MOSHE SHILO (1920– 1990)

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Moshe Shilo, the first Microbiologist to pioneer the study of Microbial Ecology in Israel, is probably best known for his work in Aquatic Microbiology. His large volume of work includes also important contributions to the study of parasites of microbes, and original work in medical bacteriology.

Moshe Shilo was born in 1920 in Moscow, Russia, and his family emigrated to Israel in 1934.

He studied Bacteriology, Parasitology and Biochemistry in the prefaculty of Medicine of the Hebrew University of Jerusalem and worked for his master's degree with Prof. Manfred Ashner on the Ecology of Marine Luminescent Bacteria in the Mediterranean and concluded his doctoral thesis on Heat Labile Antigens of Shigella with Prof. Olitzky in 1947.

Before summarizing Shilo's contributions to science, it might be of interest to try to understand the origins of his specific intellectual attitude towards the study of micro-organisms. In his doctoral thesis he dealt with some aspects of medical microbiology - which is the study of bacteria as agent of disease. Still, in his life's work in research and teaching, he dealt with the study of bacteria themselves and their extraordinary ability to interact with their changing environment.

This approach is very much in the spirit of what is known as the "Delft School" of Microbiology. The founder of the "Delft School" was Martinus W. Beijerinick who worked and taught, at the beginning of the last century, at the Technical University of Delft in the Netherlands.

The objective of his studies was the bacteria themselves and their role in the cycle of matter in nature. He applied biochemistry to understand the great variety of bacterial metabolism. His student, A.J. Kluyver, continued the broad comparative study of microbial metabolism from which he derived the principle of the unity of biochemistry.

Shilo probably encountered this approach when he worked for his master's degree with Prof. Manfred Ashner on luminescent bacteria of the

Mediterranean Sea. "Beijerinick, Collected Works" was always on Ashner's desk and considered by his students to be "The Bible".

Shilo had a second encounter with the Delft School when he attended the famous course in general microbiology in 1955 given by C.B. van Niel in California. It was the Delft School philosophy presented by Ashner and Van Niel which had some crucial influence on Shilo's future work.

Shilo's first serious encounter with aquatic microbiology and with an acute applied problem, came when he started to study the reasons for a severe outbreak of fish deaths which occurred in 1947, some years after the first fish ponds were established in Israel. The fish epidemic was attributed by Reich and Ashner to the growth of a toxic unicellular phytoflagellate – *Prymnesium parvum*. But no close relationship could be established between the number of phytoflaglellates in the water and fish deaths, and therefore some doubt remained as to the cause of the fish mortality.

Working first with Ashner and then with Mira Shilo, Shilo was able to grow the algae in axenic cultures and study their toxin production, which was stimulated by light and phosphate depletion. He showed that the growth of the algae and the synthesis of their toxins have different optimal requirements which explained the lack of correlation between numbers of phytoflagellates and the toxity of the ponds. Toxicity to fish is a function of the changing equilibrium between the various factors affecting toxin production and do not necessarily reflect the size of the phytoflagellate population. They also noted that in lab cultures, NH_4 sulfate salts killed the Prymnesium at relatively low concentrations. These studies led to the determination that molecular ammonia is the toxic agent to the flagellates. The use of aqueous ammonia as an almost convenient control measure is now widely used, but its effect is of short duration only.

Isolation and purification of the *Prymnesium* toxins was another achievement.

The toxin turned out to be a proteolipid containing 15 amino acids, fatty acid and phosphates. This is one of the first algal toxins to be isolated and its toxic effects studied.

This study by Shilo of the phytoflaggelate *Prymnesium*, as an algal bloom leading to fish disease by the excretion of a toxin, is a model system for the study of algal ecology, algal bloom and the understanding of the various factors effecting such populations and their toxicity.

The combination of basic research on the regulation and mode of action of the Prymnesium toxins, together with the development of field tests and the appropriate treatment of fish ponds by ammonia and copper – eliminated this problem in aquaculture in Israel. As a result of their thorough interdisciplinary studies, Shilo's laboratory became an internationally recognized center for the study of algal blooms.

While the above studies are of great importance to aquaculture, Shilo's more fundamental contribution to aquatic microbiology are his studies on the solar lake, the Dead Sea bloom and the biology of cyanobacteria.

In 1967, the most exciting habitats of Sinai became available for scientific investigation, including a small unique hypersaline body of water 20 km. south of Eilat - the Solar Lake.
Shilo initiated an interdisciplinary and international research program coordinated by Yehuda Cohen which was to continue for the next 15 years. The Solar Lake was established as a model for microbial interactions in extreme environments. The limnological setting of the lake, the annual development of its photosynthetic communities, the microbial heretotrophic production, the sulphur cycle and the structure and function of cyanobacterial mats developing in the solar lake were described. Some of the major achievements of these studies are:

1. The discovery of anoxygenic photosynthesis in cyanobacteria as a major evolutionary stage in the evolution of photosynthesis, a study initially carried out in cooperation with Yehuda Cohen Etana Padan and Bo Jorgensen.
2. Fine regulation of oxygenic photosynthesis, anoxygenic photosynthesis and sulfate reduction in diurnal changes occurring in the Solar Lake (with Bo Jorgensesn, Gijs Kuenen and Yehuda Cohen).
3. The study of Microbial Mats - these stratified benthic microbial communities develop all over the bottom of the Solar Lake dominated by cyanobacteria. These microbial mats are modern analogs to stromatolites: Precambrian laminated sedimentary rocks which are the oldest known microfossils (dated to be 3.5 billion years old).

One group of microorganisms which were of special interest to Shilo were the cyanobacteria. They were isolated often from extreme environments and could thrive under conditions of rapid and extreme environmental change.

One example of such extreme adaptation is the cyanobacterium Oscillatoria limnetica isolated from the Solar Lake.
This organism is able to carry out an aerobic photosynthesis. It could also grow well under anaerobic conditions using anoxygenic photosynthesis in which H_2S replaced H_2O as electron donor. Anaerobic photosynthesis of this kind is known in photosynthetic Bacteria under strictly anaerobic conditions but has not been shown in cyanobacteria. This is the first instance in which the same organism can carry out both aerobic and anaerobic photosynthesis. Since cyanobacteria are considered to be the oldest known group of organisms and because life is thought to have evolved under anaerobic conditions, these observations are of special interest. Shilo speculated that the organisms could represent a very early adaptation from anaerobic to aerobic photosynthesis.

An additional aspect of cyanobacterial physiology, investigated in Shilo's laboratory, is the mechanism of diurnal vertical migration in ponds. Cyanobacteria seem to have an ability to accumulate on the surface of lakes, or to be distributed through the water column, or sink to its bottom. They therefore seem to have a mechanism which regulates vertical migration in water. These phenomena have been correlated in literature with the function of gas vacuole which these organisms contain and by which they apparently control their buoyancy. On closer examination of the cytology of cells in Shilo's lab, it turned out that no changes occurred in the gas vacuoles of these organisms during the daily cycle of migration. Therefore another factor had to be responsible for such daily changes in buoyancy. Shilo found that during photosynthesis, large amounts of carbohydrates are accumulated by these micro-organisms, leading to an increase in weight. Consequently, the cells lose buoyancy rapidly. Such effects could be simulated in laboratory experiments where it was shown that cyanobacteria exposed to light in nitrogen-free medium - sink. On the bottom of the lake they stop photosynthesizing. Metabolic degradation of carbohydrates in the dark and in the presence of ammonia explains the increase in buoyancy which occurs at night.

One of the subjects which occupied Shilo during the last decade was the study of benthic cyanobacteria.

Working with Ali Fattom, Yeshayahu Bar-Or, Martin Kessel and Yehuda Cohen, Moshe's team discovered some of the unique features of these organisms. All benthic cyanobacteria had hydrophobic cell surfaces as opposed to hydrophilic surfaces of planktonic cyanobacteria. This was measured by partitioning - in a biphasic hexadecane and water system developed by Eugene Rosenberg. Hydrophobicity explained the strong non-specific adherence of cyanobacteria to submerged surfaces, and was shown to be located in the outer surfaces of the cell wall which could be removed by enzymes, making the cell hydrophilic.

Attached micro-organisms must be able to detach themselves from their substratum at some stage in their life cycle in order to spread and colonize a new surface. Two mechanisms of release from attachment were discovered, both involving changes in cell surface properties, from hydrophobic to hydrophilic. The first mechanism involves the formation of special cells, so called hormogonia, which detach themselves from the mother cells and have a hydrophilic surface and float away.

A second mechanism which occurs in organisms which have no biphasic life cycle is the production and excretion by hydrophobic mature cells of a high molecular weight compound which covers the cell surface and temporarily masks the hydrophobicity of the mature cell, enabling it to detach itself form the surface: this occurred in the cyanobacteria *Phormidium*.

Some of Shilo's work on the adaptability of cyanobacteria is of actual interest in the framework of the recent debate on global climate change.

Marine photosynthetic communities are consumers of CO_2 and their efficiency to remove excess carbon dioxide in the atmosphere is a crucial factor in the calculation of future excesses of carbon dioxide in the atmosphere and its influence on the climate. Such calculations are usually based on actual measurements of primary production of the ocean and the large lakes. As a life-long student of rapid adaptation of microbial communities to changes in the environment, Shilo was rather skeptical about our ability to predict such adaptive changes for future periods.

From Shilo's writing the clear message which he left on this subject is that we should not underestimate the adaptability of photosynthetic microorganisms and that our knowledge of such adaptability is certainly incomplete and sometimes inaccurate.

Between the various factors controlling microbial populations in nature there is one less explored than others - the parasitism of bacteria by bacteria. A small parasitic bacteria bdellovibria bacteriovorum was first discovered in 1962 by Stolb and Peterhold in Germany. They considered it a typical exoparasite which supposedly created a hole in the cell wall of bacteria and got its nourishment by sucking bacterial protoplasm. A very different picture of Bdellovibria lifestyle emerged when Shilo, working in cooperation with Sid Rithenberg in California and Shilo's students Mazal Varon, Aviva Hurwitz and Martin Kessel, investigated their life cycle.

It was shown that the various Bdellovibria strains were host specific and their specificity was determined by bacterial cell wall structure. It was also shown that Bdellovibrio, three minutes after its attachment to the bacterial cell, entered the cell through an opening created by enzymes of Bdellovibrio and that the opening through which it entered, closed again, creating the so called Bdelloplast. After penetration, the Bdelloplast grows rapidly in the cell without dividing, creating a large filament which undergoes multiple fusions - generating many Bdellovibria cells. An additional very significant contribution to the study of Bdellovibria and its understanding of its life cycle by Moshe's group, was their success in growing this intercellular parasite on a host-free medium containing host extracts.

Bdellovibrio growth in this condition was dependent on factors in host extract which were DNAse stable but RNAse and pronase sensitive. Multiple fission in the in vitro culture could be induced by addition of RNAse indicating that an RNA molecule might prevent cell division in vivo.

Shilo also isolated a variety of Bdellovibrio strains from the marine environment and established their specific marine nature by showing that their growth depended on the right concentration of sodium potassium, calcium and magnesium.

Another parasite of microbes which was studied intensely in Moshe's lab was cyanophage. The existence of viruses of cyanobacteria have been described before, but never studied. While the bacterophages of E. Coli, were the subject of intense studies and constituted one of the cornerstones of the beginnings of molecular Biology, there was no systematic study of cyanophages. Shilo and his students Eitana Padan, Moni Elitzur and others, were the first to describe their life cycle which turned out to be significantly different from the life cycle of classical bacteriophages. Working with the cyanobacterium Plectonema boryanum, infected by cyanophage LPPI or LPPIC they showed that the time between infection and appearances of new viruses in the infected cell, which is minutes in bacteria, was between 7--30 hours in cyanophage. The virus turned out to be a DNA virus, and its assembly which starts with a hexagonal head formation occurs apparently at a single site in the bacterium - because cyanophage were released in large aggregates. Also while bacterophage development depends largely on external nutrients, the LPP cyanophage seem to depend in its growth completely on nuclear acids and proteins which preexisted in cyanobacterial cells before infection.

Lately the interest in cyanophage has been renewed when it was found that a significant percent of DNA extracted from marine plankton is actually phage DNA - indicating a major role of phages in the population dynamic of marine plankton - which consists partly of cyanobacteria.

Shilo's contributions to Science in Israel are not limited to his research achievements and the training of a significant number of inspired and creative students, who continue his work on microbial ecology but also included some significant contributions to Israel's scientific infrastructure. His major contribution in this field was of course the establishment of the Department of Microbial Molecular Ecology - in the Faculty of Science at Hebrew University in Jerusalem, the base of his research and teaching. He also established the laboratory for the study of fish disease in Nir David in 1949. In 1964 he chaired a national committee which recommended the establishment of a Marine Research Laboratory in Israel. This recommendation led to the establishment of "The Israel Oceanographic and Limnological Research Corporation" in 1966, one of the major research institutes investigating the Mediterranean Sea. Shilo was chairman of the Board of this institution for 10 years. He was also involved in the establishment and direction of the Steinitz Marine Biological Laboratory in Eilat, the first modern laboratory to investigate the Gulf of Aquaba, and was the first Chairman of its Board of Directors. The Moshe Shilo Minerva Center for Marine Biochemistry was established after his death, and is directed by Prof. Yehuda Cohen.

Shilo was very much a part of the international community of microbiologists and one of the founders of modern microbial ecology.

He was a life-long member of the American Society of Microbiology and was honored by being appointed their 1967 ONR lecturer, and by receiving the 1978 Fisher Award. He was member of the Marine Biological Laboratory in Woods Hole from 1970, and spent several summers there doing research. He was also an instructor of the Microbial Ecology course at M.B.L. in 1970, 1975 and 1981.

Moshe Shilo taught at the University of Berkley, Rutgers and UCLA. His scientific judgment was respected by the scientific community, and he was a member of many editorial boards of international scientific journals. He was the founding member of the editorial board of "Microbial Ecology", and a member of the editorial boards of "Annals de l'Institut Pasteur", "Advances in

Microbial Ecology", "Mircen Journal of Applied Microbiology and Biotechnology" of UNESCO, as well as the "F.E.M.S. Journal for Microbial Ecology." He was also one of the founders of the three annual "Symposia on Photosynthetic Prokaryotes".

Fifteen years after his death he is still very much missed by his friends with whom he was always willing to share his keen sense of observation, highly original thought and endless enthusiasm for science.

His legacy is still very much alive with all those who study the biology of bacteria.