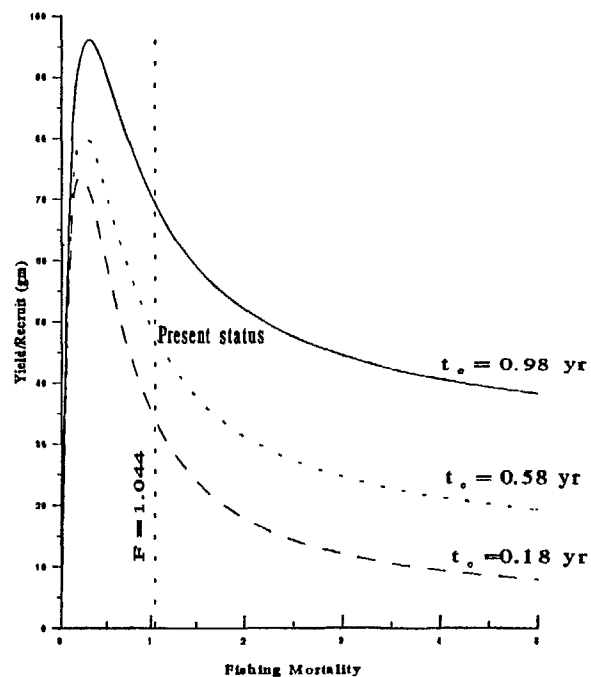


Fig.(14) : Yield per recruit(gm) of *Oreochromis niloticus* as a function of fishing mortality (F) and different ages at first capture(t_c).

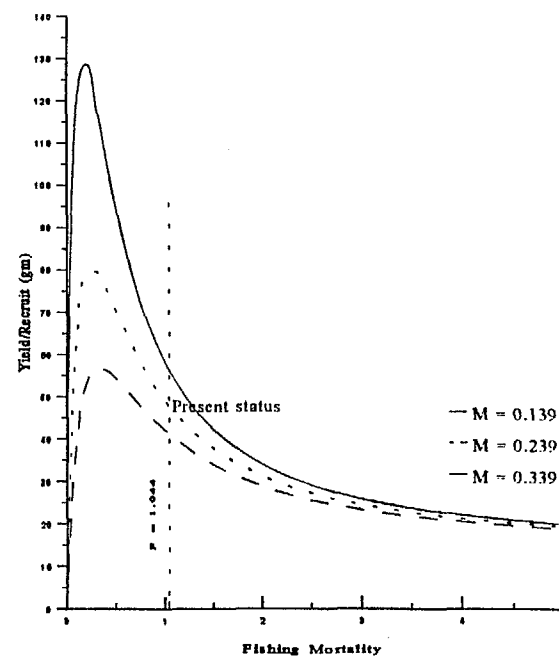


Fig.(15): Yield per recruit(gm) of *Oreochromis niloticus* as a function of fishing mortality (F) and different levels of natural mortality (M).

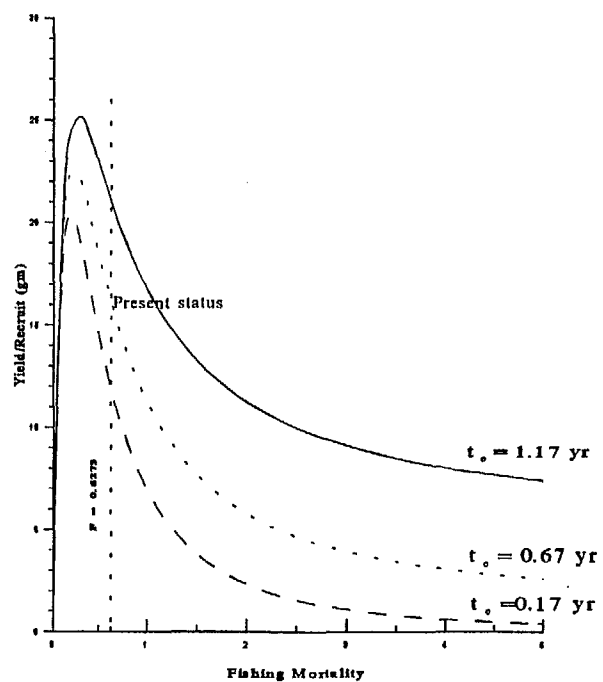


Fig.(16) : Yield per recruit(gm) of *Tilapia zillii* as a function of fishing mortality (F) and different ages at first capture(t_c).

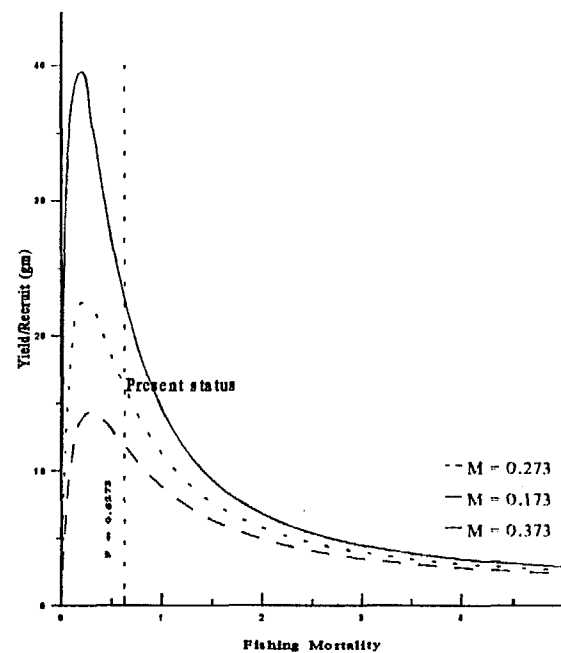


Fig.(17): Yield per recruit(gm) of *Tilapia zillii* as a function of fishing mortality (F) and different levels of natural mortality (M).

**(6.) Seafood Safety and Fish Decontamination - Technical
and Health Implications**

Third Annual Report

**Department of Environmental Health, University of Michigan (U.S.A.)
and
National Institute of Oceanography and Fisheries (Egypt)
and
The Hebrew University of Jerusalem (Israel)**

Executive Summary

This report includes concise accounts of main research activities which took place in 1994-95 project year. This includes surveys of the safety of fish marketed in Egypt and Israel, experiments on the rates of depuration of toxic chemicals, and legislation dealing with seafood safety in Egypt.

Microbiological and parasitological surveys of fish catches from different locations in Egypt have been conducted by a research team from Cairo University, under the direction of Dr. Mohyi S. Easa. These surveys included locations at (a) the High Dam Reservoir and the Nile at Aswan, (b) the Nile at Giza, (c) Lake Manzala, and (d) El-Max and Abokir at Alexandria. We have reported earlier on surveys conducted in the Aswan region.

The selected sites from Aswan in the south, to Lake Manzala and Alexandria in the north of Egypt, represented stark differences in the degree of water pollution, and the quality of the fish harvested from these waters. In the extreme south, the level of pollution was minimal and the quality of sampled fish were relatively high. In contrast, with increasing population density and waste discharges in the north, the polluted waters of Lake Manzala and the Alexandria region contained diseased fishes, infested with parasites and bacterial pathogens. Clearly, the prevalence of parasite encysted metacercarial in fish samples increased northward from Aswan (2.5 percent infection) to Cairo (17 percent infection), to Lake Manzala (100 percent infection). Similarly, bacterial counts of fecal coliform increased from low levels in Aswan to very high levels in Lake Manzala and Alexandria.

The public health significance of these results indicate that fish harvested from the polluted regions is unsafe if consumed raw. More significantly, the health risk is quite high for persons involved in fish handling, e.g. fishing, marketing, and food preparation.

Microbiological surveys of fish marketed in Israel gave the same results. In Israel, fishponds are generally supplemented with animal manure, and in some farms, with sewage. Bacterial and viral pathogens introduced in the fishponds may pose a health hazard for fish handlers, and consumers.

The rates of elimination of bacterial pathogens from fish were investigated by Dr. Mohamad El-Samra and his research team, at the laboratories of the National Institute of Oceanography and Fisheries, in Suez, Egypt. Research findings indicate that it will take several days to achieve 95% depuration of bacterial pathogens from fish. These are ongoing investigations. Research results will be useful in assessing the time required for fish depuration practices.

Investigations on the survival of bacterial and viral pathogens in natural water at the Hebrew University, Israel, provided very interesting information. This included a variety of pathogens, e.g. Hepatitis A. Virus (HAV), Poliovirus 1, F+ Bacteriophages, and E. coli. Different microorganisms exhibited different die-off rates. However the startling results were that virus can survive for several months, especially at low temperatures in the winter.

Studies on the uptake, accumulation, and elimination of pesticides by fish are being conducted by Dr. Mohamed Sherief, Ain Shams University, Cairo, Egypt. In this report, we are presenting research done on an organophosphorus pesticide, commonly used in Egypt. These studies were conducted with the Nile Tilapia. Research findings will provide valuable information

on pesticide depuration rates, which will be used in the development of advisories for seafood safety.

Finally, this report includes a section on the legislation pertaining to seafood safety. This includes fresh fish and processed fish marketed in Egypt. A discussion on the limitations of some of these regulations is also included.

Our plans for the fourth and final year of this project include providing a comprehensive account of (a) the microbiological and parasitological conditions of fish marketed in Egypt and Israel, (b) surveys of chemical contaminants in fish, (c) depuration rates of chemicals and pathogens from fish and their health and technical implications for fish farming practices, and (d) guidelines for improving seafood safety management, which includes procedures for assessing health risk, protection of consumers, and specific elaborations on pertinent legislation in Israel and Egypt.

**(6.a) Microbiological and Parasitological Surveys of Fish at
Aswan, Giza Province, and Lake Manzala**

Third Annual Report

Principal Investigator: Dr. Mohi S. Easa

Cairo University, Egypt

I. Purpose of Study

The purpose of this study was to investigate the parasitic and microbial contamination of fish from three regions along the River Nile. Due to the fact that fish are important vectors in the transmission of many microorganisms to man, the results of this study are invaluable in assessing the extent of human exposure to these pathogens.

Indicator microorganisms established for water quality, mainly bacteria such as total coliforms and fecal coliforms (WHO, 1971, Jazrawi *et al.*, 1988), have generally been used to evaluate the potential health hazards associated with contaminated food and seafood (West and Coleman, 1986 and Daw *et al.*, 1990). These microorganisms survive and multiply in the gut, mucous and tissues of fish. Both quantitative and qualitative bacterial assays of important microbial indicators in skin, gills and alimentary canal of fish were conducted. Information about several microorganisms including *Aeromonas hydrophila*, *Klebsiella*, *Salmonellae*, *Shigellae*, *Staphylococcus aureus* and *Streptococcus faecalis* are provided in the Appendix of this report. General information concerning parasitic transmission to man through fish is also provided in the Appendix.

II. Procedures of Study

Live *Tilapia* species were collected from each of the three test sites along the River Nile from 1) the Aswan area, northward to 2) the Giza Province (Helwan, El-Maidy and Giza areas) near Cairo, and at 3) Lake Manzala in the Nile delta. Pieces of one gram in weight of viable metacercarial cysts, from various fish muscles (head, trunk and tail regions) were extracted, then submitted again to the digestive technique to determine motility and viability (Ilan Paperna, 1980). Each type of active metacercariae obtained from these muscles was fed to 10 albino rats at a rate of 30 metacercariae per rat. This procedure was also conducted on herons, puppies, suckling kittens and chickens. After 3 days of infection, daily fecal samples were examined for trematodes eggs to determine the prepatent period by the sedimentation technique. Each day two animals from each treatment group were sacrificed, their intestines were separated and the content of each part of the intestine examined separately for collection of mature worms. Worms and encysted metacercariae were fixed, Carmine-stained, mounted and identified according to Yamaguti (1958).

For microbiological examination of fish samples the procedures were as follows:

1- Surface swab:

Over prescribed area (10 cm²) and collected from fish directly after being caught. Each swab was taken in 10 ml of 0.1% peptone water.

2- Muscle samples:

After the fish were disinfected with a piece of cotton moistened with alcohol, a 5 gram sample of muscle was taken from dorsal areas under aseptic conditions in 45 ml 0.1% peptone water.

3- Intestine samples:

Under complete aseptic conditions intestines were cut and transferred to a test tube containing 10 ml peptone water as an enrichment.

Determination of aerobic plate count and Coliform count was conducted according to ICMSF (1978). The techniques are as follows:

Aerobic plate count:

By surface spread plate. Total aerobic bacteria was determined using plant counting agar and the pour-plate technique.

Coliform count:

By multiple tube method. Total coliforms and fecal coliforms were quantified by the five-tube most probable number (MPN) technique using Mac-Conkey purple broth (APHA, 1985). The bacterial numbers were determined per square centimeter for external skin surface, and per gram for gills and alimentary canal.

Salmonella and Shigella:

- Pre-enrichment in peptone water
- Direct isolation on Mac-Conky, S.S. agar at 37°C for 24 hours.
- In direct isolation for *Salmonella* by transferring 1 ml of sample to 10 ml of sterile F broth for 18 hr at 37°C then culture on Mac-Conky or S.S agar.
- Non bacteria fermenting colonies tested biochemically and serologically.

Yersinia enterocolitica:

- 1 ml of sample inoculated to modified Rappaports broth and incubated at 22°C for 48 hours.
- A loop full of enrichment broth was streaked onto *Yersinia* selective agar medium (Oxoid) and incubated at 25°C for 48 hours.
- Typical colonies of *Yersinia enterocolitica* developed as a dark red (bull's eye) surrounded by a transparent border. These were tested biochemically.

III. Results

A. Aswan area

i. *parasitological examination*

Table 1: The prevalence of infection with the encysted metacercariae in *Tilapia* species at Aswan.

Type of Infection	Total Examined Fish	Number of Infected Fish	Percentage
Single Infection	40	16	40.0
Mixed Infection with two types of encysted metacercariae	40	1	2.5
Mixed Infection with more than two types of encysted metacercariae	40	1	2.5

Data in Table 1 indicate that the prevalence of single infection of e.m.c. was more than mixed infection in contrast with the results of e.m.c. infested *Tilapia* sp. in Cairo area where mixed infection was higher than single infection.

Table 2: The prevalence of the encysted metacercariae in the *Tilapia* species at Aswan.

Types of Encysed Metacercariae	Number of Examined Fish	Number of Infected Fish	Percentage
<i>Diplostomatid</i>	40	16	40.0
<i>Heterophyid</i>	40	1	2.5
<i>Clinostomatid</i>	40	2	5.0
<i>Euclinostomatid</i>	40	3	7.5

Data displayed in Table 2 show that the percentage of infestation by *Heterophyid* metacercariae was considered low, at 2.5 percent. In addition to the information provided in

Table 2, the *Clinostomatid* metacercariae was commonly found dorsal to bronchial cavity encysted with fibrenoid capsule. Also, *Euclinostomatids* were commonly found in posterior kidney, especially around the dorsal area and were sometimes seen attached to vertebral column and adjacent muscle.

Table 3: The prevalence of the encysted metacercariae in different regions of *Tilapia* species at Aswan area.

Type of Encysted Metacercariae	Head Region		Trunk Region		Tail Region	
	Number of Infected Fish	Percent	Number of Infected Fish	Percent	Number of Infected Fish	Percent
<i>Diplostomatid</i>	13	32.5	4	16.0	3	7.5
<i>Heterophyid</i>	1	2.5	0	0.0	0	0.0
<i>Clinostomatid</i>	0	0.0	0	0.0	0	0.0
<i>Euclinostomatid</i>	0	0.0	0	0.0	0	0.0

ii. microbial examination

Table 4: Results of the Aerobic Plate Count (A.P.C.) in the examined fish from the Aswan area. (n = 25)

Fish Species	Surface (A.P.C./cm ²)	Muscle (A.P.C./gm)
<i>Tilapia</i>	200	0

Table 5: Results of the coliform count in the examined fish from the Aswan area. (n = 25)

Fish Species	Surface (Coliform/cm ²)	Muscle (Coliform/cm ²)
<i>Tilapia</i>	1	0

Table 6: Enterobacteriaceae isolated from fish surface from the Aswan area. (n = 25)

Microorganisms	Number of positive fish sample	Percentage
<i>Esherchia coli</i>	4	16
<i>Enterobacter aerogenes</i>	7	28
<i>Citrobacter freundii</i>	6	24
<i>Proteus vulgaris</i>	6	24
<i>Edwardsilla tarda</i>	1	4

Table 7: Bacteria other than Enterobacteriaceae isolated from fish surface from the Aswan area. (n = 25)

Microorganisms	Number of positive fish sample	Percentage
<i>Pseudomonas aerogonosa</i>	1	4
<i>Micrococci</i>	8	32

iii. intestinal examination of laboratory experimentally infested with metacercariae

The experiment resulted in the development of mature worms in the intestine of lab animals causing destruction of intestinal villi, invading intestinal crypts and atrophy of the intestinal lining (epithelium). In some cases, parasites invading the intestinal wall appeared in the intestinal glands. The reaction of the intestine against parasite was mild, characterized by aggregation of low numbers of inflammatory cells, mainly macrophages and lymphocytes. Low numbers of fibroblasts were seen at areas of parasitic infestation.

At early stages, intestinal reaction to the worms was characterized by dilation of blood vessels, hyperplasia and/or hypertrophy of mucous glands, with extensive accumulation of tissue debris in the intestinal lumen. This development explains the clinical lesions found in mammals, including humans, infested with encysted metacercaria ingested with fish. Symptoms are manifested as diarrhea, abdominal discomfort, headache, vomiting, and sometimes bloody diarrhea. This syndrome is commonly referred to by fishermen as "fish dysentery".

B. Giza Province (Cairo area)

i. parasitological examination

Table 8: The encountered e.m.c. in <i>Tilapia</i> species from the Cairo area						
Number of Fish	<i>Prohemi-stomatid</i>	<i>Diplo-stomatid</i>	<i>Hetero-phyid</i>	<i>Haplor-chid</i>	<i>Clino-stomatid</i>	<i>Euclino-stomatid</i>
1	+	-	-	-	-	+
2	+	+	+	-	-	+
3	+	-	-	-	-	-
4	+	+	-	-	-	-
5	+	-	-	-	-	-
6	+	-	-	-	-	-
7	+	+	+	+	-	-
8	+	+	+	-	-	-
9	+	-	-	-	-	-
10	+	-	-	-	-	-
11	+	+	-	-	-	+
12	+	-	-	-	-	-
13	+	+	-	-	-	-
14	+	+	+	-	-	+
15	+	+	-	-	-	-
16	+	+	-	-	-	-
17	+	-	-	-	-	-
18	+	-	-	-	-	-
19	+	-	-	-	-	-
20	+	-	-	-	-	-

(+) indicates that the particular e.m.c. was detected

(-) indicates that the particular e.m.c. was not detected

Table 8, continued: The encountered e.m.c. in *Tilapia* species from the Cairo area

Number of Fish	<i>Prohemi-stomatid</i>	<i>Diplo-stomatid</i>	<i>Hetero-phyid</i>	<i>Haplor-chid</i>	<i>Clinostomatid</i>	<i>Euclinostomatid</i>
21	+	-	-	-	-	-
22	+	-	-	-	-	-
23	+	-	-	+	-	-
24	+	+	+	-	-	-
25	+	+	-	-	-	-
26	+	+	-	-	-	-
27	+	+	-	-	-	-
28	+	+	-	-	-	-
29	+	-	-	-	-	-
30	+	+	-	-	-	-
31	+	+	+	+	-	-
32	+	-	+	+	-	-
33	+	+	-	-	-	-
34	+	-	-	-	-	-
35	+	+	-	-	-	-
36	+	+	-	-	-	-
37	+	-	-	-	-	-
38	+	+	-	-	-	-
39	+	-	-	-	-	-
40	+	+	-	-	-	-

(+) indicates that the particular e.m.c. was detected

(-) indicates that the particular e.m.c. was not detected

From Table 8 it can be seen that *Prohemistomatid* was detected in all 40 of the *Tilapia* species tested at Cairo. The *Clinostomatid* e.m.c. was not detected in any of the 40 fish.

Table 9: The prevalence of the encysted metacercariae in the *Tilapia* species from the Cairo area.

Types of Encysed Metacercariae	Number of Examined Fish	Number of Infected Fish	Percentage
<i>Prohemistomatid</i>	40	40	100.0
<i>Diplostomatid</i>	40	21	52.5
<i>Heterophyid</i>	40	7	17.5
<i>Haplorchid</i>	40	4	10.0
<i>Euclinostomatid</i>	40	4	10.0

Table 9 shows that three types of encysted metacercariae detected in muscle of *Tilapia* species are of zoonotic importance, transmittable to humans. The three types of metacercariae were *Prohemistomatid*, *Heterophyid* and *Haplorchid*. While the remaining two types, *Diplostomatid* and *Euclinostomatid* are not transmitted to humans. Results also documented a prevalence of *Prohemistomatid* at 100 percent, meaning that all *Tilapia* species in the area of investigation were infected with trematodes.

Table 10: The prevalence of infection with the encysted metacercariae in *Tilapia* species from the Cairo area.

Type of Infection	Total Examined Fish	Number of Infected Fish	Percentage
Single Infection	40	16	40.0
Mixed Infection with two types of encysted metacercariae	40	17	42.5
Mixed Infection with more than two types of encysted metacercariae	40	7	17.5

Table 10 displays the type of e.m.c. infection along with the total number of examined fish that were infected. Results in Table 10 indicate that:

- mixed infection was higher than that of single infection.
- polluted area of investigation may play a role of prevalence of mixed infection.

Table 11: The prevalence of the encysted metacercariae in different regions of *Tilapia* species from the Cairo area.

Type of Encysted Metacercariae	Head Region		Trunk Region		Tail Region	
	Number of Infected Fish	Percent	Number of Infected Fish	Percent	Number of Infected Fish	Percent
<i>Prohemistomatid</i>	16	40.0	17	42.5	37	92.5
<i>Diplostomatid</i>	20	50.0	10	25.0	11	27.5
<i>Heterophyid</i>	1	2.5	2	5.0	7	17.5
<i>Haplorchid</i>	0	0.0	1	2.5	4	10.0

Table 11 shows that the prevalence of infestation in different body regions of *Tilapia* species differed according to the species of parasite. In the tail region, the percentage of infestation with *Prohemistomatid* and *Heterophyid* reaches 92.5 percent and 17.5 percent, respectively. Infestation with *Diplostomatid* reaches 27.5 percent in tail region, while the head region is highly infested with *Diplostomatid* (50 percent).

ii. microbial examination

Table 12: Results of the Aerobic Plate Count (A.P.C.) in the examined fish from the Cairo area. (n = 25)

Fish Species	Surface (A.P.C./cm ²)	Muscle (A.P.C./gm)
<i>Tilapia</i>	1200	0

Table 13: Results of the coliform count in the examined fish from the Cairo area.
(n = 25)

Fish Species	Surface (Coliform/cm2)	Muscle (Coliform/cm2)
<i>Tilapia</i>	15	0

Table 14: Enterobacteriaceae isolated from fish surface from the Cairo area. (n = 25)

Microorganisms	Number of positive fish sample	Percentage
<i>Shigella dysenterae</i>	2	8.0
<i>Providencia alcalifaciens</i>	7	28.0
<i>Enterobacter aerogenes</i>	8	32.0
<i>Klibsiella pneumoniae</i>	10	40.0
<i>Esherchia coli</i>	20	80.0

Table 15: Enterobacteriaceae isolated from intestine of fish from the Cairo area.
(n = 25)

Microorganisms	Number of positive fish sample	Percentage
<i>Shigella dysenterae</i>	0	0.0
<i>Providencia alcalifaciens</i>	0	0.0
<i>Enterobacter aerogenes</i>	2	8.0
<i>Klibsiella pneumoniae</i>	3	12.0
<i>Esherchia coli</i>	22	88.0

Table 16: Enterobacteriaceae isolated from fish muscles from the Cairo area. (n = 25)

Microorganisms	Number of positive fish in sample	Percentage
<i>Shigella dysenterae</i>	2	8.0
<i>Providencia alcalifaciens</i>	7	28.0
<i>Enterobacter aerogenes</i>	8	32.0
<i>Klibsiella pneumoniae</i>	10	40.0
<i>Esherchia coli</i>	20	80.0

Table 17: Enterobacteriaceae isolated from fish species from the Cairo area.

Microorganism	Surface		Muscle		Intestine	
	Number	Percent	Number	Percent	Number	Percent
<i>Shigella dysenterae</i>	2	4.2	0	0	0	0
<i>Providencia alcalifaciens</i>	7	14.8	0	0	0	0
<i>Enterobacyer aerogenes</i>	8	17.0	0	0	2	7.4
<i>Klibsiella pneumoniae</i>	10	21.2	0	0	3	11.1
<i>Esherchia coli</i>	20	42.5	0	0	22	81.4

C. Lake Manzala

i. *parasitological examination*

Table 18: The prevalence of the encysted metacercariae in the *Tilapia* species found in Lake Manzala.

Types of Encysed Metacercariae	Number of Examined Fish	Number of Infected Fish	Percentage
<i>Heterophyid</i>	50	20	40.0

Only *Heterophyid* metacercariae were observed in the muscle of *Tilapia* growing in Lake Manzala.

Table 19: The prevalence of the infection with *Heterophyid* encysted metacercariae in different regions of the body in three species of *Tilapia* from Lake Manzala.

<i>Heterophyid</i> e.m.c.									
Type of <i>Tilapia</i> Species	HEAD REGION			TRUNK REGION			TAIL REGION		
	Num.	Inf.	%	Num.	Inf.	%	Num.	Inf.	%
<i>Tilapia nilotica</i>	3	1	33.3	3	0	0.0	3	1	33.3
<i>Tilapia zilli</i>	10	4	40.0	10	2	20.0	10	10	100.0
<i>Tilapia aurea</i>	7	1	14.3	7	1	14.3	7	6	85.7
Total Number	20	6	30.0	20	3	15.0	20	17	85.0

Num. = the total number of fish

Inf. = the number of infected fish

% = percentage

ii. *microbial examination*

Table 20: Results of the Aerobic Plate Count (A.P.C.) in the examined fish from Lake Manzala. (n = 25)

Fish Species	Surface (A.P.C./cm ²)	Muscle (A.P.C./gm)
<i>Tilapia</i>	38000	0

Table 21: Results of the coliform count in the examined fish from Lake Manzala.**(n = 25)**

Fish Species	Surface (Coliform/cm2)	Muscle (Coliform/cm2)
<i>Tilapia</i>	21000	0

Table 22: Enterobacteriaceae isolated from examined fish from Lake Manzala.**(n = 25)**

Microorganisms	Number of Isolates	Percentage of Total Number of Isolates
<i>Aeromonas hydrophila</i>	2	4.2
<i>Esherchia coli</i>	15	31.9
<i>Edwardsilla tarda</i>	5	10.6
<i>Citrobacter freundii</i>	8	17.1
<i>Proteus vulgaris</i>	6	12.8
<i>Klibsiella pneumoniae</i>	11	23.4
Total	47	100.0

Table 23: Enterobacteriaceae isolated from surface of examined fish from Lake Manzala.**(n = 25)**

Microorganisms	Number of Isolates	Percentage of Total Number of Isolates
<i>Esherchia coli</i>	9	32.1
<i>Edwardsilla tarda</i>	4	14.3
<i>Citrobacter freundii</i>	5	17.9
<i>Proteus vulgaris</i>	4	14.3
<i>Klibsiella pneumoniae</i>	6	21.4
Total	28	100.0

Table 24: Enterobacteriaceae isolated from intestine of examined fish from Lake Manzala. (n = 25)

Microorganisms	Number of Isolates	Percentage of Total Number of Isolates
<i>Aeromonas hydrophila</i>	2	10.5
<i>Escherichia coli</i>	6	31.5
<i>Edwardsiella tarda</i>	1	5.3
<i>Citrobacter freundii</i>	3	15.8
<i>Proteus vulgaris</i>	2	10.5
<i>Klebsiella pneumoniae</i>	5	26.3
Total	19	100.0

From Tables 23 and 24, *Aeromonas hydrophila* was isolated only from intestine.

Table 25: Enterobacteriaceae isolated from different parts of the examined Tilapia from Lake Manzala.

Microorganism	SITE OF LOCATION			TOTAL
	SURFACE	INTESTINE	MUSCLE	
<i>Aeromonas hydrophila</i>	0	2	0	2
<i>Escherichia coli</i>	9	6	0	15
<i>Edwardsiella tarda</i>	4	1	0	5
<i>Citrobacter freundii</i>	5	3	0	8
<i>Proteus vulgaris</i>	4	2	0	6
<i>Klebsiella pneumoniae</i>	6	5	0	11
Number of Isolates	28	17	0	47

Table 26: *Staphylococci* isolated from different parts of the examined *Tilapia* from Lake Manzala.

Microorganism	SITE OF LOCATION			TOTAL
	SURFACE	INTESTINE	MUSCLE	
<i>Staphylococcus epidermidis</i>	3	0	5	8
<i>Staphylococcus aureus</i>	1	0	0	1
Number of Isolates	4	0	5	9

Table 27: Microorganisms isolated from different parts of the examined *Tilapia* from Lake Manzala.

Microorganism	SITE OF LOCATION				TOTAL
	SURFACE	INTESTINE	MUSCLE	GILLS	
<i>Staphylococcus aureus</i>	1	0	0	0	1
<i>Staphylococcus epidermidis</i>	2	0	5	1	8
<i>Aeromonas hydrophila</i>	0	2	0	0	2
<i>Escherichia coli</i>	9	6	0	6	21
<i>Edwardsiella tarda</i>	4	1	0	3	8
<i>Citrobacter freundii</i>	5	3	0	4	12
<i>Proteus vulgaris</i>	4	2	0	3	9
<i>Klebsiella pneumoniae</i>	6	5	0	7	18
Number of Isolates	31	17	5	24	79

Table 28: <i>Staphylococci</i> isolated from examined fish from Lake Manzala.		
Microorganisms	Number of Isolates	Percentage of Total Number of Isolates
<i>Staphylococcus aureus</i>	1	11.1
<i>Staphylococcus epidermidis</i>	8	88.9
Total	9	100.0

IV. Conclusion

A. Parasitological Examination

In the southern most test site of Aswan, it was determined that 55 percent of the *Tilapia* species collected were infected with encysted metacercariae (e.m.c.). However, only 2.5 percent (1 out of 40 fish) were infected with the *Heterophyid* type, which like *Prohemistomatid* and *Haplorchid*, can be transmitted to humans. Neither *Prohemistomatid* nor *Haplorchid* were detected. The highest percentage of the e.m.c. recorded (40 percent) was of the *Diplostomatid* type.

Near Cairo, although the prevalence of *Diplostomatid* increased to about 53 percent, more of the fish (43 percent) were infected with two or more types of e.m.c., compared to only 5 percent in the Aswan region. The major contributor to this increase was the 100 percent infection of *Prohemistomatid* in the 40 fish which were examined. It should be considered that the polluted aquatic conditions of the area of investigation play a significant role in the prevalence of mixed infection.

Of the 50 *Tilapia* which were collected from Lake Manzala, the twenty which were infected with e.m.c., were only infected with the *Heterophyid* type. Clearly, the prevalence of this e.m.c. type increases northward from Aswan (2.5 percent infection), to Cairo (17 percent infection), to Lake Manzala (100 percent infection).

B. Microbial Examination

At Aswan, the average count of A.P.C. was 200 per square centimeter (Table 4). This indicates that the water from which the fish originated had low bacterial count and was not overly polluted. With reference to Table 5, the average coliform count on the skin surface of the fish sampled was 1 coliform per square centimeter. This very low coliform count indicated that there was very little fecal pollution. The presence of non-significant numbers of coliform may be a result of a high level of fishing activity.

In the Nile waters of the Cairo area, a surface A.P.C. of 1200 per square centimeter (Table 12) and a surface coliform count of 15 per square centimeter (Table 13) were recorded. At Lake Manzala, the surface A.P.C. per square centimeter was 38,000 (Table 20). As is displayed in Table 21, the surface coliform count was 21,000. These values

indicate that the bacterial count and fecal pollution levels found on *Tilapia* increase in the areas of the Nile downstream from Aswan. Compared with the A.P.C. and coliform counts on the fish surfaces of Aswan, the levels recorded near Cairo and at Lake Manzala were very high.

At Aswan, the failure of findings to detect bacteria in muscles of living fish agrees with earlier researchers who stated that the flesh and body fluids of newly caught fish were sterile. It seems probable that infection in living fish is kept down by continuous secretion and sloughing of slime, which act as good mediums for bacterial growth.

From results shown in Tables 6 and 7, it can be determined that there is no isolation of primary pathogens, although some Entrobacteriaceae are present. The presence of this bacteria may be due to the waterbird population in the area and the previously mentioned fishing activity.

Fish and shellfish do not naturally carry wide varieties of pathogens. Furthermore, those pathogens contaminating marketed fish products are normally derived from the environment, chiefly, pollution of the water in which they live and improper handling after the fish were removed from their natural habitat.

The flora of fish are influenced quantitatively and qualitatively by both environment and season and reflect the flora of the water in which they are caught. If the water is polluted, the fish will almost certainly be polluted as well.

V. APPENDIX

A. Parasites Transmitted to Man through Fish

Numerous fish parasites are harmless to man, being digested when eaten with their host fish. However, some species of parasites in their larval stages encyst in the muscles and viscera of fish (encysted metacercariae of trematodes and plerocercoid of cestodes) and are capable of maturation to adult stages in the alimentary canal of man and other animals. Examples of several of these parasites are presented below.

Clonorchis (opisthorchis) sinensis (its cercariae) infest in fish flesh. This encyst (metacercariae) will infect man. They live in bile ducts and causing cirrhosis or cancer of the biliary tract. In Southern China, human clonorchiasis reached to 40% in some villages and in rural South Korea, recent surveys show 11% of school children infected, with even higher prevalence in adults (Rim, 1982).

Other species, *Opisthorchis viverrini*, infects 25% to 46% of village populations in Northeast Thailand (Sadun, 1955). Belov and Feiginova (1969) stated that acute opisthorchiasis is divided into latent and clinical symptoms of varying degree with typhoid-like variant, hepatocholangitic, and a gastro-enterochoelic variant. Also, they added that chronic opisthorchiasis is associated with cholangitis, cholangiocholecystitis, cholangiohepatitis, hepato-pancreatitis and cholangitic cirrhosis of the liver. Symptoms of a third category designated residual (post-opisthorchiasis) have also been described, including infection of bile ducts, biliary peritonitis, and primary liver cancer.

Heterophyid are other trematodes commonly found in the intestinal tract of man and transmitted by ingestion of fish flesh infested with encysted metacercariae. Seo et al., (1981) found 8 people to be infected with *Heterophyid* eggs in their feces. However, no symptoms of the infection were observed. Concerning *Heterophyes* infection in Egypt, Khalil and Abdel-Baki (1966) reported inflammation and ulceration of the intestinal mucosa resulting in abdominal colic with mucous and blood together with diarrhea. Moreover, they stated that deeply embedded worms infiltrate ova into the general blood circulation through lymphatics, where egg emboli are arrested in capillaries in the heart, brain and elsewhere, including tissue reaction.

B. Bacteria Transmitted to Man from Fish

i. Aeromonas hydrophila

a. Infections in human

Although *Aeromonas hydrophila* is not considered a normal inhabitant of the human gastrointestinal tract and less than 1% of healthy adults carry aeromonads in their guts (von Graevenitz and Mensch, 1968), the role of *Aeromonas hydrophila* in humans is receiving a great deal of attention. Many strains of *Aeromonas hydrophila* have been found to be responsible for septic infections, diarrhea, corneal ulcers, skin infections, wound infections (George et al., 1985), meningitis and fatal infections in compromised cancer patients (Heckerling et al., 1983; Karam et al., 1983) or as a result of AIDS or other chronic diseases (Flynn and Knepp, 1987).

b. Infections in fish

Ventura et al. (1988) described two types of *Aeromonas hydrophila* infection. One caused systemic infection in which *Aeromonas hydrophila* was isolated in the skin and subjacent muscles only. The other type caused cutaneous lesions. These researchers added that biochemical characteristics of the bacteria isolates and source of the fish were not consistently different in cutaneous and systemic infections. Skin lesions in fish with systemic and cutaneous infections were similar.

Soliman et al. (1989) reported that infection of striped mullet (*Mugil cephalus*) with *Aeromonas hydrophila* resulted in an acute septicaemia. This disease is characterized by early inflammatory and proliferative changes and later by narcotic changes in different organs. This study documented that enteritis and hepatic necrosis were consistently found, but surface lesions were not pathognomic for these infections in mullet.

c. Pathogenicity of *Aeromonas hydrophila*

Pathogenicity of *Aeromonas hydrophila* may be attributed to its toxigenic properties. The production of these toxins varies among *Aeromonas hydrophila* isolates. A number of potential factors may influence the virulence of the organism, including enterotoxins, cytotoxin, hemolysins, proteases, lipases, and leucocidins (Gloriose, 1974; Trust *et al.*, 1979; Allan *et al.*, 1981 and Thune *et al.*, 1982a, 1982b).

However, Janda *et al.*, (1985) studied the virulence markers of *Aeromonas* species and found that there is no correlation between highly virulent *Aeromonas* isolates and phenotypes associated with enterotoxigenicity, haemolytic activity, and cytotoxin production.

d. Motile *Aeromona Septicaemis* in Egypt

Motile *Aeromonas Septicaemis* (MAS) caused by *Aeromonas hydrophila* was reported for the first time in Egypt among catfish (*Clarias lazera*) by Amin and Eisa (1982). The disease caused considerable losses in both private and governmental fish farms as well as hatcheries in Upper and Lower Egypt (Ahmed, 1983; Enany, 1983; Amin *et al.*, 1985).

The clinical signs reported by Ahmed (1983) from observations on *Tilapia nilotica* naturally infected with *Aeromonas hydrophila* included redness of the skin, especially at the ventral part of the abdomen, also extending to the caudal area including the anal orifice. The skin was dark in color with roughness of scale and ulcer formation. All visceral organs were congested with slight abdominal distension and there was an accumulation of small amounts of sanguinous fluid in the peritoneal cavity. In fish that survived for one week post-inoculation, their intestines were congested and filled with a pink, yellowish mucous. The liver was enlarged with minute hemorrhages and multiple necrotic foci. The kidney appeared hyperemic and the spleen was enlarged. Most of these symptoms and post-mortem lesions were noticed in naturally infected carp by Enany (1983) and in *Tilapia nilotica* inoculated with *Aeromonas hydrophila* (Soliman, 1984).

From observations on *Tilapia nilotica* raised in ponds in Upper Egypt and naturally infected with *Aeromonas hydrophila*, Amin *et al.*, (1985) reported two main forms of MAS, acute and chronic. The acute form was characterized by redness and inflammation of the skin and membranous portion of the fins, marked abdominal distension and bilateral exophthalmia. The chronic form was marked by various haemorrhagic patches all over the body, dark discoloration of the skin, together with roughness of the scales and their detachment from some part of the body. Not uncommonly, ulceration of the epidermis and slight abdominal distention were observed.

Faisal *et al.*, (1989) reported that in Egypt the bacterium was responsible for losses between 10% and 70% among farmed *Tilapia*. The LD₅₀ of 17 isolates ranged from 10³ to 10⁷ bacteria.

ii. *Klebsiella*

Klebsiella species were found to be opportunistic pathogens. *Klebsiella* is widely distributed in nature, as it has been isolated from lakes, rivers, sea water, sewage and various waste water, drinking water, soil, and animals, including humans. (Siedler *et al.*, 1975; Campbell *et al.*, 1976; Knittel *et al.*, 1977; Brynhildsen *et al.*, 1988).

Nabilah Mohamed (1975) examined 265 fish, especially the *Tilapia* species, and isolated 231 bacterial strains including *Klebsiella* sp. from the intestine.

Knittel *et al.*, (1977) concluded that water polluted with botanical material served as a potential reservoir for multiplication of the opportunistic *Klebsiella* pathogens.

a. Morphological and Biochemical Characters

Klebsiella colonies appear pink to red on the agar surface within 24 hours of incubation at 37° C. On Rimler-Schotts (1973) medium colonies were greenish yellow to green. They showed the following biochemical reactions: are indole negative (Krieg and Holt 1984), do not utilize citrate or produce H₂S, and are urease negative.

iii. *Salmonella*

In man, all forms of salmonella infection enter via the oral route and may produce either clinical or subclinical infection. Salmonellae may produce three main types of disease, but mixed forms are frequent.

a. The enteric fever [*S. typhia* (typhoid), *S. paratyphi*, *S. schottmulllesi*, etc. (paratyphoid)]

Organisms ingested with contaminated food or drink reach the small intestine and enter the intestinal lymphatics. These ingested organisms then travel via the thoracic duct into the blood stream and are disseminated into many organs including the intestines where organisms multiply in lymphoid tissue and are excreted in the stool.

The outstanding lesions are hyperplasia and necrosis of lymphoid tissue, focal necrosis in the liver and inflammation of the gall bladder.

b. Septicemias (*S. cholera suis*)

Early invasion of the blood stream follows infection by the oral route, although intestinal involvement is often absent. The organisms are widely disseminated and tend to cause focal suppuration, abscesses, meningitis, osteomyelitis, pneumonia and endocarditis, especially in debilitated hosts.

c. Gastroenteritis (*S. typhimurium*, *S. enteritidis* and *S. derby*)

Symptoms appear after only 1 to 3 days incubation which suggests that ingestion of a large number of organisms results in a violent irritation of mucous membranes. Usually invasion of the blood stream and dissemination of infection to other organs does not occur.

Fish became carriers when salmonella is excreted in feces of infected animals contaminating water from which fish contract this microorganism and transmit it to man without gaining any disease.

iv. *Shigella*

Shigella is obtained by fish from water which is contaminated with the feces of infected animals. Infected fish then act as a passive vector in the transmission of this microorganism to man.

In man, after a short incubation period (1 to 4 days), there is a sudden onset of abdominal pain, cramps, diarrhea and fever. Stools are liquid and contain mucus and blood after the first few movements. Their passage is accompanied by much straining and tenesmus. Spontaneous recovery generally occurs in a few days, but small children sometimes succumb to dehydration and acidosis. The disease caused by *Shigella dysenteriae* is particularly severe.

v. *Staphylococcus aureus*

In man, *Staphylococcus aureus* causes 1) abscess formation (localized and painful) which undergoes suppuration from the center, and 2) food poisoning due to its toxin. Food poisoning caused by *Staphylococcus aureus* has a short incubation period (1 to 8 hours), with symptoms of gastro-intestinal disturbance, nausea, vomiting and diarrhea with no fever.

In fish, the causative pathogen grows and produces high potent endotoxins in improperly processed fish meat. After ingestion, this meat can cause human disease due to poisoning rather than infection.

Three studies document that members of the genus *Staphylococcus* occasionally produce disease in fish (Shotts and Teska, 1989). Oghondeminu and Okaeme (1986) isolated *staphylococcus* sp. from African aquaculture pond water. In Egypt, Akealah (1987), Laila et al., (1986) and Nabilah (1975), isolated and identified *Staphylococcus aureus* and *E. coli* from intestinal tract of *Tilapia* species.

vi. *Streptococcus faecalis*

Streptococcus faecalis can cause food poisoning in man which is characterized by gastro-intestinal disturbances, such as nausea, vomiting, abdominal pain and diarrhea. Although no transmission of *streptococcus* sp. from fish to man has been documented, the potential for human infection does exist among individuals who handle diseased fish. Many fish isolates are lancifield group B and D serotypes (Shotts and Tesha, 1989). Outbreaks produced by members of the genus *streptococcus* among fresh water and salt water fish in the USA and Japan were reported by Frerichs and Roberts (1989). In Egypt, Laila et al., (1986) and Hefnawy et al., (1989) isolated *Staph. faecalis* from

intestinal tract of *Tilapia* sp. Lesions were not specific and appeared as raised inflamed areas behind the dorsal fin extending ventrally, hemorrhage around the anus, congestion in the intestine, swollen kidneys and congestion of the liver.

vii. *Yersinia enterocolotica*

In last few years, *Yersinia enterocolotica* have been described as a new pathogen causing enteritis mainly in children. This disease is characterized by fever, diarrhea, abdominal pain, and sometimes vomiting. *Yersinia enterocolotica* also causes septicaemia, erythema nodosum, polyarthritis, and other diseases in adults.

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**(6.b) Microbiological and Parasitological Surveys of Fish at
Alexandria: El-Max and Abokir**

Third Annual Report

Principal Investigator: Dr. Mohi S. Easa

Cairo University, Egypt

SUMMARY

MICROBIOLOGICAL AND PARASITOLOGICAL ASPECTS

Alexandria Area

a. El-Max

b. Abokir

Section A. - Results of parasitological Examination of Muscle of Tilapia, Mugil, Siganus and Sharagesh Species

Two parasitic species found in the fish of El Max and Abokir are *Pygidiopsis genata* and *Haplorchis pumilio*. El Max had a higher incidence of heterophyids which may be attributed to its high contamination of human sewage. The heterophyid metacercariae were mainly located in the superficial muscle of the head region, and less often in the tail region. Depending on upon the trematode species involved, some preference of location within the muscle of the fish host was observed, however it is difficult to differentiate which species of parasite was present by this method. The presence of the heterophyid metacercariae in the muscles causes concern as it presents a hazard to man when improperly cooked fish is eaten.

Fish who hosted these parasites had inflammatory reactions associated with abnormal pigmentation at the site of metacercariae. The pigmentation may be a result of the metabolic products released from the developing metacercariae, which stimulate the surrounding host muscle tissue to produce pigmentation (melanin). This also explains the presence of pigment around old metacercariae and its absence surrounding the young parasites.

Morphological changes of *P. genata* and *H. pumilio* generated in this study are similar to those mentioned by Yamaguti, 1971. Tables 1 and 2 indicate monthly changes in the prevalence of *P. genata* and *H. pumilio*. This correlates with the higher incidence of infection during the warmer months, and warmer waters. Higher temperature in water can also be attributed to contamination of human sewage. Snails, the intermediate hosts to these parasites, flourish in warmer waters, which allows for more *P. genata* and *H. pumilio*. The snail population, snail species and nutrients in the water found in El Max and Abokir explains the incidence of the two types of heterophyids, and the lack of *H. heterophyes*.

Part B - Results of Histopathological examination of the intestine of laboratory animals Histopathological studies of lesions in the intestine of lab animals (mice, puppies, and cats)

The parasites were identified after the lab animals (puppies, cats and mice), were orally inoculated via stomach tube with 200-300 viable encysted metacercariae. Animals were sacrificed after 3, 7 or 10 days respectively, after examination of their feces, for ova and mature parasites. Fixation and staining tests were carried out on worms obtained from the small intestine of the lab animals.

Adult parasites were discovered in the intestinal lumen or attached to its wall 7-10 days after inoculation of the animals. They caused destruction of intestinal mucosa and sloughing of their epithelium. Necrotic debris, mucus, connective tissue and numerous inflammatory cells replaced the affected epithelium.

Fig (1 & 2).

In most cases the parasites invaded the intestinal wall and appeared between intestinal glands Fig (3). Mild tissue reaction and excessive metaplastic proliferation of intestinal epithelium to mucus cells were noticed. Changes in the intestinal crypts were characterized by hyperplasia of mucus glands, connective tissue proliferation, aggregation of numerous inflammatory cells, and a few melanin carrying cells.

Early stages of the parasite reaction were characterized by vascular dilation, hyperplasia and hypertrophy of mucus cells. Excessive accumulation of tissue debris over the mucosa of the lower intestine was also observed. After ten days of moderate tissue reaction characteristics included the atrophy and decrease in number of mucous glands in the area of parasitic elements, fibroblastic proliferation which appeared to replace the intestinal mucosa, numerous inflammatory cells - mainly macrophages, lymphocytes and small eosinophilic cells. Point of which the parasite entered the intestinal crypts revealed destruction of the mucosa and edema. When the intestinal wall had a significant number of parasitic symptoms, more than one species of heterophyids were detected Fig (4).

The histopathological changes observed within the intestine wall of the lab animals fed fish metacercariae explain the clinical lesions developed in mammals and human beings who ate undercooked or raw fish contaminated with transmissible parasitic cysts.

Part C - Results of Bacteriological Examination

A wide variety in bacterial count exist between El Max and Abokir. This variation may be attributed to the salinity of Abokir and/or the high human sewage found in El Max. The high contamination of El Max may decrease the die-off of bacteria.

El Max revealed a high Aerobic Plate Count (APC), Coliform count/cm² of fish surface and grams of muscle in their fish population. (Tables 1 & 2). For Abokir, APC/ cm² and APC/gm of muscle coliform counts are lower than El Max. (Tables 3 & 4). Staphylococci count isolated in fish of El Max were higher (Table 5 & 6) when compared to the fish of Abokir (Table 7 & 8). *Staphylococci epidermides* were usually greater in number than that of *Staphylococci Saprephyticus*.

Bacteria were isolated from dead fish obtained from fishermen from El Max and Abokir. From Tilapia, Mugil, Siganus and Sharagesh species, bacteria was isolated from the surface of the fish and from the muscle, liver, kidneys and intestine, which is due to bacteria invading the muscle and other organs from the intestine after the death of the fish. Tables 1-15 reveal that the total number of bacteria was consistently higher in species from El Max as compared to Abokir. Higher numbers of isolates coincided with higher numbers of isolates of enterobacteriaceae from fish of El Max than Abokir (Tables 11-14). This is believed to be a result of the human sewage contamination of El Max.

A- Results of Parasitological examination of Muscle of Tilapia, Mugil, Siganus and Sharagesh Sp:

The parasites were identified after experimental inoculation in Lab animals (puppies, cats and mice), orally via stomach tube with 200 - 300 viable encysted metacercariae.

Animals were sacrificed after 3, 7, and 10 days respectively and after examination of their faeces, for ova and mature parasite. Sometimes the worms were obtained from small intestine of the laboratory animals. Fixation and staining of the worms were carried out.

Only two Sp. of heterophyids were identified From El-max and Abokir the prevalence of two parasites was comparatively high at El-Max. (Table 1) than at Abokir (Table 2) this may be as a result of high pollution of max with human sewage.

Heterophyid metacercariae were seen mainly in the muscle show site preference in the fish host depending upon the trematode Sp. involved. However, it is difficult to use this suggestion to differentiate Sp. of the parasites even within the same host species. In the present study the metacercariae of *P. genata* and *H. Pumilio* were mainly seen in superficial muscle, especially in the head region and less frequently in the tail. The presence of these metacercariae mainly in the muscle is of considerable importance and presents a hazard for man when improperly cooked fish is eaten especially in Tilapia and Mugil Sp.

The inflammatory reaction in fish is associated with abnormal pigmentation at the site of metacercariae, pigments may be as a result of metabolic products of the developing metacercariae stimulating the surrounding tissue to produce pigmented substance (melanin). This may explain the presence of the pigments around old metacercariae and their absence in area

of the young ones. The morphological characters of *P. genata* and *H. pumilio*. in this study are similar to those of mentioned (Yamaguti, 1971) the monthly changes of the prevalence of metacercariae of *P. genata* and *Hablorchis pamelio* in fish explain the high incidence of infection during warm months (High water temperature). Coincided with time of emergence of cercariae from snail vectors which is usually affected with temperature of environment. Moreover in high temperature the water is more liable to be contaminated with human sewage or of the reservoir hosts- this agrees with the view of some authors that the snail intermediate host which is flourished better in warm water.

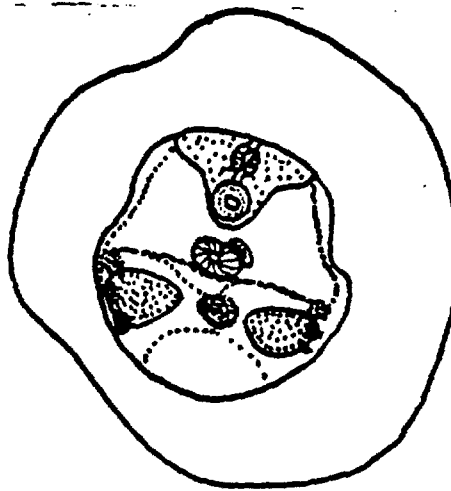
Two types of heteroplyids only identified from El-Max and Abokir, can explain that is due to snail population, species of snail (no *Pironella conica* ----> No *H. heterophyes*), variation in nutrient content of water.

Table 2: Prevalence of fish infection with Heterophid (*Pygidiopsis genata* and *Haplorchis pumilio*) metacercari in Abokir.

Sample Time	No. of ex. fish	Mugil Sp.		No. of ex. fish	Siganus Sp.		No. of ex. fish	Shargesh Sp.	
		P. g	H. P		P. g	H. P		P. g	H. P
October 1994	274	170	27	54	10	8	3	1	0.0
November 1994	69	42	0.0	34	17	14	10	3	0.0
December 1994	99	23	0.0	10	1	0.0	6	0.0	0.0
Total	442	235	27	98	28	2.2	19	4	0.0
% infected		53.2	6.1		28.6	22.0		21.1	0.0

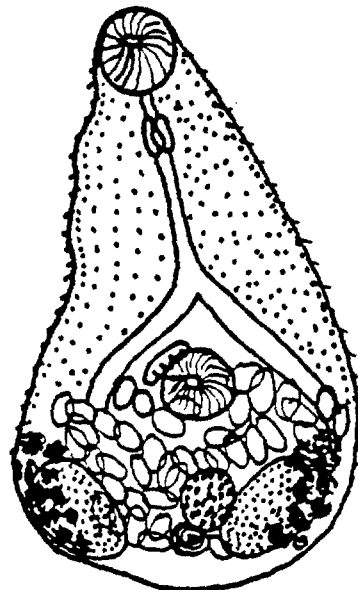
Table 1: Prevalence of fish infection with Heterophyid (*Pygidiopsis genata* and *Haplorchis pumilio*) in El-Mox

Sample Time	No. of Exam. fish	Tilapia Sp.		No. of ex. fish	Mugil Sp.		No. of ex. fish	Siganus Sp.		No. of ex. fish	Shargesh Sp.	
		P. g	H. P		P. g	H. P		P. g	H. P		P. g	H. P
October 1994	151	85	12	161	126	18	58	25	8	36	13	4
Novamner 1994	97	2	0.0	169	47	0.0	94	10	0	47	5	0.0
December 1994	100	8	0.0	150	13	4	10	0.0	0.0	10	2	0.0
Total	247	95	12	490	186	22	162	3.5	8	93	20	4
% infected		38.5	4.9		37.9	4.5		21.6	5.0		21.5	4.3



0.1 mm.

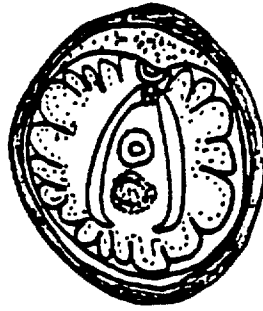
Pygidiopsis metacercariae.



0.1 mm.

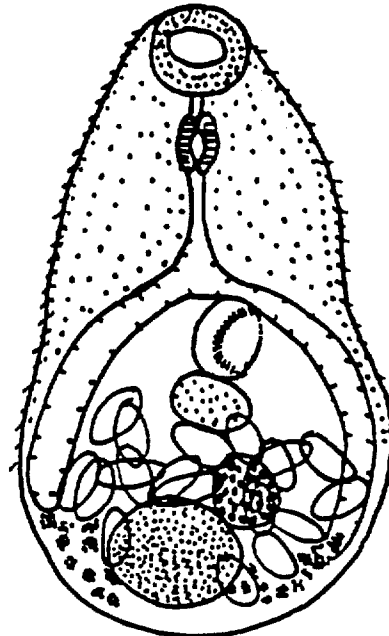
Pygidiopsis genata.

0.5 mm.



Haplorchid.

0.1 mm.



- Haplorchis pumilio.

B - Results of Histopathological examination of intestine of lab. animals:

Histopathological studies of Lesions in the intestine of laboratory animals (mice, puppies and cats):

Mature worms were developed in intestinal tract of laboratory animals. Adult parasites were seen in the intestinal lumen or attached to its wall 7-10 day after inoculation of metacercariae. Parasites caused destruction of the intestinal mucosa and sloughing of their epithelium. Epithelium replaced by necrotic debris, mucus, connective tissue and numerous inflammatory cells Fig. (1 & 2).

In most examined cases the parasites invaded the intestinal wall and appeared between intestinal glands Fig. (3). Mild tissue reaction and excessive metaplastic proliferation of intestinal epithelium to mucous cells were determined. The reactive changes were seen mainly in the intestinal crypts characterized by hyperplasia of mucous glands, connective tissue proliferation, aggregation of numerous inflammatory cells mainly Lymphocytes and macrophages. Few numbers of melanin carrying cells also seen.

At early stages of development of the parasite reaction of the intestine was characterized by vascular dilation, hyperplasia and hypertrophy of mucous cells. Excessive accumulation of tissue debris over the mucosa of the lower intestine was also seen.

In lab. animals that sacrificed after 10 days moderate tissue reactions were noticed characterized by atrophy and decrease of number of mucous glands in area of parasitic elements, fibroblastic proliferation which appeared replace the intestinal mucosa, numerous inflammatory cells mainly macrophages, Lymphocytes, and small size eosinophilic cells. Point of entrance of the parasite in intestinal crypts revealed destruction of the mucosa and edema. The infestation of the intestinal wall with great number of the par-

asitic elements, more than one species of the parasite can be detected (*P. genata* and *H. pamilio*). Fig (4).

The histopathological changes in the intestinal wall of lab. animal fed on fish metacercariae explain the clinical lesions developed in mammals and human being eat undercooked or raw fish, handle or gut the fish containing transmissible parasitic cysts.



Fig. 1: Histological section in the intestine of dog infected with metacercariae, Showing: mature parasite (*H. pumilio*) invade intestinal crypt lead to destruction of mucosa, proliferation of fibroblasts and great number of inflammatory cells aggregation. Stain: H & E. (10 x 10).



Fig. 2: High power of Fig. 1- Showing internal structure of parasite and its ovary that containing unstained characteristic parasitic eggs Stain: H & E (12,5 x 40) .

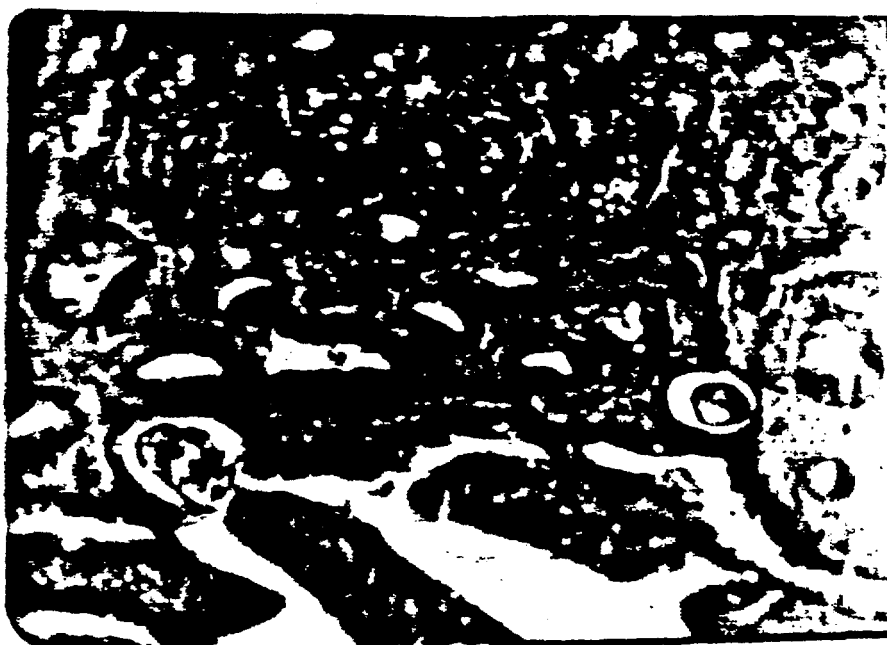


Fig. 3: Section in intestine of cat infected with fish flesh metacercariae showing: early invasion of the intestinal crypts with mature parasite. Other parasites were deeply located in intestinal wall (*P. genata*) Stain: H & E. (10 x 10).



Fig. 4 : Section in the intestinal wall of cat experimentally infected with fish flesh metacercariae. showing: Inflammatory cells around parasitic elements (right - *H. pamilio*, left - *P. geuata*). Stain : H & E. (12,5 x 25)

C- Results of Bacteriological examination:

Table (1 , 2) Revealed high Aerobic Plate Count (APC) and coliform count/ Cm^2 of fish surface and gm. of muscle in El-max area while in Abokir area the APC/ cm^2 and gm of muscle and coliform count (Table 3 , 4) are low.

This wide range variation in bacterial count between the two areas may be due to contamination of water with sewage or high salinity in Abokir area. The high contamination of water with human Sewage and Freshness of water In El-Max may decrease the die-off of bacteria.

Staphylococci isolates from El-max region were higher (22 isolates, Table 5 , 6) than that isolated from Abokir area (13 isolates, table 7, 8). *Staphylococci epidermidis* were usually greater in number than that *Staphylococci saprophyticus*. This may be related to the high degree of pollution in El-Max than that in Abokir area.

Bacteria were isolated from dead fish obtained from fishermen, at El-Max and Abokir areas. So isolation of bacteria were marked from the muscle and visceral organs of fish in both areas.

Bacteria were isolated from surface of the fish (Tilapia, Mugil, Siganus and Sharagesh Sp.) and from Muscle, Liver, Kidneys and intestine in high accounts. This is due to bacteria after fish deaths may invade the muscle and other organs from intestine. Results achieved from table 1-15 revealed that the total number of bacterial isolates from El-Max area were higher if compared with that isolated from Abokir area. This may be due to higher contamination of El-Max area with Sewage. This result coincided with higher number of isolates of enterobacteriaceae from fish of El-Max area (53 isolates table 11, 12) than that of Abokir area (28 isolates, table 13 and 14).

Table (1): Results of Aerobic plate count in examined fishes from El-Max region.

Fish species	Surface APC/cm²	Muscle APC/gm
Mugil Sp.	7×10^3	2×10^2
El-Sharagesh	7×10^3	1×10^2
Siganus Sp.	8×10^3	5×10
Tilapia Sp.	1×10^4	2×10^2

No. of examined fish samples were 10 fish of each Sp.

Table (2): Results of Celiform count in examined fishes from El-Max region.

Fish species	Surface Coliform/cm²	Muscle Colif. /gm
Mugil Sp.	70	9
El-Sharagesh	40	6
Siganus Sp.	30	5
Tilapia Sp.	90	20

No. of examined fish samples were 10 fish of each Sp.

Table (3): Results of Aerobic plate count in the examined fish from Abokir region .

Fish species	Surface APC/cm²	Muscle APC/gm
Mugil Sp.	4 x 10³	3 x 10
El-Sharagesh	2 x 10³	2 x 10
Siganus Sp.	1 x 10³	1 x 10²

No. of examined fish samples were 10 fish of each Sp.

Table (4): Results of Celiform count in the examined fish from Abokir region .

Fish species	Surface Colif-orm/cm²	Muscle Coliform/gm
Mugil Sp.	40	7
El-Sharagesh	30	4
Siganus Sp.	20	3

No. of examined fish samples were 10 fish of each Sp.

Table (5): Staphylococci isolated from examined fishes from El-Max region .

Fish species	Total No. of isolates	Stoph. epi-dermidis	Stoph. sapro-phyticus
Mugil Sp.	3	0	3
El-Sharagesh	6	6	0
Siganus Sp.	9	7	2
Tilapia Sp.	4	4	0
Total	22	17	5

No. of examined fish samples were 10 fish of each Sp.

Table (6): Staphylococci isolated from different parts of the examined fishes from El-Max region .

Site of isolation	Total No. of isolates	Stoph. epi-dermidis	Stoph. sapro-phyticus
Surface	11	9	2
Liver	0	0	0
Kidney	2	2	0
Intestine	9	6	3
Total	22	17	5

No. of examined fishes 10 of each Sp.

Table (7): Staphylococci isolated from examined fishes from Abokir region .

Fish species	Total No. of isolates	Stoph. epidermidis	Stoph. saprophyticus
Mugil Sp.	8	6	2
El-Sharagesh	0	0	0
Siganus Sp.	5	1	4
Total	13	7	6

No. of examined fishes 10 of each Sp.

Table (8): Staphylococci isolated from different parts of the examined fishes from Abokir region .

Site of isolation	Total No. of isolates	Stoph. epidermidis	Stoph. saprophyticus
Surface	9	5	4
Liver	0	0	0
Kidney	1	0	1
Intestine	3	2	1
Total	13	7	6

No. of examined fishes 10 of each Sp.

Table (9): Staphylococci isolated from examined fishes from Abokir region .

Fish species	El. Max region		Abokir region		Total
	Staph. epi- dermidis	Staph. sap- rophyticus	Staph. epi- dermidis	Staph. sap- rophyticus	
Mugil Sp.	0	3	6	2	11
El-Sharagesh	6	0	0	0	6
Siganus Sp.	7	2	1	4	14
Tilapia Sp.	4	0	ND	ND	4
Total	17	5	7	6	35

No. of examined fishes were 10 of each Sp.
ND Not done

Table (10): Staphylococci isolated from different parts of the examined fishes from Abokir region .

Site of isolation	El. Max region		Abokir region		Total
	Staph. epi- dermidis	Staph. sap- rophyticus	Staph. epi- dermidis	Staph. sap- rophyticus	
Surface	9	2	5	4	20
Liver	0	0	0	0	0
Kidney	2	0	0	1	3
Intestine	6	3	2	1	12
Total	17	5	7	6	35

No. of examined fishes were 10 of each Sp.

Table (11): Enterobacteriaceae isolated from examined fishes from El-Max region .

Fish species	Total No. of isolates	Shigella dysenteriae	E. coli	Proteus vulgaris	Klebsiella pneumoniae	Citrobacter freundii
Mugil Sp.	12	0	7	0	4	1
El-Sharagesh	15	0	9	2	1	3
Siganus Sp.	9	1	4	2	2	0
Tilapia Sp.	17	0	10	1	4	2
Total	53	1	30	5	11	6

No. of examined fishes were 10 of each Sp.

Table (12): Enterobacteriaceae isolated from different parts from the examined fishes from El-Max region .

Site of isolation	Total No. of isolates	Shigella dysenteriae	E. coli	Proteus vulgaris	Klebsiella pneumoniae	Citrobacter freundii
Surface	23	1	14	2	4	2
Liver	3	0	3	0	0	0
Kidney	8	0	4	0	4	0
Intestine	19	0	9	3	3	4
Total	53	1	30	5	11	6

Table (13): Enterobacteriaceae isolated from examined fishes from Abokir region .

Fish species	Total No. of isolates	E. coli	Klebsiella pneumoniae	Citrobacter freundii	Enterobacter aerogenes
Mugil Sp.	7	4	0	1	2
El-Sharagesh	8	3	1	4	0
Siganus Sp.	13	7	2	1	3
Total	28	14	3	6	5

No. of examined fishes were 10 of each Sp.

Table (14): Enterobacteriaceae isolated from different parts from the examined fishes from Abokir region .

Fish species	Total No. of isolates	E. coli	Klebsiella pneumoniae	Citrobacter freundii	Enterobacter aerogenes
Surface	13	7	1	3	2
Liver	2	1	0	1	0
Kidney	4	1	0	1	2
Intestine	9	5	2	1	1
Total	28	14	3	6	5

Table (15): Other types of bacteria isolated from the examined fishes in Alexandria .

Fish species	Total No. of isolates	El-Max region		Abokir region
		Aeromonas hydrophila	Vibrio alginolyticus	Pseudomonas aeruginosa
Mugil Sp.	1	0	0	1
El-Sharagesh	1	0	1	0
Siganus Sp.	1	1	0	0
Total	3	1	1	1

*No. of ex. fish were 10 of each Sp.

*From table "16" we notice that one isolate of *Aeromonas hydrophila* and one isolate of *vibrio alginolyticus* were isolated from El-Sharagesh and siganus Sp. at El-Max area, while one isoalte of *Pseudemonas af-rugnosa* was isolated from Mugil Sp. at Abokir area, all of these three isolates are of human origin and usually causing gasters-intestinal disorders.

(6.c) Testing Marketed Fish in Israel for Different Microorganisms

Third Annual Report

Principal Investigator: Dr. Badri Fattal and Prof. Hillel Shuval

The Hebrew University of Jerusalem

Introduction

Fishponds are generally supplemented with animal manure and, in some farms, with wastewater. However, animal and untreated human wastes can be loaded with pathogenic bacterial and viral agents and their introduction into fishponds may pose a health hazard for fish handlers and consumers. The impact of microbiological loading of growth waters on the levels of contamination of various fish organs has been studied previously (Buras et al., 1987; Slabbert et al., 1989; and Fattal et al., 1992). It can be concluded from these studies that fish grown in contaminated waters may not be considered safe for human consumption.

Results:

Ten tilapia sp. and 2 Mugill (sp) (Mullet) were examined for E. coli, Aeromonas and Coliphages.

The results for Aeromonas in tilapia sp in different fish organs are presented in Figures 1 and 2.

Figure 1 shows the number of aeromonas colonies found in the digestive tract (D.T.), skin, liver, spleen and muscle, in each fish separately, while Figure 2 shows the log mean of the 10 tilapia fish. It can be seen that the highest concentration of aeromonas was found in the D.T (mean of 3.5 logs with std of ± 0.32). The lowest concentration was in the muscle (mean of 0.6 log). It is worth mentioning that the variations of aeromonas counts in the muscle ranged between 0 to more than 1 log. However, only 3 out of 10 fish were positive.

The results of coliphages for 2 mullets and 10 tilapia are as follows:

Mullets

Digestive tract (DT), spleen and liver were negative; muscle were positive (!) and the skin was positive in 1 out of 2 mullets.

Tilapia

DT	= 6 out of 10 were positive	(60%)
Muscle	= 3 out of 10 were positive	(30%)
Spleen	= 2 out of 10 were positive	(20%)
Liver	= 2 out of 10 were positive	(20%)
Skin	= 1 out of 10 was positive	(10%)

For technical reasons the results of E. coli are not reported.

Enteroviruses data are in process.

In the coming months we are planning to continue testing more fish from the open market for the above mentioned microorganisms and also to test some fish for chemical contaminants. At a later stage we shall analyze the data for public health implications and seafood safety guidelines.

Methods:

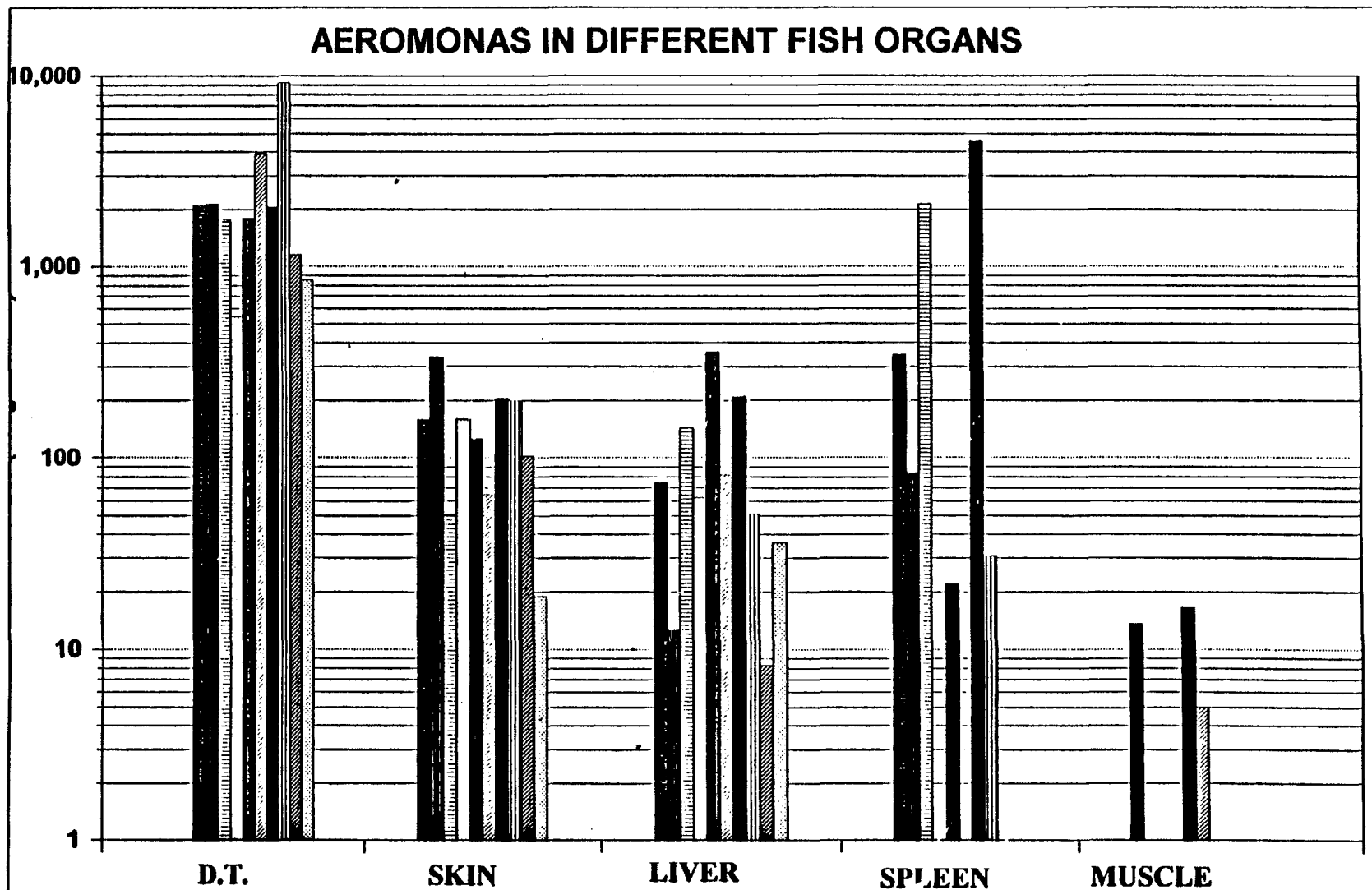
Ten tilapia sp. and 2 Mugill sp.(Mullet) freshly harvested fish (refrigerated with ice before marketing) were obtained from the main open market in Jerusalem.

The fish were dissected aseptically within 1 hour of arrival at the laboratory. Samples of the digestive tract, liver, spleen, skin and muscle were excised and homogenized in an Omni Mixer (Sorvall, Newton, CN, USA) with 0.01M PBS pH 7. The homogenates were analyzed for *E. coli* and *Aeromonas* on the same day and the remainder of the samples were stored at -20°C for the later of F⁺ coliphages and enteroviruses.

The levels of *E. coli* and *Aeromonas* in the various fish tissues were determined by the membrane filtration technique (Dufour et al. 1981; Bisson and Cabelli 1979). Briefly, blended samples were diluted in PBS and then passed through a 0.45 µm Millipore filter. Filters were then placed either on mTEC agar for the detection of *E. coli* or on M-*Aeromonas* agar. To enhance the recovery of *E. coli*, mTEC plates were incubated for 2h at 37°C and then transferred to a 44.5°C incubator. M-*Aeromonas* plates were incubated at 37°C. *E. coli* and *Aeromonas* colonies were counted after 16 - 24h incubation.

F⁺ coliphages were detected by the double-agar layer technique (Adams 1959). Briefly, 1ml of blended tissue sample was mixed with 3ml molten soft agar containing the bacterial host. The mixture was then poured onto a suitable bottom agar (Havelaar and Hogeboom 1984).

Enteroviruses were analyzed for digestive tract only. Blended samples were centrifuged at low speed and then seeded on confluent monolayers of BGMK cultures. After incubation for 1h at 37°C to allow adsorption, the liquid was withdrawn from the culture plates to prevent cytotoxicity. RPMI-1640 medium (5 ml) supplemented with 2% fetal calf serum was applied. The cultures were incubated at 37°C and observed daily for cytopathogenic effect (CPE). Samples showing the development of CPE were passed to freshly prepared BGMK cultures to ascertain that cell destruction was caused by viral infection.



¹
Figure 3. The Concentration of Aeromonas in Tilapia in Different Fish Organs
 (each represent one fish; CFU=Colony Forming Unit; DT=digestive tract)

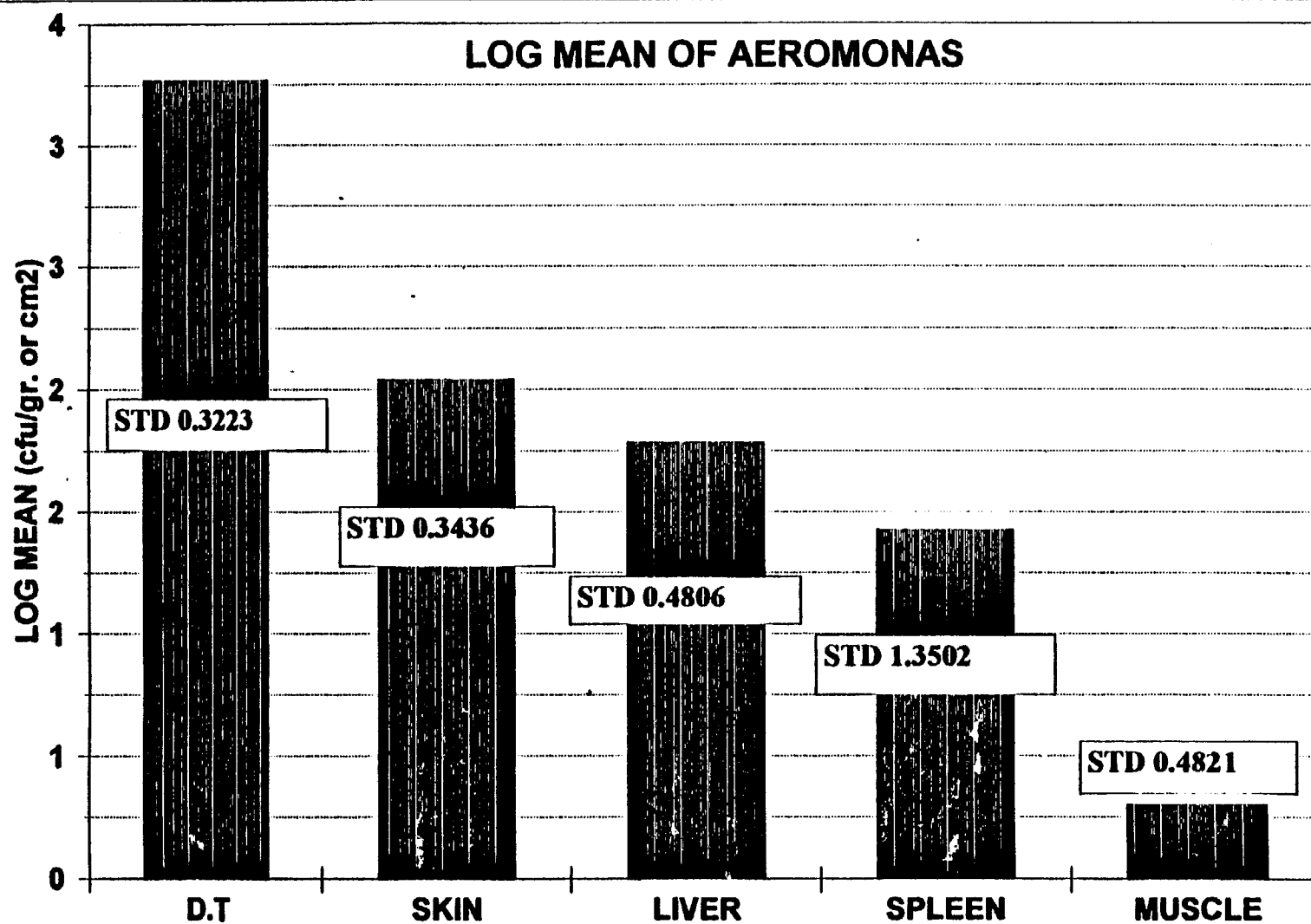


Figure 2. Log Mean Concentration of Aeromonas in Tilapia in Different Fish Organs (CFU=Colony Forming Unit; DT=Digestive tract)

**(6.d) Pathogenic Bacterial Indicators in Contaminated
and Decontaminated Fish**

Third Annual Report

Principal Investigator: Dr. M El-Samra

National Institute of Oceanography and Fisheries (Suez, Egypt)

PATHOGENIC BACTERIAL INDICATORS IN CONTAMINATED AND

DECONTAMINATED FISH:

**TOTAL AEROBIC BACTERIA, TOTAL COLIFORMS
AND FECAL COLIFORMS**

The main way of human expose to pathogenic micro-organisms is the consumption of contaminated water and food. The indicator micro-organisms, mainly bacteria such as total coliforms and fecal coliforms, which established for water quality (WHO, 1971, Jazrawi et al., 1988), have generally been used to evaluate the potential health hazards associated with the contaminated food and seafood (West and Coleman, 1986 and Daw et al., 1990).

OBJECTIVE:

The work in task 2:g of the project is devoted to make bacterial quantitative and qualitative assays of some microbial indicators in skin, gills and alimentary canal of fish.

EXPERIMENT:

Alive fish, *Tilapia nilotica*, were collected and acclimatized to in-door condition for one week. After then, the experiments were started by putting groups of active and healthy fish (each group consists of 5 fish) in glass tanks

containing raw sewage water. The initial total aerobic bacteria, total coliforms and fecal coliforms were quantified in the raw sewage water and in skin, gills and alimentary canal of fish. Contamination of fish by bacteria was measured also after 12 hours exposure to the raw sewage. The rest fish (alive, active) were transported into tanks containing clean brackish water and the bacteria counting was determined, thus elimination experiment was started. Static renewal of clean water was done every 48 hours. Total aerobic bacteria was determined using plate counting agar and the pour-plate technique. Total coliforms and fecal coliforms were quantified by the five-tube most probable number (MPN) technique using Mac-Conkey purple broth (APHA, 1985). The bacterial numbers were determined per cm² for external skin surface, and per gram for gills and alimentary canal.

RESULTS:

Figures (1-3) and Table (1) show the contamination of total aerobic bacteria (TAB), total coliforms (TC) (*Escherichia*, *Klebsiella*, *Entrobacter*, *Serratia* and *hafnia* species) and fecal coliforms (FC) (Fecal *Escherichia coli*) during 12 hours of exposure to the raw sewage and elimination of these bacteria during the next 180 hours.

TAB increased by 4.5, 41.6 and 4.1 fold over initial numbers in skin, gills and alimentary canal, respectively, during the contamination period, then reduced to about there initial numbers after 84 hours of elimination period. On the other hand, TC increased by 105, 65 and 38 fold while FC by 20, 42 and 13 fold over initial numbers in the corresponding organs during the contamination period, then decreased to less than the initial numbers after 180 hours of elimination period, except in gills where slightly higher values were recorded.

Further investigations is necessary to get precise and useful data in long term experiments, also the concentration and/or presence of pathogenic indicators bacteria (*Salmonella* sp., *Shigella*, *Campylobacter*, *Yersinia enterocolitica* and *Staphylococcus aureus*) are to be considered.

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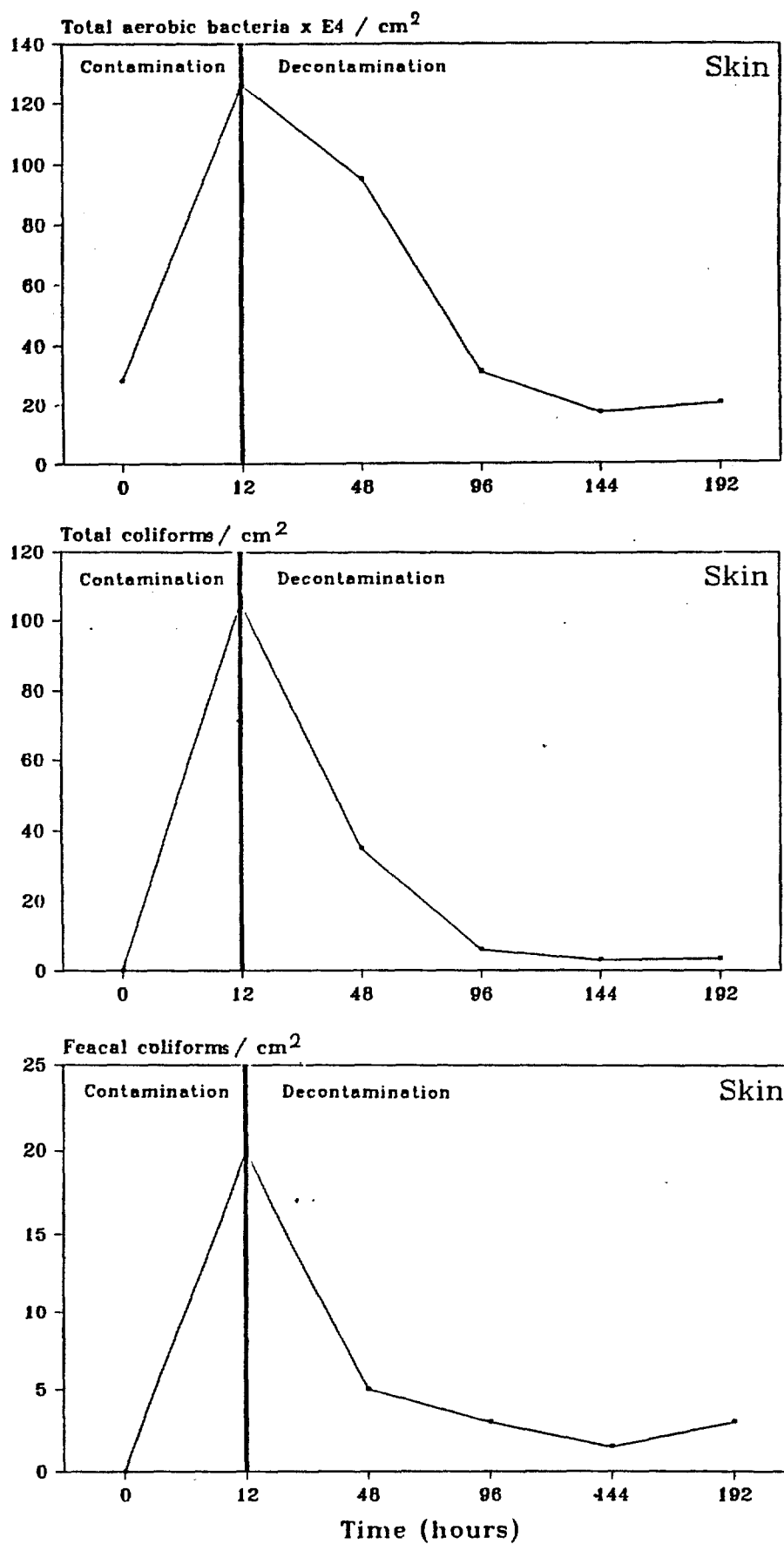


Fig. (1) Contamination and decontamination of bacteria

in skin of *Tilapia nilotica*

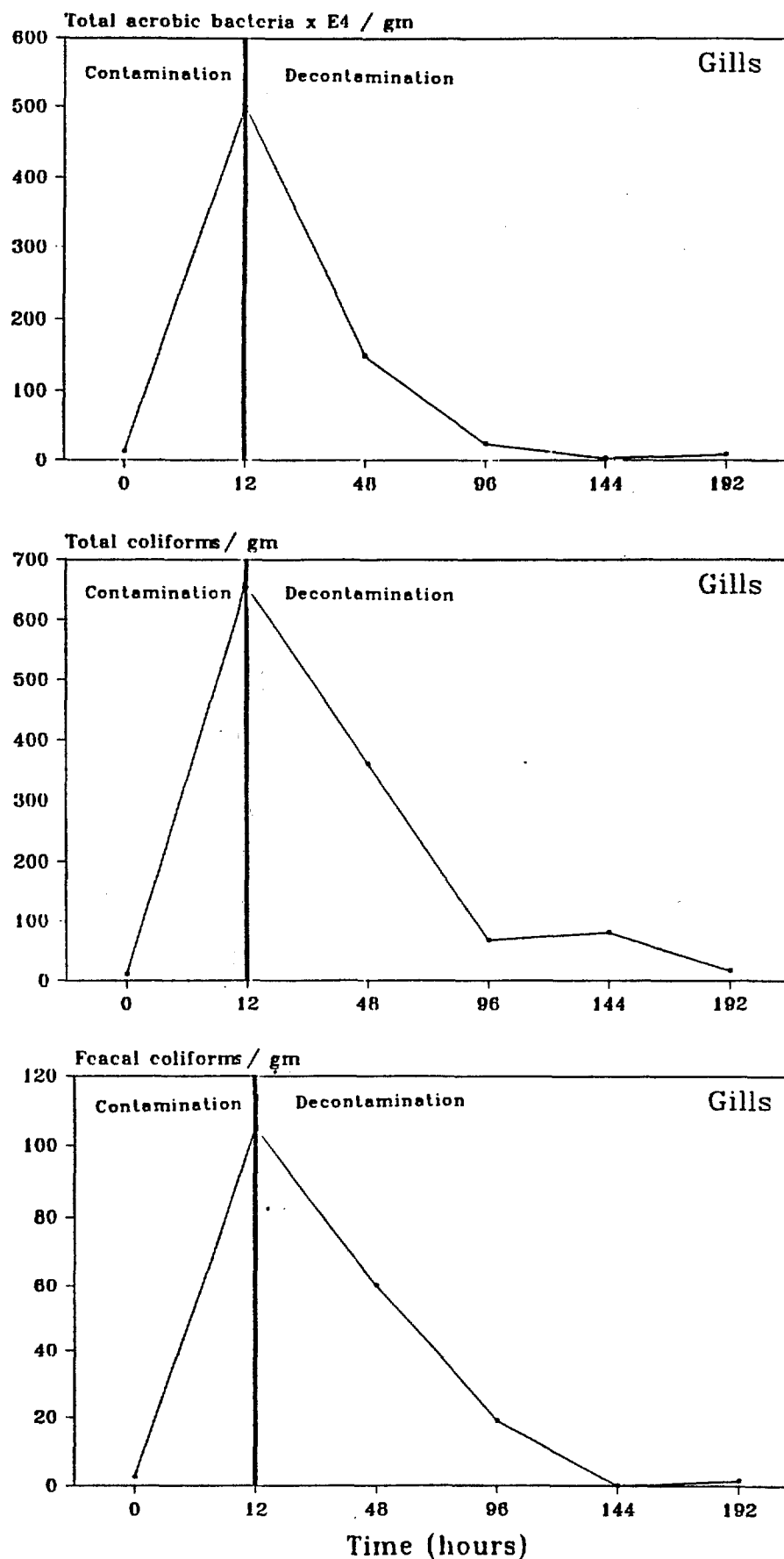


Fig. (2) Contamination and decontamination of bacteria

in gills of *Tilapia nilotica*

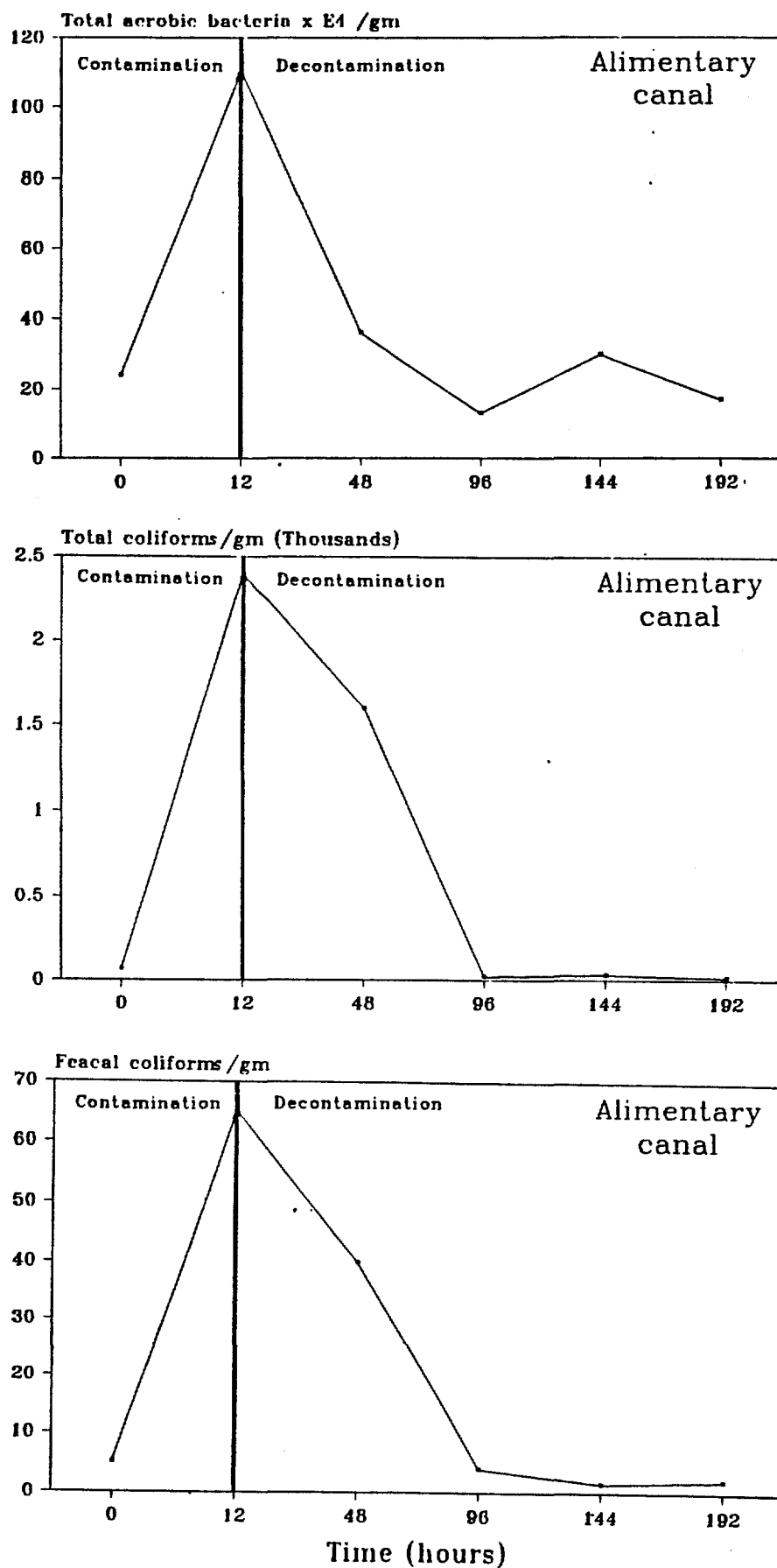


Fig (3) Contamination and decontamination of bacteria
in alimentary canal of *Tilapia nilotica*

Table (1): Contamination and decontamination by bacteria in organs of *Tilapia nilotica*

Time (hours)	Water			Skin			Gills			Alimentary canal		
	TAB	TC	FC	TAB	TC	FC	TAB	TC	FC	TAB	TC	FC
0	80×10^6	7500	210	28×10^4	0	0	12×10^4	10	2.5	24×10^4	63	5
12	—	—	—	126×10^4	105	20	500×10^4	657	105	110×10^4	2400	65
48	20×10^3	7	0	95×10^4	35	5	150×10^4	360	60	36×10^4	1600	40
96	1×10^3	3	0	31×10^4	6	3	24×10^4	67	19	13×10^4	21	4
144	1.5×10^3	0	0	17×10^4	3	1.5	3×10^4	80	0	30×10^4	32	1.5
192	1.1×10^3	0	0	20×10^4	3.5	3	8×10^4	17	1.5	17×10^4	15	2

TAB: Total aerobic bacteria

TC : Total coliform

FC : Fecal coliform

**(6.e) Survival of Viral and Bacterial Pathogens in Groundwater and
Wastewater as it Pertains to Seafood Safety**

Third Annual Report

Principal Investigators: Dr. Badri Fattal and Prof. Hillel Shuval

The Hebrew University of Jerusalem

Introduction

Hepatitis A Virus (HAV) is a major waterborne disease agent with worldwide distribution (see Naser 1994, a review of the literature on the prevalence and fate of HAV in water). The main transmission route of HAV is direct person-to-person contact. However, an outbreak of hepatitis caused by the consumption of raw oyster was described by Jean-Claude et al. (1991), and also by Portnoy et al. (1975). Shellfish can filter, accumulate, and retain viruses from surrounding waters. It is possible that shellfish concentrate the hepatitis virus from surrounding water in a manner similar to that observed for other enteroviruses (Goyal et al., 1979). Therefore, it is very important to study the die-off and the survival of HAV in waters in order to analyze the public health implication and seafood safety guidelines.

Materials and Methods

Four different water types were used. Raw wastewater samples were obtained from the wastewater treatment plant of Jerusalem after primary sedimentation; Sterile wastewater was prepared by autoclaving the raw wastewater for 30 min. at 121°C ; Groundwater samples were obtained from a well in Tira, a small town in the central part of Israel (this well is 55m deep and is used as drinking water); PBS 0.01M, pH- 7.2 served as a control.

One liter of each water type was seeded with various microorganisms to reach an initial titer of 10^6 cfu/ml *E. coli* and 10^4 - 10^5 pfu/ml of the viruses. After thorough mixing each sample was divided into 20ml portions in sterile plastic tubes. The tubes were kept, in the dark, at 4°C , 10°C , 20°C , 30°C and 37°C in covered water bath or incubator. Samples were removed at (0 time) and after 1, 5, 10, 20, 30, 60, and 90 days of incubation. The samples for *E. coli* were assayed on the same day and the remainder of the samples stored at -20°C for later virus assay.

Virus and virus-assays

Poliovirus, Lsc strain, was cultivated in Buffalo Green Monkey Kidney (BGMK) cultures. Enumeration of poliovirus 1 was performed according to Guttman-Bass and Nasser 1984.

Hepatitis A virus (HAV), a cell culture-adapted HM-175 strain, was obtained from M. D. Sobsey, the University of North Carolina, Chapel Hill, USA. HAV was cultivated in FRhK-4 cell. Enumeration of HAV was performed according to Cromeans et al.1987.

F⁺bacteriophages were concentrated from raw wastewater by the polyethylene glycol (PEG)-precipitation method (Lewis and Metcalf 1988). A specially constructed host bacterium, *Salmonella typhimurium* WG49, was obtained from L. Havelaar, Netherlands. The F⁺bacteriophages used in this study are an endogenous group which grows in *Salmonella typhimurium* WG49 host bacterium. Enumeration of F⁺bacteriophages was performed by the double-agar method (Adams, 1959).

E. coli strain resistant to ampicillin and streptomycin was received from V. Cabelli, Rhode Island University. Enumeration of *E. coli* was done by the membrane filtration method on mTEC agar containing ampicillin and streptomycin (Dufour et al.1981).

Results

The results of the survival of HAV, poliovirus1, F⁺ bacteriophages and E. coli in PBS solution, Wastewater (sterile and raw) and groundwater are summarized in figure 1 (A & B).

Figure 1A shows the die-off of these microorganisms after 30 days and Figure 1B - after 90 days incubation period at 10°C, 20°C and 30°C. Figure 2 shows the die-off coefficient $\log N_t/N_0 \times \text{day}$ of these microorganisms in PBS, groundwater and wastewater at temperatures of 4°C, 10°C, 20°C, 30°C and 37°C. It can be seen from these two figures that the die-off of HAV polio1 and F⁺bacteriophage were similar at 4°C in PBS, groundwater and wastewater. The die-off averaged from 0 to 1 \log_{10} after 3 months of incubation. The decay of E. coli was greater than those of the viruses and reached 2 to 6 \log_{10} .

In all water samples at 20°C, the decay of F⁺bacteriophages was lower than that observed for poliovirus 1 and HAV. The die-off of F⁺bacteriophages was incubation. No decay of E. coli was recorded at 20°C in PBS or groundwater, whereas in raw wastewater the E.coli die-off reached 3 \log_{10} .

The highest decay rates of the various microorganisms was recorded at 30°C and 37°C. After one month of incubation at 37°C, HAV and poliovirus 1 were undetectable in the various water samples. E. coli die-off was lowest in PBS and was similar to HAV and poliovirus 1 in raw wastewater and groundwater.

highest in groundwater and wastewater and reached about 2 \log_{10} after 2 months of

At 20°C the decay rates of HAV and poliovirus 1 were highest in wastewater.

The results presented in this study indicate that both HAV and poliovirus 1 persist in groundwater and wastewater for several months at lower temperatures, and that transmission of these pathogenic viruses during the winter months is highly possible. This may also explain some of the groundwater outbreaks caused by HAV (Graun 1985).

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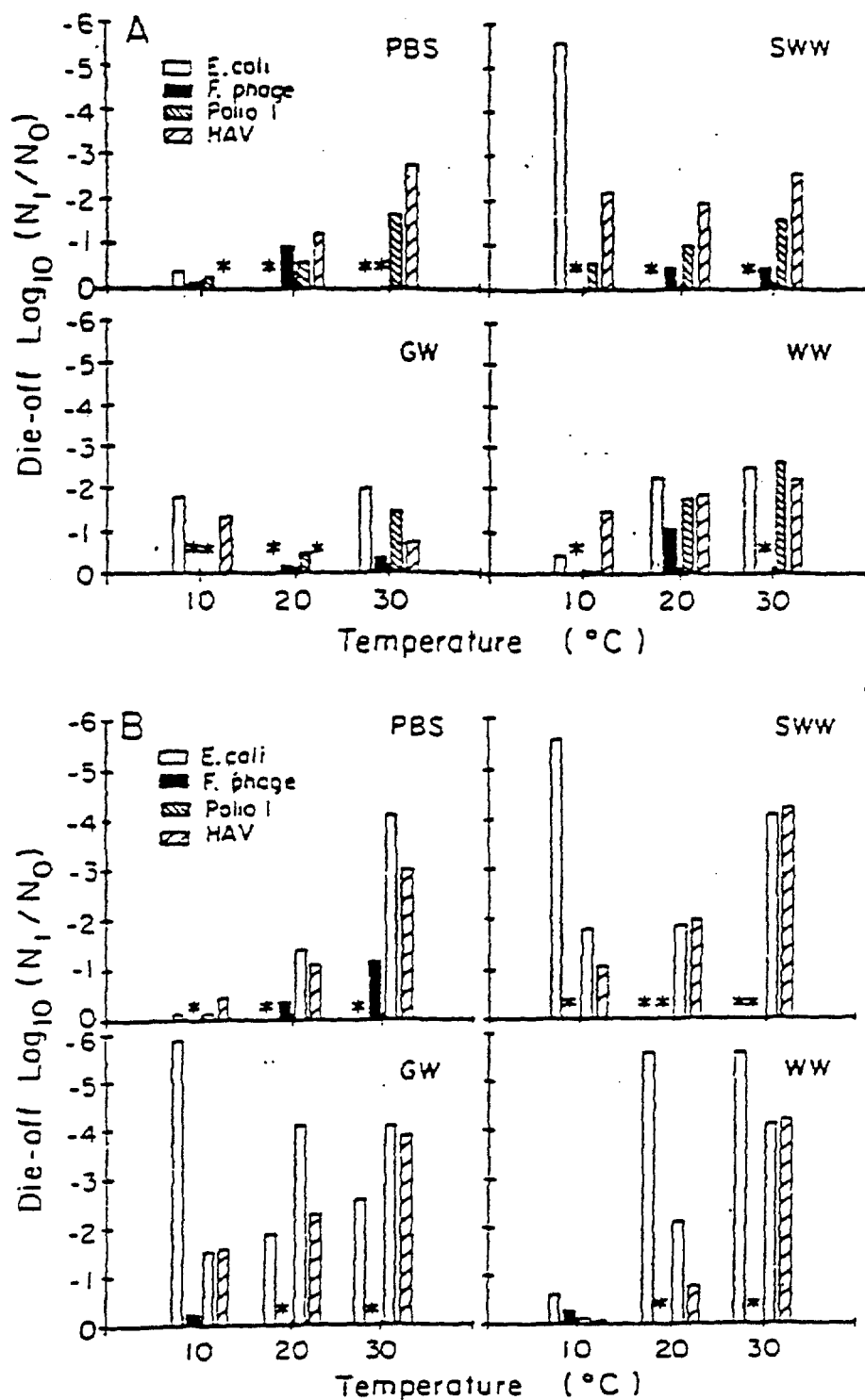


Figure 1. Die-off *E. coli*, F⁺ bacteriophages, Poliovirus 1 and Hepatitis A Virus (HAV) After 30 Days (A) and 90 Days (B) Incubation at 10°C, 20°C and 30°C in Phosphate Buffered Saline (PBS), Groundwater (GR), Sterile Wastewater (SWW) and Raw Wastewater (WW). (The initial titer of tested microorganisms was 10⁶ CFU/ml for *E. coli* and 10⁴-10⁵ PFU/ml for viruses; * No die-off was observed).

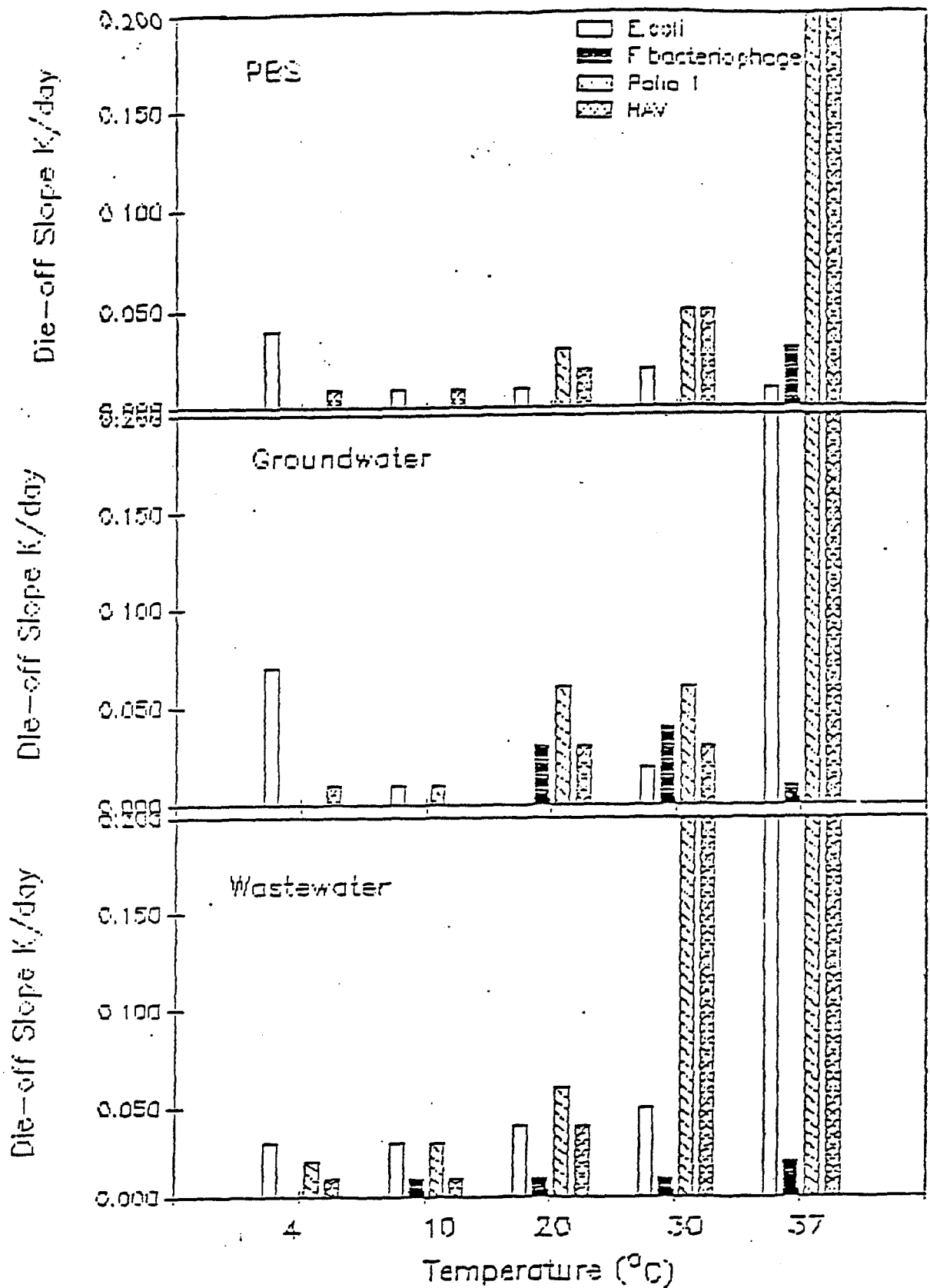


Figure 2. Die-Off coefficient $\text{Log}_{10} N_t/N_0 \times \text{day}$ of Hepatitis A Virus (HAV), Poliovirus 1, F⁺bacteriophages and E. coli in Phosphate Buffered Saline (PBS), Groundwater (GR) and Wastewater (WW) at 4°C, 10°C, 20°C, 30°C and 37°C (The initial titer of tested microorganisms was 10^6 CFU/ml for E. coli and 10^4 - 10^5 PFU/ml for viruses)

**(6.f) Uptake, Accumulation, and ELimination of Organophosphorus
Insecticide by Nile Tilapia**

Third Annual Report

Principal Investigator: Dr. M.M. Shereif

Ain Shams University

SUMMARY

Uptake, Accumulation and Elimination of Organophosphorus Insecticide Fenitrothion (Sumithion) by the Nile Tilapia (*Oreochromis Niloticus*)

M.M. Shereif -- Department of Plant Protection, Faculty of Agriculture,
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ABSTRACT

The uptake and elimination of the organophosphate fenitrothion (Sumithion) by the Nile tilapia (*Oreochromis niloticus*) were investigated under laboratory conditions. Two fish sizes were exposed to a sublethal concentration of fenitrothion, in a renewal exposure system, for 15 days and then transferred to clean water for another 15 days. The uptake of the insecticide gradually increased until day 10 and nearly reached equilibrium at day 15 of exposure. The larger fish accumulated greater concentrations of fenitrothion than did the smaller fish. When transferred to clean water, fenitrothion concentrations in the fish gradually declined up to the end of the 15 day elimination period.

INTRODUCTION

Most pesticides could reach the aquatic environment directly or indirectly. The excessive use of different types of pesticides in agriculture increase the possible contamination of water. Waterbodies, including fish ponds, closer to agricultural areas usually have higher residues from these chemicals. When pesticides enter the aquatic environment they can be acutely lethal or chronically toxic to the aquatic organisms and have deleterious effects on the indigenous populations of fish and other species.

Fenitrothion (Sumithion) is one of the organophosphate insecticides used extensively to control agricultural and forest pests. The aquatic environment could be contaminated with fenitrothion by aircraft, atmospheric drift, erosion and surface runoff. Fenitrothion, as an organophosphate, inhibits the activity of acetylcholinesterase (AChE) and is lethal to different fish species at concentrations ranging from 1 to 5 mg/L (Johanson, 1980). This study is an attempt to evaluate, under laboratory conditions, the processes of uptake and elimination of insecticide fenitrothion (Sumithion) by the fish tilapia (*Oreochromis niloticus*). The relation between these processes and fish body size will be studied.

MATERIALS AND METHODS

The fish *Oreochromis niloticus* were obtained from one of the recommended hatcheries near Cairo. Two sizes of the fish were used (small fish: 6.5 g (ave. weight), 3.5 cm (ave. length) and large fish: 9.7 g (ave. weight), 6.5 (ave. length)) and acclimated for two weeks in the laboratory. During this period, fish were fed a commercial dry pellet food, which was the same food used in all experiments. The fish were acclimated

for another week to the glass aquaria (50 x 35 x 35 cm) used in the experiments at a constant temperature of 27 C.

The median lethal concentration (LC 50) of fenitrothion EC 50% was measured in a static test (USEPA, 1982) using a series of concentrations with three replicates. Separate water control was used. Fingerlings of *O. niloticus* were placed in the aquaria and no food was provided during the test (96 hours). Mortalities were recorded at four hour intervals for the first day and at 24 hour intervals for each day thereafter. Fish were considered dead when there was no movement of the operculum, and dead fish were removed. The value of 96-hr LC 50 was calculated using the binomial test method with no partial kills (Stephan, 1977). This method allows for calculation of both the LC 50 and the confidence level with the following equation:

$$LC\ 50 = (AB)^{1/2}$$

Where:

A = Concentration where no fish die.

B = Concentration where all fish die.

Confidence level = $100 (1 - 2 (1/2)^n)$

Where:

n = number of fish exposed.

The uptake and elimination experiments were carried out in glass aquaria filled with aqueous solutions comprised of the 1/100 of the LC 50 of fenitrothion. The renewal exposure system was used to conduct such experiments. The test solutions and control water were renewed periodically (usually at 24-hr intervals) by transferring the test organisms to different chambers with freshly prepared material or by removing and replicating the material in the original containers. 15-20 fish were placed in each aquarium, with three replicates, and reared for 15 days, then transferred into clean water for another 15 days. Commercial dry feed was given once a day (4% of fish body weight) throughout the experiments.

At each sampling time (1, 3, 7, 10 and 15 days), two fish were taken, washed with running tap water, weighed and analyzed for chemical residues. Water samples were taken at the same sampling time and subjected to residue analysis.

The extraction and clean-up procedures of fish and water samples followed those developed by Kanazawa (1975) and USEPA (1974), respectively. A varian 3700 GLC equipped with flame ionization detector (FID) was used for the detection of fenitrothion in water and fish samples. A standard calibration curve was constructed for fenitrothion from different concentration levels of standard solutions following injections of appropriate volume in the GLC-FID. The average percent recovery in spiked samples was 96.7.

RESULTS AND DISCUSSION

The acute toxicity data of fenitrothion to *O. niloticus* are shown in Table (1). There were no partial kills in any treatment, so the binomial test method was used to calculate a 96 h LC 50 of 400 ug/L. The confidence limits of this estimate were 200 and 800 ug/L at a confidence level of 99.90%.

Table (1): Acute toxicity data of fenitrothion to *O. niloticus*

Parameter	Value
96 h LC 50	400 ug/L
Confidence limits	200 & 800 ug/L
Confidence level	99.90%

Duangasawadi and Klaverkamp (1979) pointed out that the LC 50 value for fenitrothion was 600 to 1000 time less than that for acephate to fish rainbow trout. This difference in toxicity is probably due to their solubility tendency in water and fat where fenitrothion is a lipid-soluble chemical and acephate is a water-soluble.

The uptake of fenitrothion by the two sized of the fish exposed to 40 ug/L (1/100 of LC 50) of fenitrothion are shown in Table (2). Increased concentrations of the insecticide were observed to increase gradually until day 10 and nearly reach equilibrium at day 15 of exposure, and fish muscle tissue showed residues of 2.41 mg/kg wet weight (w.w.) in the small fish and 3.05 mg/kg w.w. in larger ones. Thus the accumulation factor (the residue concentration in the fish tissue divided by that in the water) was 60 for the small fish and 76 for the large.

Table (2): Mean residues (mg fenitrothion/kg fish w.w.), uptake and bioconcentration factor of fenitrothion by *O. niloticus* exposed to 40 ug fenitrothion/L for 15 days.

Fish Size	Time (days)					Accumulation factor
	1	3	7	10	15	
Small	0.75	1.02	1.90	2.40	2.41	60
Large	1.05	1.80	2.45	3.04	3.05	76

Sasaki et al (1982) reported 50 for the accumulation factor of fenitrothion for killifish exposed to 800 ug fenitrothion/L for 10 days, while it was 16 for coho salmon exposed to 560 ug fenitrothion/L for one day (Bull, 1974). Lokhart et al (1973), in their studies on stream-caged rainbow trout exposed to 275 g/ha fenitrothion spray, reported that residues in rainbow trout averaged 0.5 mg/kg in the 24 -hour period after spraying. Peak residue values in the fish were as high as 1.84 mg/kg, but these declined to less than 0.02 mg/kg in four days.

For this study, there was a positive relationship between the size of *O. niloticus* and uptake of fenitrothion from water. A similar relationship was observed between age and residue concentrations in pike from the Richelieu River in Canada (Boileau et al, 1979). Shannon (1977) found that large catfish accumulated greater concentrations of dieldrin in muscles than did small catfish after 28 days of exposure to that insecticide.

When the fish transferred to clean water, fenitrothion concentrations in fish declined gradually (Table 3). At the end of the 15 day elimination period, the small fish contained 0.03 mg/kg w.w. of fenitrothion, while the large fish contained 0.04 mg/kg w.w. Differences in residues were insignificant ($p < 0.05$) between large and small fish at the end of the elimination period (Table 3). Figure (1) summarized the uptake and elimination phases in relation with the two sizes of *O. niloticus*. In similar laboratory studies with another organophosphate insecticide, Geen et al (1984) found that the uptake of acephate by rainbow trout from water was rapid and reached equilibrium concentrations in 5 to 8 days. Elimination of 50% of acephate from the fish required 1.37 to 2.43 days.

Table (3): Mean residues (mg/fenitrothion/kg fish w.w.) and elimination of fenitrothion by *O. niloticus* in clean water following 15 days of withdrawal from exposure to 40 ug fenitrothion/L.

Fish Size	Time (days)				
	1	3	7	10	15
Small	2.08	1.06	0.58	0.19	0.03
Large	2.42	1.75	0.85	0.28	0.04

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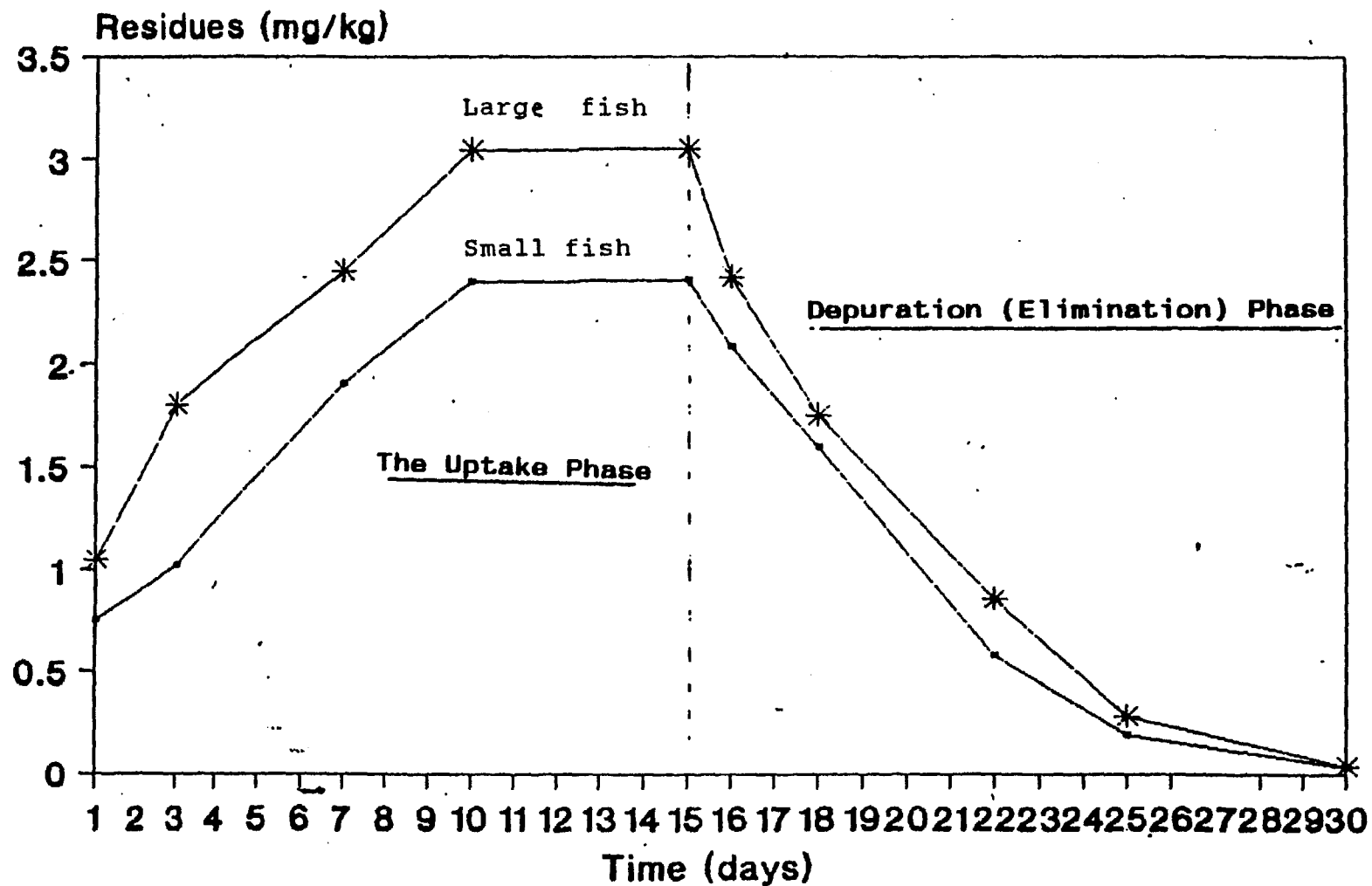


Fig. (1): The uptake and elimination phases of fenitrothion by two sizes of *O. niloticus*.

**(6.g) Legislation and Legal Restraints Pertaining to Seafood
and Fishery Products Safety in the
Arab Republic of Egypt**

Third Annual Report

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I. Introduction

Fish are nutritionally recognized for their high protein content and are considered relatively cheaper than most other food. Fish meat consists of a higher percentage of amino acids than any other animal meat. In addition, fish have a low carbohydrate content and are a rich food source for many of the important minerals.

The protein content in fish is easily digestible and presents a healthy alternative for meat consumption.

The edible portions of fish vary according to species, size, sex, and the fishing season. It ranges usually between 40% - 60% of the total fish size.

Fish are handled either as a fresh or processed food. Since fish are a highly perishable food product, man had to discover different means of preservation to prolong the shelf-life of fish and maintain its nutritional value as an important food source. The first methods used for preservations were refrigeration and freezing in cold countries and salting and drying in tropical warm countries.

The Ancient Egyptians were considered pioneers in preserving fish by salting. With the advancements made in technology, many processing techniques were introduced such as smoking and canning.

The following study will include detailed information concerning fresh and processed fish and portrays the current legislation and recommended code of practice in Egypt for seafood and fishery products whether they are handled as fresh or processed foods.

II. Fresh Fish

Signs For Assurance Of Fresh Fish

Until today, there are no reliable and standardized specifications to ensure ' degree of freshness ' in fish.

In general, the following methods of quality assurance are adopted:

a- Organoleptic Tests (Subjective Tests)

Ensuring the freshness of fish depends primarily on the consumer's judgment or any trained person through subjective inspection of the fish product. Organoleptic inspections include:

- 1- General appearance of fish
- 2- Brightness of eyes
- 3- Structure
- 4- Color of gills
- 5- Smell
- 6- Adherence of scales to skin
- 7- Condition of blood
- 8- Color of flesh meat on the backbone
- 9- Floating test of fish in water

b- Chemical Tests

This method depends on the estimation of some components in the fish such as T.M.A (Trimethylamine), U.B. (volatile organics), T.U. (Tyrosine number?), T.U.N. (volatile Nitrogen), PH.U. (acidity number), U.F.A.? (volatile organic acids), and V.R.S.

c- Microbiological Tests

This test relies on the estimation of microbial load per gram of fish size or per 1 cm² of fish surface. A relation exists between the number of fish

and the degree of spoilage; i.e., the less the number of fish the longer shelf-life of the fish.

It is important to recognize generally the following information regarding the freshness of fish:

- 1- Flat bodied fish such as sole remain fresh for longer periods than others.
- 2- White fish meat remain fresh for longer periods than red fish meat .
- 3- Benthic fish can often be stored for longer periods than surface fish.
- 4- Fish that have their intestines and gills removed directly after fishing can be stored for longer periods than others.
- 5- Fish with higher fat content spoils more quickly than those with lower fat content.
- 6- Some species of fish remain naturally alive for 12 - 24 hours after being caught. Such fish remain fresh longer than those that die directly after being caught.

Safety Assurance Of Fresh Seafood

Fresh seafood destined for commercial sales should have no unpleasant smell. The structure of the fish should be firm and lean, not soft and flaky, and the scales should cling tightly to the skin. The eyes of fresh whole fish should be bright and clear, not cloudy or sunken. Gills should have a bright red color and be clean with an acceptable smell.

A more general approach to differentiate between fresh and spoiled fish is to immerse the fish in water-filled bucket. Fresh fish will eventually sink to the bottom, whereas; spoiled fish will float to the surface.

Some fishermen and retailers resort to fraud practices to conceal spoiled fish. Some common practices include painting gills with red coloring, removal of

fish eye, and mixing of fish with large amounts of ice to conceal the smell or mixing fresh fish with stale ones.

The main problems encountered with fish manufacturing is assurance of quality and safety to consumers with regard to the rapid spoilage of fish.

In Egypt, the problems are more prominent due to lack of advanced storage systems in fishing vessels and boats, length of fishing shores, and unavailability of highway transport.

It has been proven that the gills' area of fish is the first site for microbial invasion. The next areas of bacterial attack, in order of progression, are: kidneys, peritoneal lining, outer surface of skin, flesh surrounding the skeletal bones.

It is well known that not all fresh fish are suitable for consumption. Certain kinds of fish are poisonous and can harbor or accumulate natural toxins in specific areas of the fish such as eggs, stomach, liver, skin or even sometimes in the fish meat. Such toxins are heat resistant and remain after cooking. Poisonous fish are spread across coral reef areas, and in some tropical areas such as the West Indian Ocean and equatorial regions of the Pacific Ocean and the Red Sea. The severity of poison in such fish can vary according to area, seasonal variations, and specific locations.

Eliminating poisonous fish from entering commercial markets depends greatly on the expertise of fisherman who can recognize them and remove them upon sorting.

III. Egyptian Legislation and Statutes For Fresh Fish

In Egypt, there are no comprehensive, specific legislation regarding the safety of fresh fish, but rather a very general, crude legislation applicable to all foods destined for human consumption. Established laws for foods in general are quite insufficient for the protection of fish consumers, and enforcing the law is limited, unreliable, and sometimes unfeasible. The following food laws have been established:

Law No. 48 of 1941, established for the prevention of cheating and fraud; and Law No. 10 of 1977, established for the oversight of foods and its distribution. Both laws have been amended with Law no. 106 of 1980. Penalties for violation of these laws are lax and insufficient.

Currently, there are no standardized specifications for fresh fish, in contrast to processed fish.

IV. Processed Fish

Fish refrigeration, fish freezing, salting, and canning are various processing techniques in use today for fish preservation.

1- Fish Refrigeration: The refrigeration temperature ranges between 0 °C and 7 °C. The temperature should be adjusted as to not allow glazing to occur in fish cells. To retard spoilage, the preferred temperature range is - 3 °C to - 2 °C.

2- Fish Freezing: The temperature ranges between -10 °C and -40 °C. Rapid freezing is more advantageous than slow freezing as it does not allow for spoilage and the product is of a better quality. Frozen fish can be stored for 1-2 months at -14 °C, for 2-4 months at -20 °C, and for 6-8 months at -23°C.

3- Salting: It is one of the oldest and cheapest method for fish preservation. Salt can be applied as soft or dry salt.

4- Canning: It is the best way for preservation as it retains the fish's natural flavor, provides ease of transportation of fish, and cans can be kept at room temperature without spoiling. Salmon, tuna, mackerel, sardine, and cod are some of the important types of seafood being processed by the canning industry.

V. **Egyptian Statutes and Standards For Processed Fish**

The General Egyptian Organization for the Unification of Standards and Quality Assurance (GEOUS&QA) has issued a number of standardized specifications' codes for fish and seafood that include: canned salmon, canned anchovy, salted fish, canned sardines, frozen fish, canned tuna, canned shrimps and crab meat, frozen shrimps, dried shrimps, canned mackerel, frozen squid, and smoked fish. The GEOUS has issued a quality of assurance mark: ES which stands for Egyptian Standards. This mark on commercial products designates that the product complies with Egyptian standardized specifications.

A. Canned Salmon: Code No. 1472-1980

Section 3 (Sect.3) of the code provides general requirements for proper preservation of the shucked fish by removal of internal organs, head, and fin, packing the specified species in properly sealed cans, and applying appropriate temperature for the destruction of microbes responsible for spoilage and diseases especially Clostridium botulism. Initially, the salmon fish should be of good quality and be cleaned thoroughly before processing.

Sect. 4 & 5 discusses specifications for canned salmon such as:

- Hydrogen (H₂) content not to exceed 6.7.
- Salt content not to exceed 2% by weight.
- Total volatile Nitrogen not to exceed 30 mg Nitrogen per 100 g of fish meat.

- Cans destined for packing food in should have identical specifications according to the Republic's decision No. 798 of 1957 and standard specifications No. 153/1978 for machine-produced cans that will be filled with food.
- Internal negative pressure to be maintained in the can.
- Proper labeling of cans with manufacture's name and address, name of type of canned fish, net weight of contents, production and expiration date.

Sect. 6 of the code describes proper physical inspection procedures of canned fish, sample preparation for chemical testing, examination procedures for determination of chemical or microbial constituents.

B. Canned Anchovy: Code No. 808-1988

Specifications contained in this code replace previous standard code No. 808/1966 "Canned Anchovy".

Sect. 2 of the code defines the difference between anchovy fillets, rolled anchovy, and anchovy paste.

Sect. 3 covers the general requirements:

- Anchovy has to be of good quality with head, internal organs, scales, and tails removed. The boneless product has to be thoroughly cleaned.
- Ingredients that may be added such as salt, oil, vinegar, or spices have to comply with standard specifications specified for each.
- Fillets contained in the same can have to have similar length size as much as possible.

Specifications for can contents, can design and labeling are stated in Sect.5.

Some of these are:

- Moisture content should not exceed 60%.

- Percentage of added oil should not exceed 25% of the can's contents.
- Salt content to range between 10-16%.
- Liquid that is separated from the fish should not exceed 10% of the oil contained in the fish.
- Hydrogen content (H_2) in the can should not exceed 6.
- Total volatile Nitrogen content should not exceed 30 mg/100g of estimated Nitrogen from sample.
- Fish should be free of microorganisms and their toxins that cause spoilage and diseases.
- Can design specifications and labeling are the same as those described for canned salmon. In addition, the label should specify product type (fillets - rolled fillets - paste) and ingredients.

Sect. 6 outlines recommended examination and inspection procedures which should be done according to the following standard specifications:

- No. 889 " Frozen Fish ".
- No. 1460 " Estimated Arsenic Content in Canned Food "
- No.50 " Chemical Examination and Analytical Procedures for Vegetable Oils and Other Oils Used in Food ".

Estimation of Lead and Microbiological examination is to be done according to Egyptian standard specifications published by the Organization.

C. Salted Fish: Code No. 1725-1989

Some of the requirements stated in Sect. 3 include the following:

- Fish to be salted can be either fresh or frozen and be clean and of good quality.
- Salt used must have specifications according to Egyptian standard specifications No. 273 (Sodium Chloride) Part I: Food Salt.

- No preservatives or artificial colorings may be used.

Specifications in Sect. 4 include:

- Moisture content in end-product should range between 50%-55%.
- Salt content (as estimated NaCl) in end-product range between 15%-22%.
- H₂ content range between 6 - 6.5.
- Product to be free of spores, toxins, Salmonella bacteria, *Vibrio Parahaemolyticus* , *Clostridium Botulinum* and its toxins, *Escherichia Coli* .
- Number of *Staphylococcus Aureus* bacteria not to exceed 100 cells/g.
- Number of sulfite-reducing *Clostridium* bacteria not to exceed 500 /g.
- Histamine content in the product not to exceed the limit allowed.

Sect. 5 regarding can specifications and labeling requirements covers the same information covered previously.

Sect. 6 on procedures for examinations and inspections is to be done according to Egyptian standard specifications published by the Organization.

D. Canned Sardines: Code No. 287-1990

Specifications contained in this code replace previous standard code No. 287-1980.

Sect. 2 specifies the 12 species of sardines which can be used for canning.

Sect. 3 on the general requirements is similar to what has been stated earlier.

Some of the specifications listed in Sect. 4 are:

- Total moisture content should not exceed 60% by weight.
- Salt content should not exceed 2% by weight.
- H₂ should not exceed the range 6.5-6.8.
- Histamine should not exceed 10 mg/100g of the end product.

- Volatile Nitrogen should not exceed 30 mg as N estimate per 100 g of sample.
 - Heavy metals in the end product should not exceed the following limits:
Lead 1 mg/kg, Cadmium 1 mg/kg, and Mercury 5 mg/kg.
 - Product should be free of bacteria such as *Clostridium Botulinum* .
- The remaining sections of the code: Sect.5 (Can & Labeling) and Sect.6 (Procedures for Examinations and Inspections) are the same as presented previously.

E. Frozen Fish: Code No. 889-1991

Specifications contained in this code replace previous standard code No. 889-1981 for frozen fish.

Sect. 2 gives definitions of types of frozen fish (whole fish; fish with head, tail, fin, visceral removed, fish with head, visceral removed only; fish fillets either boneless and skinless; or just boneless). Fish to be subjected to rapid freezing.

Requirements in Sect. 3 include:

- Fish to be frozen should be of good quality, bacteria-free and caught from clean waters.
- The process of rapid freezing starts at a temperature not exceeding -40°C, and the freezing process is not considered to be completed until the internal temperature of fish portions reaches -18°C within a period of 4 hours.
- Storage of fish is done at a temperature not exceeding -18°C and storage period should not exceed 6 months since it was frozen until consumed.
- Transportation of fish should be done in refrigerators with a temperature not exceeding -18°C.
- Fish should not be frozen after thawing.

Some of the specifications listed in Sect. 4 are:

- H_2 should not exceed 6.2.
- Histamine should not exceed 10 mg/100g of fish meat.
- Total volatile Nitrogen should not exceed 30 mg as N of fish meat.
- Heavy metals in the product should not exceed the following limits:
Lead 1 mg/kg, Cadmium 1 mg/kg, and Mercury 5 mg/kg (same limits as canned sardines).
- Pesticides' residues should not exceed allowable limits established by
FAO (Food & Agriculture Organization) of the United Nations.
- Fish should be free of all disease causing microorganisms and parasites.
Number of parasites or worms which can be observed by the naked eye
should not exceed 100 and only 20% or less of fish sample can contain that
number.
- Total bacteria load should not exceed 100 cells/g; total aerobic bacteria
not to exceed 1000,000 cells/g; Number of *Staphylococcus Aureus* (S.
aureus) not to exceed 1000 cells/g; fish should also be free of Salmonella,
Shigella, and *Vibrio Parahaemolyticus* (V. parahaemolyticus).
- Sect. 5 on containers and labeling and Sect. 6 are similar to what have
been previously stated for other processed seafood. The date of freezing
should appear on the label of the container or package.

F. Canned Tuna and Bonito: Code No. 804-1990 with amendments

Specifications contained in this code replace previous standard code No.
804-1966 for canned tuna.

Sect. 2 describes types of tuna and bonito fish (Bluefin, Yellowfin,
Albacore, and Bigeye Tuna) to be used for canning.

Sect. 4 on general requirements consists of similar descriptions as those outlined for canned salmon.

Some of the specifications listed in Sect. 4 include the following:

- Hydrogen ion ranges between 5.9-6.1.
- Salt content not to exceed 2%.
- Total Volatile Nitrogen should not exceed 40 mg/100 g of estimated Nitrogen of sample (as amended on June 25, 1995).
- Histamine should not exceed 10 mg/100g of fish meat.
- Heavy metals in the product should not exceed the following limits:
Lead 1 ppm, Cadmium 1 ppm, and Mercury 5 ppm

Remaining sections 5 and 6 contain similar specifications as previously stated for other canned seafood.

G. Canned Shrimps Or Prawns And Crab Meat: Code No. 414-1993

Specifications contained in this code replace previous standard code No. 414-1963 for canned shrimps or prawns and crab meat.

General requirements (Sect. 3) are similar to what has been stated for other canned fish.

Sect. 4 regarding specifications include:

- Moisture content in end-product not to exceed 55%.
- Salt content in end-product not to exceed 3%.
- Sorting of fish product as large, medium, small according to size and measurement specifications indicated in the code.
- Other specifications such as Nitrogen, Histamine, Heavy Metals contents are the same as those specified for canned sardines.

H. Frozen Shrimps: Code No. 516-1993

Specifications contained in this law code replace previous standard code No. 516-1969 for frozen shrimps.

Requirements in Sect. 3 are similar to ones outlined for frozen fish

Sect. 4 specifications include:

- Salt content in boiled frozen shrimp should not exceed 1.5% in final product.
- Trimethylamine content should not exceed 40 mg/100g of shrimp meat.
- Total volatile Nitrogen should not exceed 65 mg/100g of shrimp meat.
- Heavy metals in the product should not exceed the following limits:
Lead 1 mg/kg, Cadmium 1 mg/kg, and Mercury 5 mg/kg.
- Total number of bacteria should not exceed 100,000 cells/g.
- Number of *Staphylococcus Aureus* (*S. aureus*) bacteria not to exceed 500 cells/g.
- Product to be free of *E. Coli* and other disease causing bacteria.
- Sorting of frozen shrimp according to quality (excellent), measurement, and size (very large, large, medium, small, very small) specifications is described in Sect. 4 of the law.

Other sections are similar to sections described for frozen fish.

I. Dried Shrimps: Code No. 546-1993

Specifications contained in this law code replace previous standard code No. 546-1964 for dried shrimps.

Sect. 2 identifies dried shrimp as fresh shrimp which had been boiled in saline solution or not boiled and dried either naturally or artificially.

General requirements listed in Sect. 3 include:

- Dried shrimp has to be of good and fresh quality.

- Product has to retain its natural color (red) for boiled shrimps and should not have dark or variations in color which may occur due to an increase in temperature during drying, or due to enzymatic or Microbiological reactions.
- Product in one container has to be of equal size and type .
- Moisture content in product should not exceed 8%.
- Salt content in end product should not exceed 5%.
- Histamine should not exceed 10 mg/100g of fish meat.
- Total Volatile Nitrogen should not exceed 65 mg estimated as N per 100g of sample.
- Heavy metals in the product should not exceed the limits prepared by the Organization.
- Total bacteria should not exceed 100,000 cells/g; total aerobic bacteria not to exceed 10 cells/g; number of bacterial colonies should not exceed 10 cells/g; number fungi or yeast bacteria should not exceed 10 cells/g.

Sect. 5 on containers and labeling outlines that the product should be packaged in moisture and vapor proof packages made of polyethylene or other heat resistant material and then be placed in large corrugated cartons or reinforced wooden boxes. Labeling specifications are the same as other stated processed fish.

J. Canned Mackerel: Code No. 1521-1995

Specifications contained in this law code replace previous standard code No. 1521-1982 for canned Mackerel.

Sect. 3 identifies the species to be used for canning:

- Mackerel: *Scombridae* , *Scomber* , or *Rastrelliger*
- Jack Mackerel: *Carangidae* , *Trachurus* , or *Decapterus*

Specifications for can contents are described in Sect.5. Some of these are:

- H₂ should not exceed 6.8.
- Salt content should not exceed 2%.
- Total fat content in the can should not exceed 10%.
- Total volatile Nitrogen should not exceed 40 mg/100g as estimated N of the sample.
- Histamine should not exceed 10 mg/100g of end product.
- Product to be free of disease causing bacteria and its toxins, aerobic bacteria, *Clostridium Botulinum* (C. Botulinum) and its toxins.
- Heavy metals in the product should not exceed the following limits:
Lead 1 mg/kg, Cadmium 1 mg/kg, and Mercury 5 mg/kg (same limits established for other processed seafood).

Remaining section 6 on packaging and labeling contains similar specifications as previously stated for other canned seafood. In addition, this section provides a defect table for canned Mackerel. The table quantifies the level of defectiveness of the product and can be used by the manufacturer to evaluate the quality of their products.

Defect Table : Canned Mackerel

Defect Description	Classification		
	Serious	Major	Minor
1- Cutting and Trimming:			1
Parts of Head or Tail	—	—	—
Parts of Viscera (large amount)	4	—	—
Parts of Viscera (small amount)	—	2	—
2- Non-characteristic Pieces: (fillets or flaked pieces of fish flesh or skin or bones or disintegrated flesh pieces, expressed as % of drained fish solids Materials) :			
> 10 %	4	—	—
> 7 % < 10 %	—	2	—
3- Flesh Discoloration :			
Severe	—	2	—
Slight or Localized	—	—	1
4- Odor and Flavor of Mackerel:			
Distinctly objectionable odor or flavor	6	—	—

Defect Table : Canned Mackerel (Continued)

Defect Description	Classification		
	Serious	Major	Minor
5- Texture:			
Rotten Flesh	6	—	—
Hard Bones (not easily friable using thumb and forefinger)	—	2	—
Excessive Mushy Flesh	4	—	—
6- a) Exuded Water: (oil packs only)			
Water content expressed as % of declared net contents of can			
Fish Packed in Oil > 8 %	4	—	—
Fish Packed in Oil > 6 % < 8 %	—	2	—
6- b) Exuded Water: (oil with own juices)			
Fish Packed in Oil With Own Juice > 12 %	4	—	—
Fish Packaged in Oil With Own Juice 10 % - 12 %	—	2	—
7- Separation of Sauces: (Sauces separated into solid and liquid)			
Severe Separation	—	2	—
Partly (objectionable appearance)	—	—	1
8- Foreign Matter:	6	—	—

A sample shall be considered defective if it exceeds the total defective points according to the following table:

Classification of Points	Large Canned Mackerel in Oil	Large Canned Mackerel in Other Mediums and in Oil	Large Canned Mackerel in Other Mediums
Serious	4	4	4
Serious + Major	10	8	6
Serious + Major + Minor	14	12	10

K. Frozen Squid: Code No. 2800-1995

Sect. 3 on definitions identifies the species of the following families:

Loliginidae and *Ommastrephidae*.

Sect. 3 on general requirements describes the following:

- Squid product shall be free of objectionable odors, unnatural colors which may indicate spoilage or freezer burns, and additives.
- Re-freezing the squid should not be done after thawing.

Sect. 4 on standard specifications lists the following:

- Rapid freezing of squid shall reach a temperature of -18 °C and storage temperature should not exceed this temperature.
- Squid to be free of parasites and *V. parahaemolyticus* bacteria.
- Also, total number of bacterial colony in product should not exceed 100 cells/g.
- Total number of aerobic bacteria should not exceed 100,000 cells/g.
- Number of *S. aureus* should not exceed 500 cells/g.
- Product shall be free of *Salmonella* and *Shigella* bacteria in 25g of sample.

- H₂ not to exceed 6.8.
- Histamine should not exceed 10 mg/100g of squid meat.
- Total volatile Nitrogen should not exceed 65 mg/100g of squid meat.
- Limits for heavy metals are the same as cited earlier.
- Pesticides residues shall not exceed the recognized limits published by FAO and the Egyptian specifications distributed by the Organization.

Other sections remain the same as those cited for other processed fish.

L. Smoked Fish: Code No. 288-1991

Specifications contained in this code replace previous code No, 288-1985 for smoked fish.

Sect. 2 identifies three practiced methods for smoking of fish:

1. Cold Smoking: The process involves treating fish with rising smoke from incomplete combustion, and the temperature ranges between 28-32 °C for 20 hours or may be as long as 3-5 days.
2. Hot Smoking: This involves treating fish with rising smoke from incomplete combustion, and the temperature shall not exceed 120 °C for 4 hours.
3. Partial Hot Smoking: This involves treating fish with rising smoke from incomplete combustion, and the temperature shall not exceed 100 °C for 4 hours.

Sect 3 regarding general requirements states similar requirements as for other processed fish such as:

- Fish shall be either fresh or frozen fish with scales removed.
- Product shall be of soft texture .
- Usage of natural and artificial colorings in product is not recommended.

Sect. 4 specifies the following:

For fish treated with cold smoking:

- Salt content should not exceed 15 % of dried weight.
- Moisture should not exceed 45 % in the product.
- Storage of product shall be done at a temperature 4 °C for a period of not more than 2 months since date of production.

For fish treated with hot smoking:

- Salt content should not exceed 4 % of dried weight.
- Moisture should not exceed 65 % .
- Storage of product shall be done at a temperature 4 °C for a period of not more than 7 days since date of production.

For fish treated with partial hot smoking:

Salt content in end product should not exceed 10 % of dried weight.

- Moisture content in product should not exceed 55 % .
- Storage of product shall be done at a temperature 4 °C for a period of not more than 7 days since date of production.
- Histamine should not exceed 10 mg/100g of end product.
- Total volatile Nitrogen should not exceed 30 mg/100g of fish meat estimated as Nitrogen.
- Limits for heavy metals are the same as cited earlier.
- Product shall be free of parasites, fungi growth and their toxins, E. Coli, Salmonella and Shigella bacteria of 25g of sample, *V. parahaemolyticus* .
- Total number of aerobic bacteria should not exceed 100,000 cells/g.
- Total number of bacterial colony should not exceed 10 cells/g.
- Number of *S. aureus* should not exceed 1000 cells/g.
- Total number of aerobic bacteria should not exceed 100 cells/g.

VI. Conclusions

- 1- Legislation and legal restraints applicable for fish and seafood in Egypt are inadequate and contain many loopholes for ensure the safety of marine food.
- 2- Lack of qualified trained food inspectors is a major problem in ensuring compliance. Also, the limited number of inspectors available have divided responsibilities with a number of ministries such as Supplies, Health, Industry, Agriculture and others. Food Inspectors are not acquainted with the scientific standards and methodology for fish examination.
- 3- Current established penalties are still inadequate and not enforceable. In addition, there are numerous opportunities for violators to evade noncompliance and proving occurrence of violations may be difficult.
- 4- It is important to publicize and make more accessible all legal restraints governing the safety of food and the risk involved. This will create pressure and awareness to control and prevent handling of spoiled fish and deter fraud practices.
- 5- Lack of qualified laboratories and trainees for performing necessary analytical techniques is another problem.
- 6- The length of judicial proceedings hinder enforcement of the law.
- 7- It is vital to expedite the setting of additional standard specifications for fresh fish and other marine food.

8- The importance of relating specifications with the water quality of the marine environment should be recognized because seafood are caught from the marine environment. In addition, recognizing the impact of polluted environments on various fish is vital.

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