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PRODUCTION EXPERIMENT OF HALIBUT FRY IN LARGE ENCLOSED WATER COLUMNS.

by

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ABSTRACT

Halibut eggs were hatched in 21 floating enclosures and the larvae stored through the yolk sac period under 5 different environmental conditions, each with 4 parallels. The effect of the different environments was examined as survival rates of larvae and on morphometric measurements at day 31 and day 49, when one of each type was emptied.

Half of the initial number of enclosures, with their content of remaining larvae, were continued in a first feeding trial to bottom settling of the metamorphosed fry. An estimated number of 300 larvae reached metamorposis, and at the end of the experiment a total of 68 were captured and transferred to tanks at the station.

Light exposure was shown to be unfavourable both during hacthing and development of larvae.

Antibiotic treatment of the water had a positive effect on survival the first days of the experiment, but had a negative effect on the development of postlarvae later in the yolk sac period.

The microbiological survey indicated that yolk sac larvae are susceptible to stress caused by microorganisms and their activity. This activity was particulary large near the bottom layer.

INTRODUCTION

A domestication of the atlantic halibut (Hippoglossus hippoglossus L.) implies both basic biological research as well as developing rational farming techniques.

The yolk sac period of Atlantic halibut is long; 50 days at 5.3 ^{0}C (Blaxter et al., 1983), and in this stage the larvae are developing functionality and depending on the environment for proper storage through this fragile part of life and also for transport to the photic zone where first feeding will occur.

The yolk sac stage can generally be regarded as a training period to succeed in catching of external feed later. In addition this stage repre-sents, especially for species with long yolk sac stages, an important developmental period, and great changes in the larval body must take place before first feeding is possible.

Eggs from halibut hatch when the larvae still are at an early and undeveloped stage. According to Blaxter et al. (1983.), the jaw reached a functional stage 25 days post-hatching and by that time the gut started to bend. After 5 to 6 weeks the larvae had the first pigmentation and also the lens and retina were fully developed by that time.

In order to avoid nutritional shortage during development the swimming activity must be kept at the lowest possible rate, thus securing the limited content of the yolk to be used in building the different organs of the larva. In the laboratory the yolk sac larvae will be almost continuously disturbed by environmental factors, the physical limitations of the tanks and by each other. This leads to spontaneous activity and a too fast resorption of the yolk.

The impact of eventually pathogenic bacteria present will not be considered in this work. The activity of bacteria nonpathogenic to halibut may on the other hand cause problems as such. The

effect of nonpathogenic bacteria will be due to organic and inorganic metabolic products, some of which are toxic to fish. These effects will depend on the composition and amount of bacterial sub-strate present, the concentration and activity of the bacteria, and the metabolic processes involved.

The proteolyttic effect of exoenzymes of epibacteria growing on eggs and larvae may represent an intermediate situation between commensalism and parasittism.

The above mentioned assumptions have led to a hypothesis called "the physical and microbial stress hypothesis" which will be investigated in the present report.

MATERIALS AND METHODS

Since 1984, approximately 20 mature halibut, caught on longline, have been kept in 7 m diameter circular tanks at Austevoll Marine Aquaculture Station. The 24th and 26th of february 1986, four females with newly ovulated eggs were stripped and each batch fertilized with milt from 2 males. The stripping was done on unanesthetised fish where eggs, milt and seawater were mixed simultaneously in a wet-fertilization method at a ratio of 100:1:100.

After 15 minutes the eggs were rinsed and incubated in a slow upstream in conical incubators with a volume of 260 litres and a constant flow of approximately 3 l/min.

The day before hatching the eggs were transferred to the experimental units floating in the sea outside the hatchery. The eggs were carried in buckets with water which were emptied into the experimental units.

The number was calculated by counting representative buckets randomly chosen from the buckets emptied into the enclosures. Three rounds of 23 buckets each were filled with equal volumes of water containing eggs. For each round three buckets (totally 3x3) were counted, and the mean used for estimating the number of eggs in each bucket.

According to this, 5070 eggs were transferred to each unit (0.5 eggs per liter sea water).

The experimental units were made of a wowen polyethylene fabric, covered with black polyethylene film on the outside, and a light grey film on the inside. They measured 2 m in diameter and had a total depth of 5 m. The enclosures had conical bottoms and a total volume of 10 000 litres. Each enclosure had a flexible hose attached to the bottom of the cone and could be drained into a submerged tank using the hose as a syphon. The enclosures were hung in floating circular frames made of 160 mm PEH.

All the enclosures were filled with water pumped from 55 m depth, filtered through sand, cartridge filter (5 µm) and UV-treated.

The eggs were allowed to hatch, and no treatment of the different groups was initiated until day three. All enclosures were supplied with 150 litres of saltwater (40 ppt) in the lowest part of the cone, and a thin layer of 12 ppt brackish water on the surface.

In 20 of these enclosures 5 different environments were established; each with 4 parallels.

Environment 1: Stagnant with 100 ppm oxytetracycline chloride.

Environment 2: Stagnant water.

Environment 3: Stagnant with an inoculum of microflagellates and ciliates.

Environment 4: Slowly circulating water.

Environment 5: Stagnant with full daylight exposure and algal bloom.

In addition one 175 m^3 six m diameter enclosure with stagnant was set up.

All the enclosures except the ones with full daylight exposure (environment 5) were covered with hemispherical black plastic roofs. Throughout the experiment the salt plug with its content was drained out every 2 or 3 days, never allowing the water near the bottom to get anoxic. Both salinity, oxygen content, temperature, ammonium and bacterial growth were monitored.

All dead or living larvae trapped in the salt plug were drained out and fixed on 4% formaldehyde.

The larval material was measured for standard length (sl) and dried at 60 ^{0}C for 48 h for dry weight measurements. At day 31 when the larvae had developed functional jaws, one enclosure from each of the experimental groups were emptied and all the larvae were utilized in a morfological characterization of the material from each environment.

In the 16 remaining enclosures, natural zooplankton, (150-300 µ m) filtered from seawater was added daily. The number of plankton organisms was kept at approximately 10 per liter.

Analysis of nutrient salts

The samples were preserved with chloroform to 0.5 % (v/v) final concentration on 100 ml dark bottles. The samples were stored refrigerated (5-6^{0}C) until analyzed.

The analysis of PO_4^{---} was preformed according to (K. Grasshoff et al.) on a Shimadzu UV-160 spectrophotometer.

The analysis of NH_4^+ was preformed according to Solorzano (1969) on the same spectrophometer. Only 1 cm light-path was used thus reducing the sensibility of the method.

Counting of microorganisms

The samples were fixed with formaldehyde to 3.5 % (v/v) final concentration on 100 ml bottles. The samples were kept refrigerated (5-6 ^{0}C) until counted.

Bacteria and protozoa were enumerated as decribed in Porter and Feig (1980). For each sample only one subsample was withdrawn, stained and filtered. The filters were enumerated on a Zeiss standard microscope with epifluorescence light source.

Bacterial activity measurements

The bacterial activity in the water was measured on freshly collected samples according to Somville and Billen (1983). Fifty µl of L-Leucyl-β-naphtylmine (Sigma) and the were added to the samples developed fluorescence (Exitation and emission wavelenghts) were 360 and 410 nm, respectively).

The fluorescence was detected on a Shimadzu RP-530, HPLC-monitor and Tarkan W+W_{600} plotter. The developed fluorescence was compared to a known standard (1 µ M) of β-naphtylamine

Cultivation of bacterial grazers

A bacterial grazer Paraphysomonas sp. was isolated from a rotifer culture at the Austevoll Marine Aquaculture Station.

The grazer was rinsed to a monoxene culture with the bacterium Vibrio natriegens through successive dilution series.

Four cultures of bacteria and grazer were made in 5 l aereated bottles at 20 ^{0}C. The medium was water from the Austevoll Marine Aquaculture Station diluted to 70 % with destilled water. Mineral salts were supplied according to Onarheim (1986) and glucose was the only carbon source. The medium was autoclaved with sea water and glucose stock solution separated. Glucose was added to a final concentration of 1176 µ M giving an estimated yield of approximately 10 bacteria/ml.

At harvest the cultures contained $4x10^{5}$ bacteria/ml and $5x10^{6}$ grazers/ml.

The cultures were acclimatized slowly to ambient temperature before supplied to the surface waters of the microflagellate enclosures.

RESULTS

Egg material

The eggs used for the production experiment showed low mortality in the incubation period till 80 day-degrees and the transfer to the enclosures.

A list of egg volumes stripped, fertilization percentages and mortalities in the egg stage are presented in table 1.

Environmental parameters

Temperatures were nearly constant throughout the period. The temperature development for the experimental period is shown in fig. 1.

Oxygen measurements showed saturated water near the surface and down to 3 m depth. Near the bottom and especially in the lower part of the cones the oxygen-tension was at times as low as 10% saturation when the salt plugs were renewed. In the daylight exposed enclosures the oxygen content was higher (up to 160% saturation) in midwater and lower in the bottom of the cones (concentrations as low as 5% were found).

The concentrations of inorganic phosphate and ammonium are given in table 3. At one sampling date (April 10th 1986) the pH in the bottom water in all the enclosures was measured. The pH ranged from 6.53 - 7.03 in collected samples.

Microbial development

The bacterial counts from the different environments are given in figures 2-7, and the counts of the bacterial grazers are given in figures 8-13. The bacterial activity measurements are given in table 2.

Yolk sac larvae in the different environments

Figs. 14-19 show the estimated reduction in numbers of larvae in the 5 different environments throughout the experiment.

At the time of functional jaws (day 31), the total sampling of one out of each environment type showed the highest survival in the one with antibiotics (2375 living larvae, 46.8%). The stagnant enclosure had 1697 remaining living larvae (35.8%), the microflagellate enclosure had 1095 (21.6%), the circulating enclosure 724 (14.3%), and the daylight-exposed enclosure 173 (3.8%).

The mean dry weights of body and yolk sac from each environment (N=100) are shown in fig. 20.

The mean standard lengths (Sl) of larvae from the five different environments are shown in fig. 22.

The fraction of live larvae with skeletal deformities at day 31, mainly abnormal mouths, are shown in fig. 24.

With the exception of the daylight-exposed larvae, all living larvae in the four other environments looked lively and ready for first feeding.

At day 49 posthatching, total sampling of another enclosure from each environment was carried out. The remaining living larvae were counted and measured for dry-weights and standard lengths as well as examined for skeletal deformities.

The different enclosures had the following numbers of living larvae: microflagellates 519 (10.2%), the antibiotic enclosure 355 (7.0%), stagnant enclosure 237 (5.0%), circulation enclosure 75 (1.5%), daylight exposed enclosure 17 (0.4%).

The dry weight measurements of the larvae at day 49 are shown in fig. 21.

Mean standard lengths at day 49 are shown in fig. 23.

The fraction of abnormal larvae at day 49 are shown in fig. 24.

First feeding results

In one of the enclosures with a slow circulation approximately 50 larvae were observed feeding on zooplankton and had grown to about 20 mm length after 100 days.

In the large (175 m^3) enclosure a substantial number was observed feeding as well. The number was estimated to more than 250. By this time the supplied zooplankton was the fraction between 200 and 500 µm.

The other enclosures had small numbers of larvae in growth, but none of these survived.

DISCUSSION

Environmental parameters

Low temperature during the yolk sac period is regarded as favourable since both basic metabolism as well as swimming activity will be increased by increasing temperature. Temperature is also regarded as important in stimulating feeding behaviour in fish larvae (Hunter J.R., 1972, 1977).

In the measured pH-range (6.5-7.0) the ammonia-ammonium equilibrium is biased towards ammonium. The ratio of ammonia to ammonium is 0.17% at pH 6, and 0.54% at pH 7.

Assuming a pH of 7, the ammonium measured April 26th in the bottom water represents 6.0 to 10.1 µ M ammonia (Table 3). This level may well be toxic if halibut larvae are exposed to it.

Microbiological survey

Some unintended features of the experimental setup should be noted.

From April 4th 1986 and later, an increasing population of methanogenic bacteria were found in all the enclosures exept the enclosures with antibiotics. The methanogens were found both in the water coloumn and in the bottom water and reached a maximum of approximately 5% of the total count April 26th 1986. The precence of methanogens means that there were anoxic "microenvironments" even if oxygen always was present in the samples. In marine anoxic environments, sulfate reducing bacteria will produce the highly toxic H_2S.

A loss of larvae was observed from day 7 to day 10 in all the enclosures. There are no support in the bacterial counts that this event was of microbiological origin. The loss/death of larvae led to an increase in bacterial numbers (figs. 2-7). This increase was followed by an increase in bacteria grazing flagellates (Figs. 8-13).

The measurements of bacterial activity (table 2) reflected the concentration of bacteria (Figs. 2-7).

It was good agreement between the measured bacterial activity and the concentrations of ammonium (table 2 and 3 respectively). When using proteins as an energy source, many bacteria will excrete surplus nitrogen as ammonia.

Antibiotic treatment caused low bacterial concentrations in both water colomn and bottom water. This was also reflected in bacterial activity (table 2) and in concentration of bacterial grazers (fig. 12).

Enclosures with daylight had a massive algal bloom after approximately one week. Concentrations of Halosphaera sp. and Chaetoceros spp. reached a maximum of approximately 10 cells/ml.

The algae maintained a large population of bacteria and grazers causing a rapid turnover of nutrients (figs. 2, 8 and table 3). Compared to the other environments it was a smaller difference in bacterial activity between bottom water and the watercolumn (table 2). The supply of bacterial grazers showed only moderat success in controlling bacterial concentrations (figs. 5 and 11). A high count of bacterial grazers in the water column after the supply slowly decreased. Much of the supply of grazers was later found in the bottom water and caused levels of ammonia comparable to the enclosures with daylight. Ciliates predating the flagellates could be an explanation to the high concentration and activity of bacteria in these enclosures.

The enclosures with stagnant water and with circulating water developed very similar (figs. 3, 4, 9, 10 and tables 2, 3).

The renewal of ca. 25% of the water per day in the enclosures with circulation showed little impact on the parameters.

It was later observed that the water pipe within and after the filters etc. was contaminated with a complete microbial society.

All the bags were therefore supplied with microorganisms well adapted to the specific water in use. Medium sized ciliates, considered to be predators on the smaller sized bacterial grazers was thus introduced.

The enclosures were not totally separated from the surrounding water. There were perforations in the walls from the sewing of the enclosures, and it was free passage through the overflow sieve. Influence from the outside waters could be traced in periods of the experiment. In the covered enclosures, debris from phytoplankters was observed.

Egg quality

Less than 13% of the eggs died during incubation. Hatching percentages varied in the different environments and were lower in the light exposed enclosures than in the others. This is in good accordance with the results found by Rabben and Jelmert (1986).

Judging from the numbers of unhatched eggs drained through the bottom hose, a hatching percentage of 90% in light enclosures, 99% in circulating enclosures, 99% in microflagellate enclosures, 93% in stagnant enclosures, and 99% in antibiotic enclosures was observed.

Yolk sac larvae

In all environments the larvae were distributed near the surface (upper 1m) the first week after hatching. On the 10th day a high mortality was observed in the enclosures exposed to daylight (14%), while in the other systems there was a less dramatic reduction in larvae (9%, 9% and 8% respectively). One of the four enclosures with antibiotics had a very high number of larvae drained out on day 10. These were alive, and the drainage was caused by a loss of the salt plug. Apart from this special event the 3 other enclosures of the same kind had a mean mortality of 9% on day 10.

The reduction in numbers indicated that the enclosures with roofs were clearly favourable for storing yolk sac larvae. Both numbers and quality of larvae indicated this. Various explanations can be discussed. The bacterial activity in light exposed enclosures was generally higher and the oxygen tension more labile. In addition the activity of the larvae tend to be higher in light which can lead to a faster resorption of the yolk as well as a higher mechanical stress.

Already at day 31 it was clearly shown that the larvae from the daylight exposed enclosures were shorter than larvae from the other enclosures (mean Sl=8.02 mm, 9.86 mm, 9.75 mm, 10.18 mm and 9.24 mm for the daylight enclosure, stagnant enclosure, circulating enclosure, microflagellate enclosure and antibiotic enclosure respectively). The differences observed were significant on a 95% level (t-test).

Larvae from the microflagellate enclosure had the highest dry weights (mean=1.12 mg), and the larvae from the antibiotic enclosure had the lowest dry weights (mean = 0.92 mg). The differences between dry weight means from 3 of the different environments were significant on a 95% level (t-test). There was no significant difference between the mean dry weights of the larvae from the circulating and stagnant enclosures.

The environment with antibiotics had the highest survival in the first period (to day 31) and an apparently positive effect of the bacteriostatic agent. This was true regarding the number of survivals, but not when quality and developmental status was measured. The bacterial numbers in the antibiotic environments were kept low and this kept the larvae from being stressed by indirect and direct actions of microorganisms.

It was not possible to see any positive effects of antibiotics on the quality of larvae during this first period. Later in the storing period and in the feeding stage a negative effect was demonstrated.

The light exposed larvae had 92.6% mouth deformities while the rest of the environments had less than 20% abnormal larvae and the antibiotic enclosure as little as 5%.

After day 49 the larvae in microflagellate enclosure and the circulation enclosure were almost equal, with 0.780 mg and 0.787 mg respectively.

The larvae in the antibiotic enclosure had slightly higher mean dry weight (0.998 mg) than in the stagnant enclosure (mean dry weight= 0.997 mg).

In the stagnant enclosure the larvae had a mean standard length of 12.68 mm and were significantly larger than larvae from the other enclosures, which measured 10.75 mm, 10.38 mm and 10.65 mm for the circulating, flagellate, and antibiotic enclosures respectively.

The stagnant enclosure had 11.2% deformities, the cirulation enclosure had 15.9%, the microflagellate 13%, and the antibiotic 48.4%. The larvae in the enclosure with antibiotics differed clearly from the others in that in addition to a very high degree of skeletal deformities, the fins had a greyish color and the larvae looked unhealthy and had a whirling swimming behaviour.

The enclosures with microflagellates as bacteriostatic agent, showed a deviation from the enclosures kept stagnant without any bacteriostatic agent. Both larval lengths and dry weights were significantly higher in the microflagellate enclosures after 31 days, but after 49 days it was opposite.

An explanation to this is not possible to find from this experiment. It was not demonstrated any significant differences in bacterial populations as effects of grazing microflagellates.

At the time of metamorphosis the number of living postlarvae were reduced as they started settling on the bottom. Dead larvae were drained out through the bottom hose the last days before the rest were captured by diving. When the larvae were ready for bottom settling, they seemingly went down into the bottom water even though this from time to time showed low oxygen tension.

REFERENCES

Blaxter, J.H.S., D. Danielssen, E. Moksness and V. Øiestad, 1983. Description of the early life development of the halibut (Hippoglossus hippoglossus L.) and attempts to rear the larvae past first feeding. Mar. Biol. 73:99-107.

Grasshoff, K., M. Erhardt, K. Kremling (Eds.), 1983. Methods of seawater analysis. Verlag Chemie Gmb H, D-6940 Weinheim.

Hunter, J.R. 1972. Swimming and feeding behaviour of larval anchovy, Engraulis mordax. U.S. Fish. Bull. 70:821-838.

Hunter, J.R. 1977. Behavior and survival of northern anchovy, Engraulis mordax larvae. Calif. Coop. Oceanic. Fish. Invest. Rep. 19:138-146.

Onarheim, A.M. 1986. Vekst og innhold av proteiner og nukleinsyrer og vekst hos V. natriegens. Thesis in general microbiology, Univ. of Bergen.

Porter, K.G. and Y.S. Feig, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25(5)943-948.

Rabben, H. and A. Jelmert, 1986. Hatching of halibut (Hippoglossus hippoglossus L.) eggs under different light conditions. Coun. Meet. int. Coun. Explor. Sea, 1986(F:17)(Mimeo).

Soloranzo, L. 1969. Determination of ammonia in neutral waters by phenol-hypochlorite method. Limnol. Oceanogr. 14:799-801.

Somville, M. and G. Billen, 1983. A method for determining exo-protolytic activity in neutral waters. Limnol. Oceanogr. 28(1):190-193.

Table I. General egg data.

Fish-number	Eggvolume incubated (ml)	Eggmortality during incubationperiod (ml)	Fertilization %
21	1000 (ml)	50 (ml)	93.8%
60	1200 (ml)	475 (ml)	74.8%
69	500 (ml)	0 (ml)	96.4%
71	1600 (ml)	965 (ml)	90.8%

Table 2 Bacterial activity in the different environments.
Activity is measured as nmol BNA/(ml x min).
Legends: W=water coloumn, B=bottom water

		Daylight		Stagnant		Circulation		Flagellates		Antibiotics		Large enclosure	
Sample		W	B	W	B	W	B	W	B	W	B	W	B
Date	4/11/86	0.049	0.388	0.010	0.219	0.018	0.389	0.016	0.532	n.d.	0.060	-	-
	4/27/86	0.104	0.839	0.007	0.120	0.006	0.151	0.003	0.210	n.d.	0.050	-	-
	5/5/86	-	-	-	-	-	-	-	-	-	-	0.273	0.571

Table 3 Concentrations of inorganic phosphorous and ammonia.
Legends:As above. Concentrations as uM.

Date			Daylight	Stagnant	Circulation	Flagellates	Large enclosure
3/14/86	W	PO4---	0.1	-	-	-	0.2
	B	PO4---	0.2	-	-	0.5	0.9
	B	NH4+	192	635	650	841	400
3/20/86	W	PO4---	0.1	0.3	-	0.7	-
	B	PO4---	2.4	2.0	4.9	6.9	4.1
	B	NH4+	1213	840	835	1128	505
3/24/86	W	PO4---	-	-	0.2	10.2	-
	B	PO4---	6.5	2.5	-	-	4.6
	B	NH4+	-	-	-	-	-
4/01/86	W	PO4---	-	-	-	0.3	0.3
	B	PO4---	7.7	3.2	5.0	13.1	15.4
	B	NH4+	1388	759	538	1266	942
4/07/86	W	PO4---	-	0.5	-	-	-
	B	PO4---	5.5	6.3	2.0	8.6	15.5
		NH4+	923	381	727	927	1516
4/10/86	W	PO4---	0.3	0.1	-	0.1	-
	B	PO4---	5.0	-	12.5	7.2	14.9
	B	NH4+	989	626	844	1335	1441
4/26/86	W	PO4---	0.6	0.2	1.0	-	0.5
	B	PO4---	7.8	12.7	10.7	8.1	15.4
	B	NH4+	1870	1116	1652	1702	1776

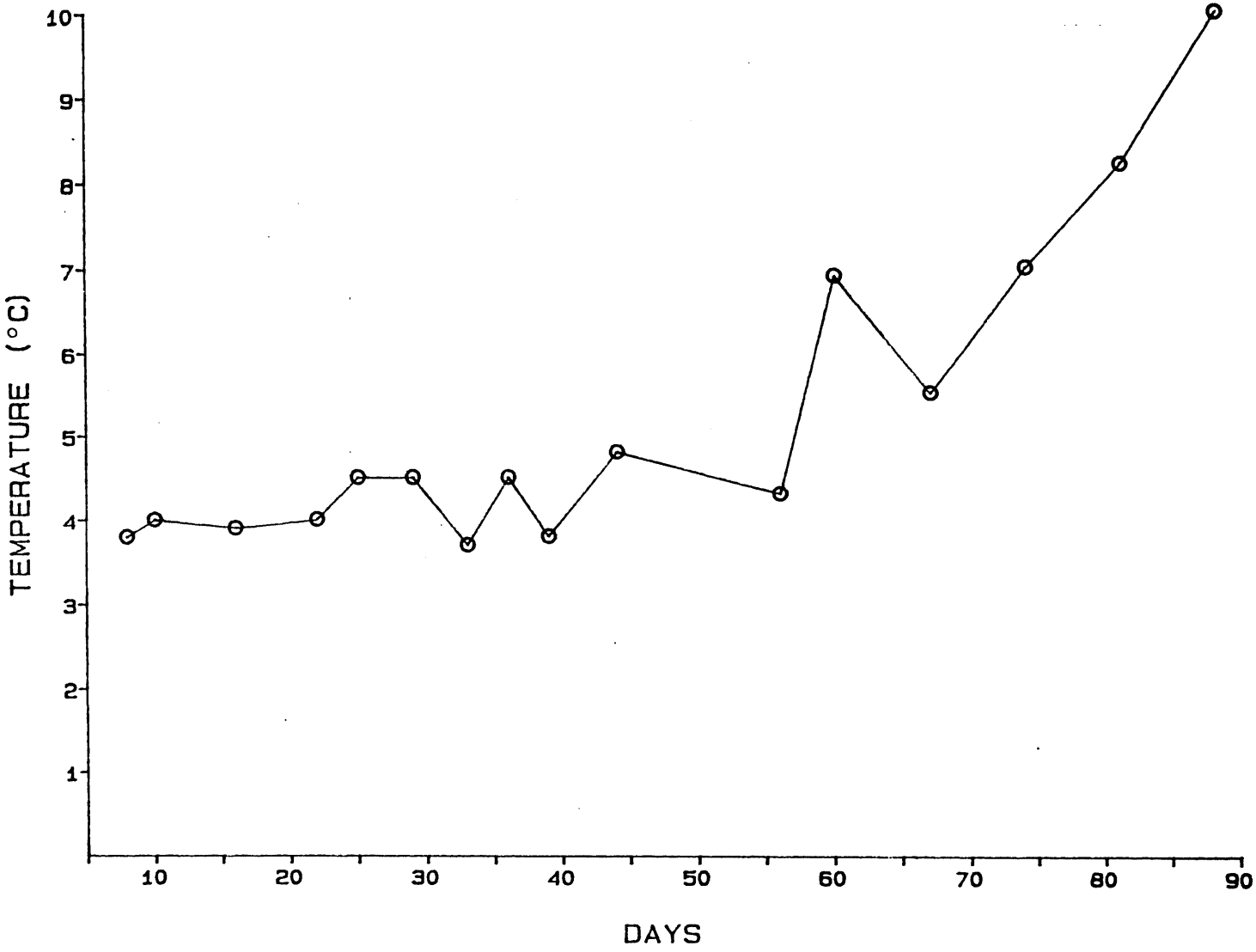

Fig.1: Temperature variations during experiment.

Fig. 2.

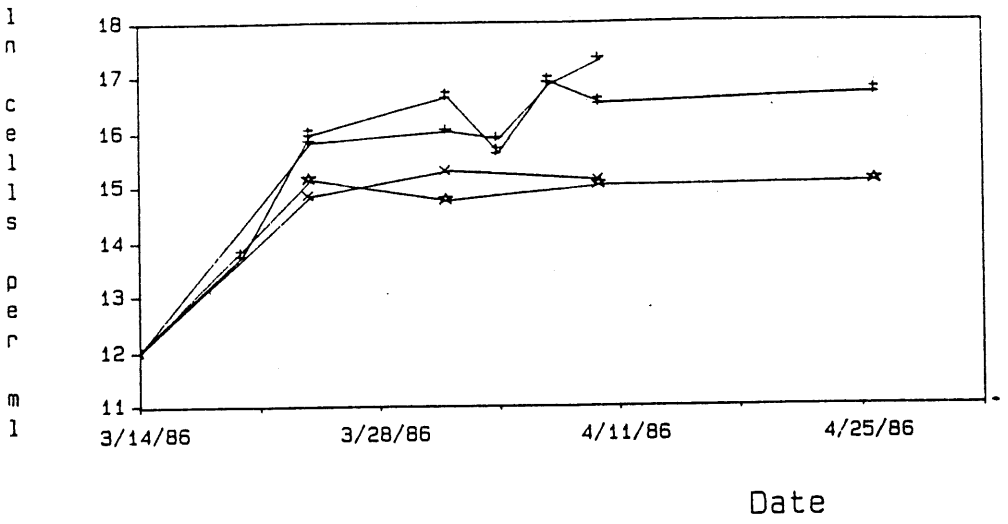

Fig. 3.

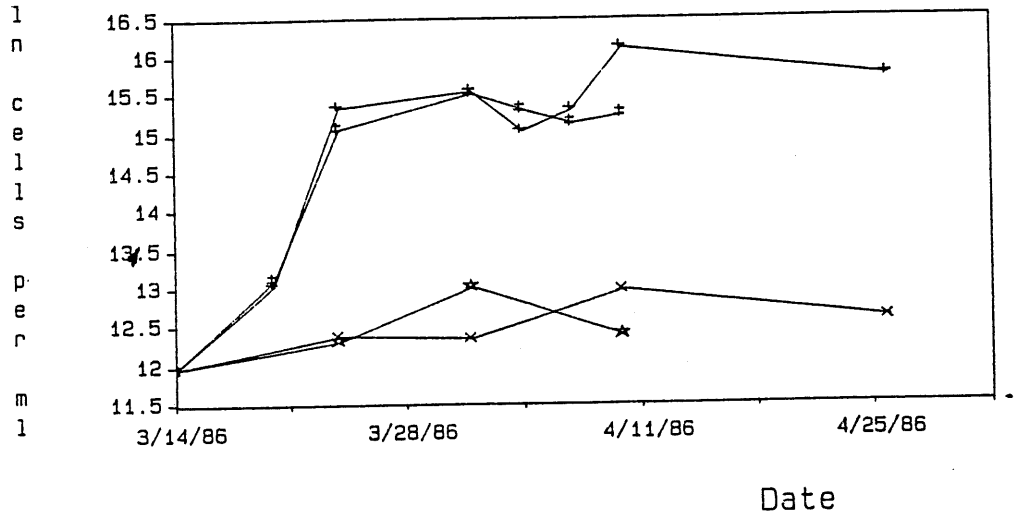

Fig. 4.

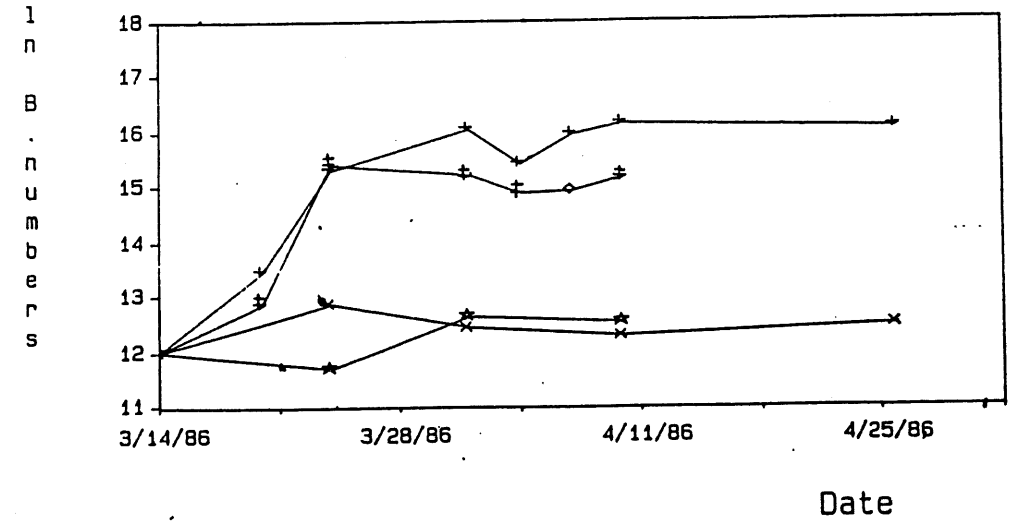

Figure 2 - 4 Concentrations of bacteria as ln cells per. ml.

Fig. 2 = Daylight, Fig. 3 = Stagnant, Fig. 4 = Circulation.

Symbols: + and ǂ bottom water (two encl.)

x and ✯ watercolumn (two encl.)

Fig. 5.

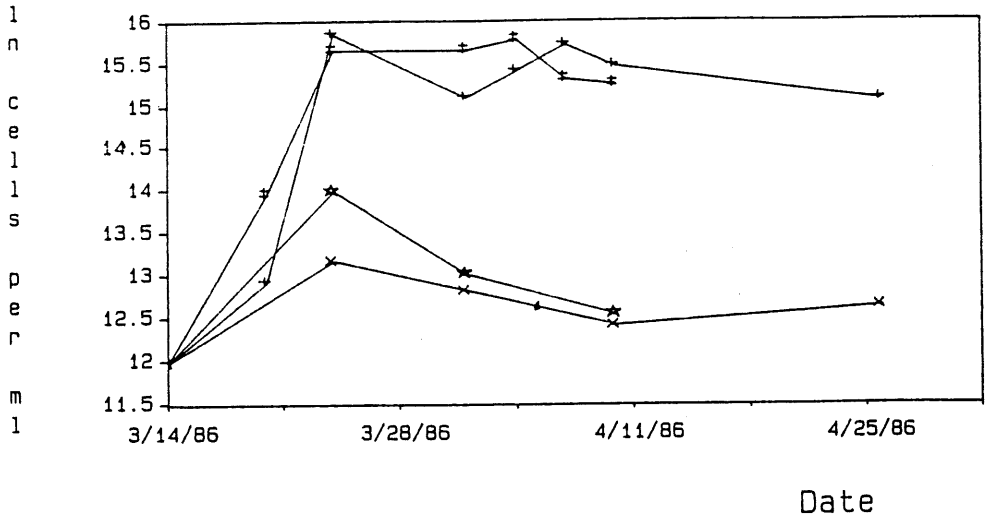

Fig. 6.

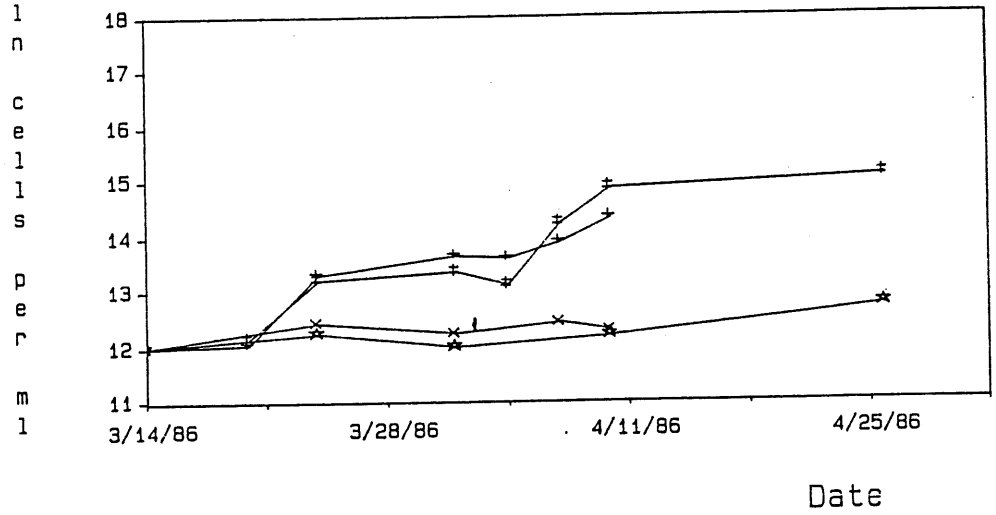

Fig. 7.

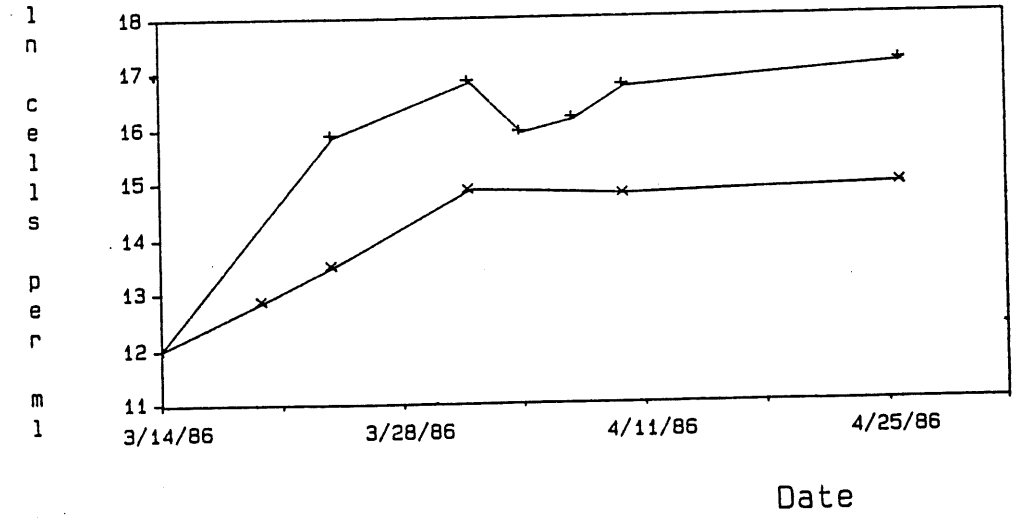

Figure 5 - 7 Concentration of bacteria as ln cells per. ml.

Fig. 5 = Flagellates, Fig. 6 = Antibiotics, Fig. 7 = Large enclosure.

Symbols: + and ‡ bottom water (two encl.)
x and ✱ watercolumn (two encl.)

Fig. 8.

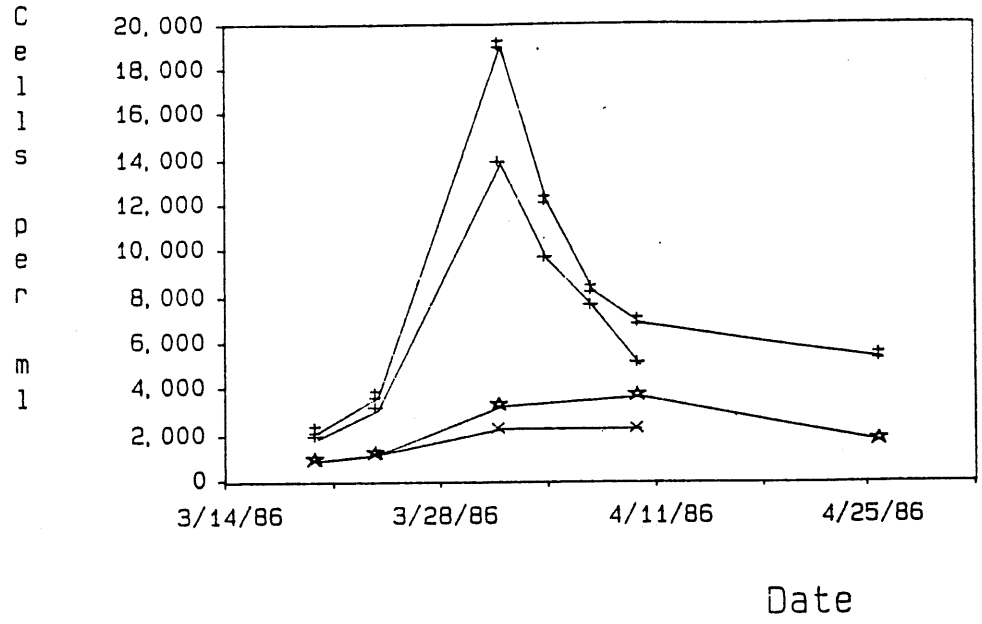

Fig. 9.

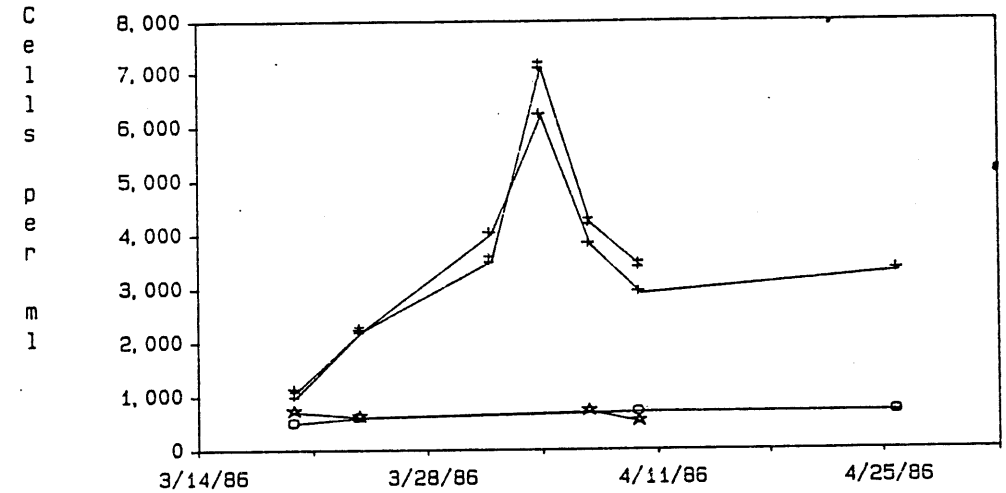

Fig. 10.

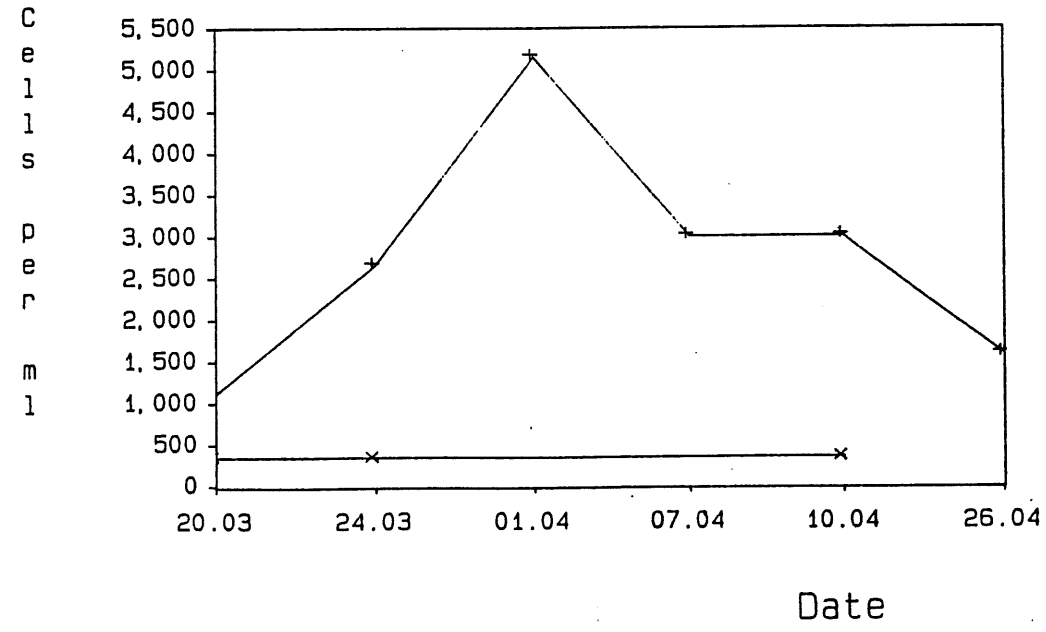

Figure 8 - 10 Concentrations of heterotrophic eucaryotic microorganisms.

Fig. 8 = Daylight, Fig. 9 = Stagnant, Fig. 10 = Circulation.

Symbols: + and ⧺ bottom water (two encl.)
x and ✡ watercolumn (two encl.)

Fig. 11.

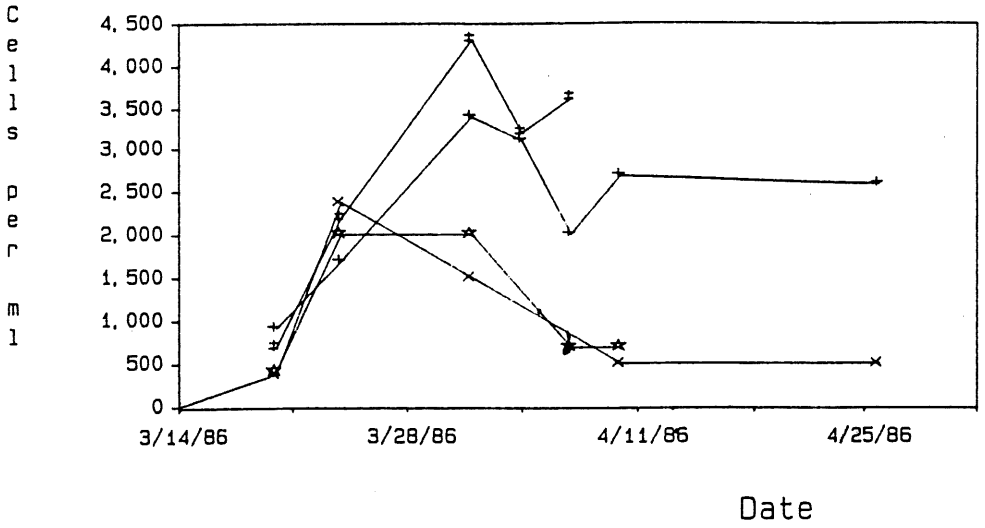

Fig. 12.

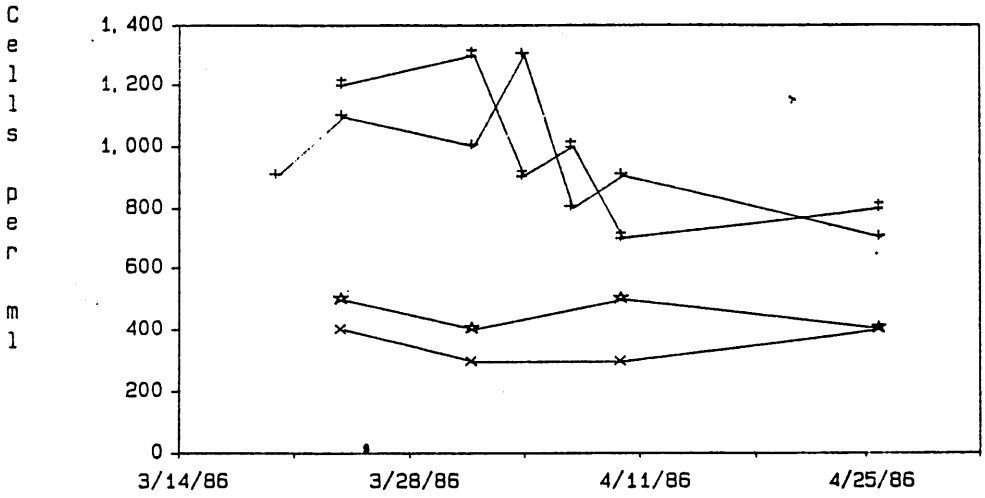

Fig. 13.

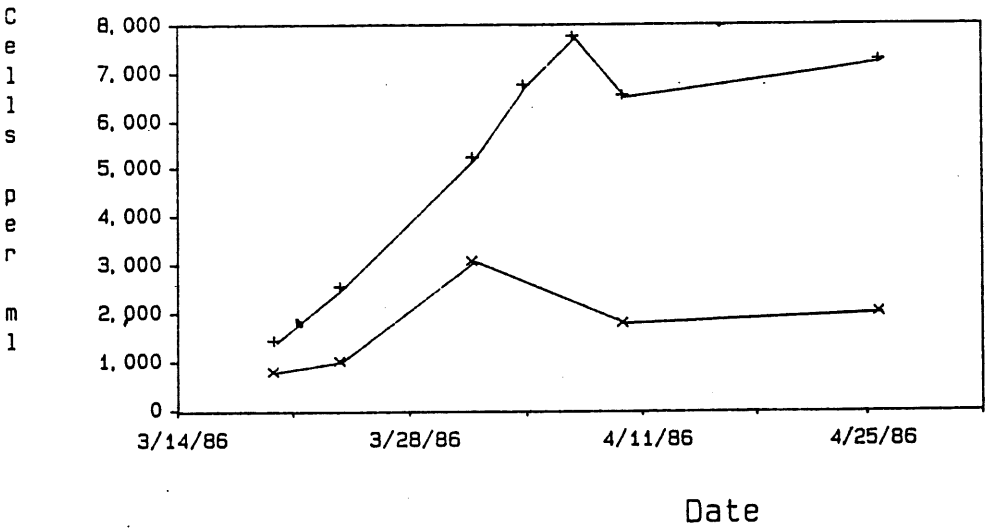

Figure 11 - 13 Concentrations of heterotophic eucaryotic microorganisms.

Fig. 11 = Flagellates, Fig. 12 = Antibiotics,
Fig. 13 = Large enclosure.

Symbols: + and ≠ bottom water (two encl.)
x and ✱ water column (two encl.)

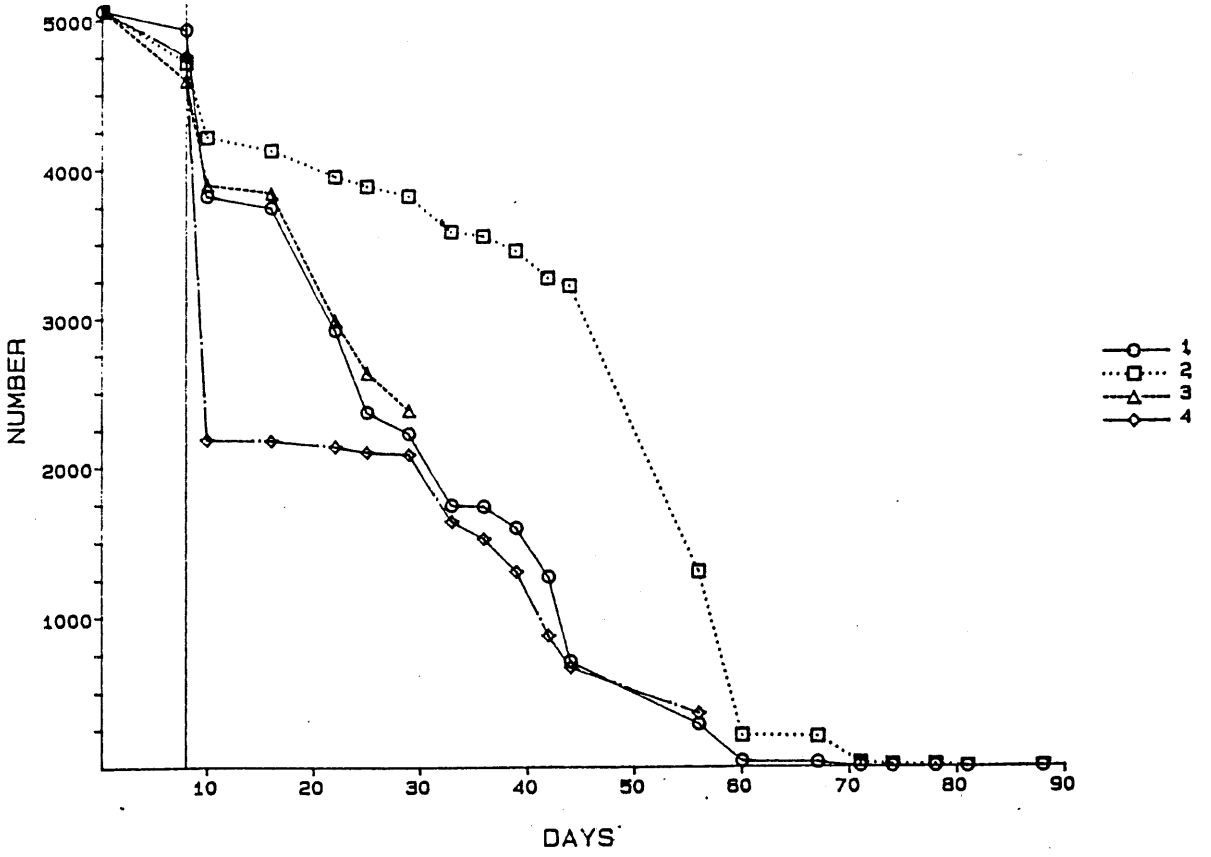

Fig. 14: Estimated survival in stagnant water columns with antibiotics added.

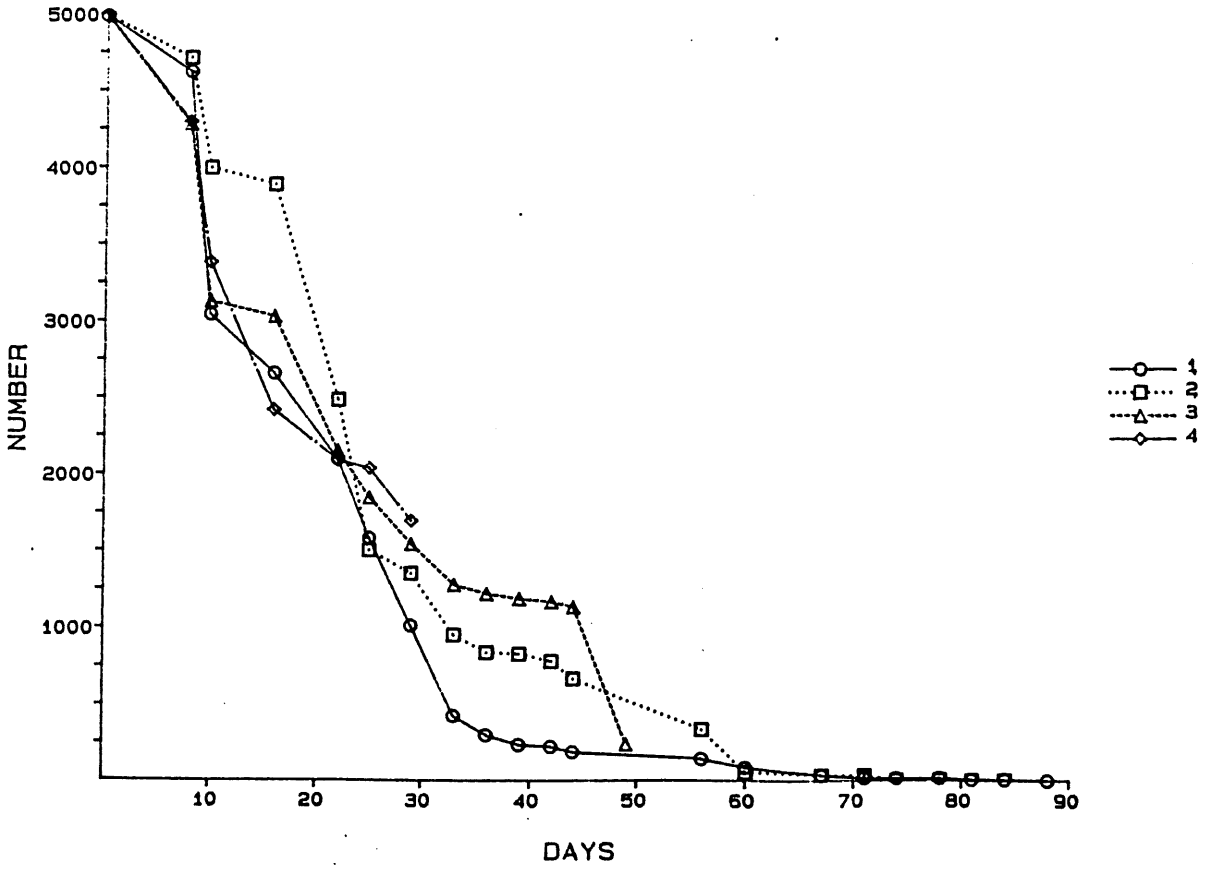

Fig. 15: Estimated survival in stagnant water columns.

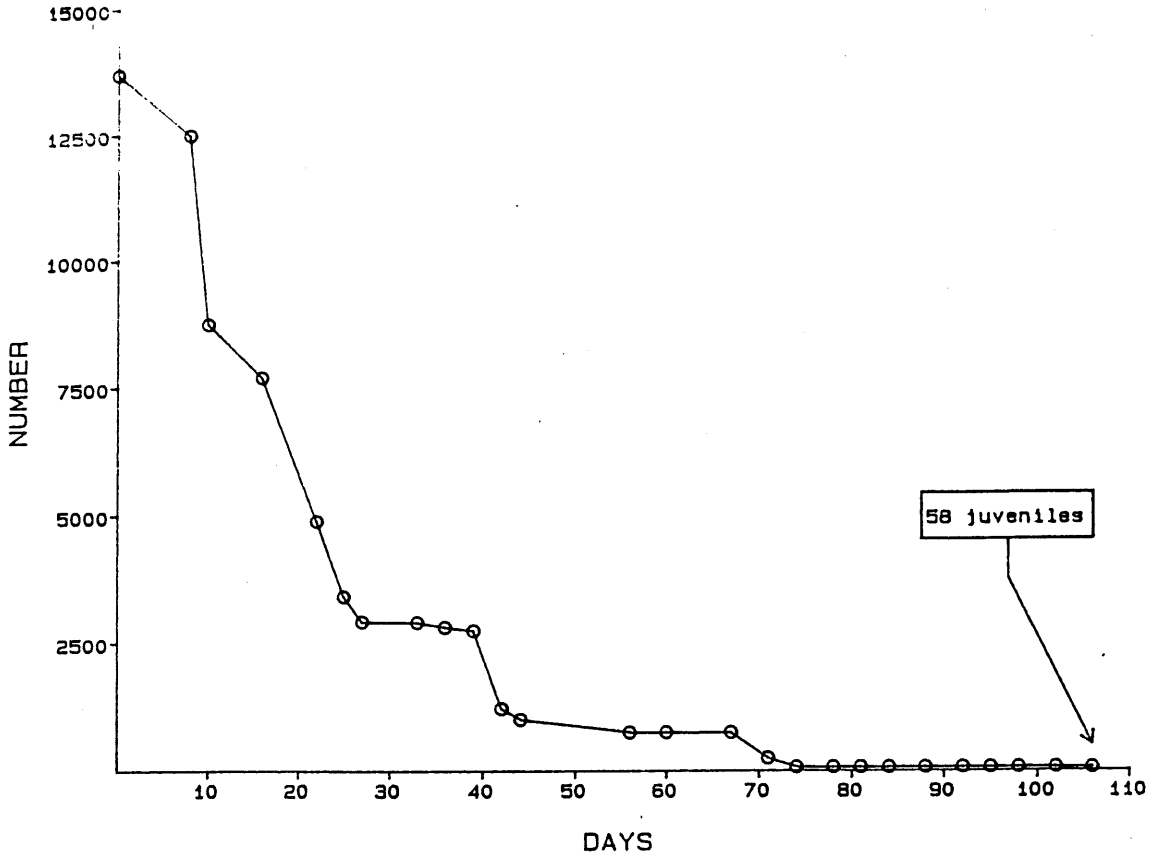

Fig. 16: Estimated survival in large (175 m^3) stagnant water column.

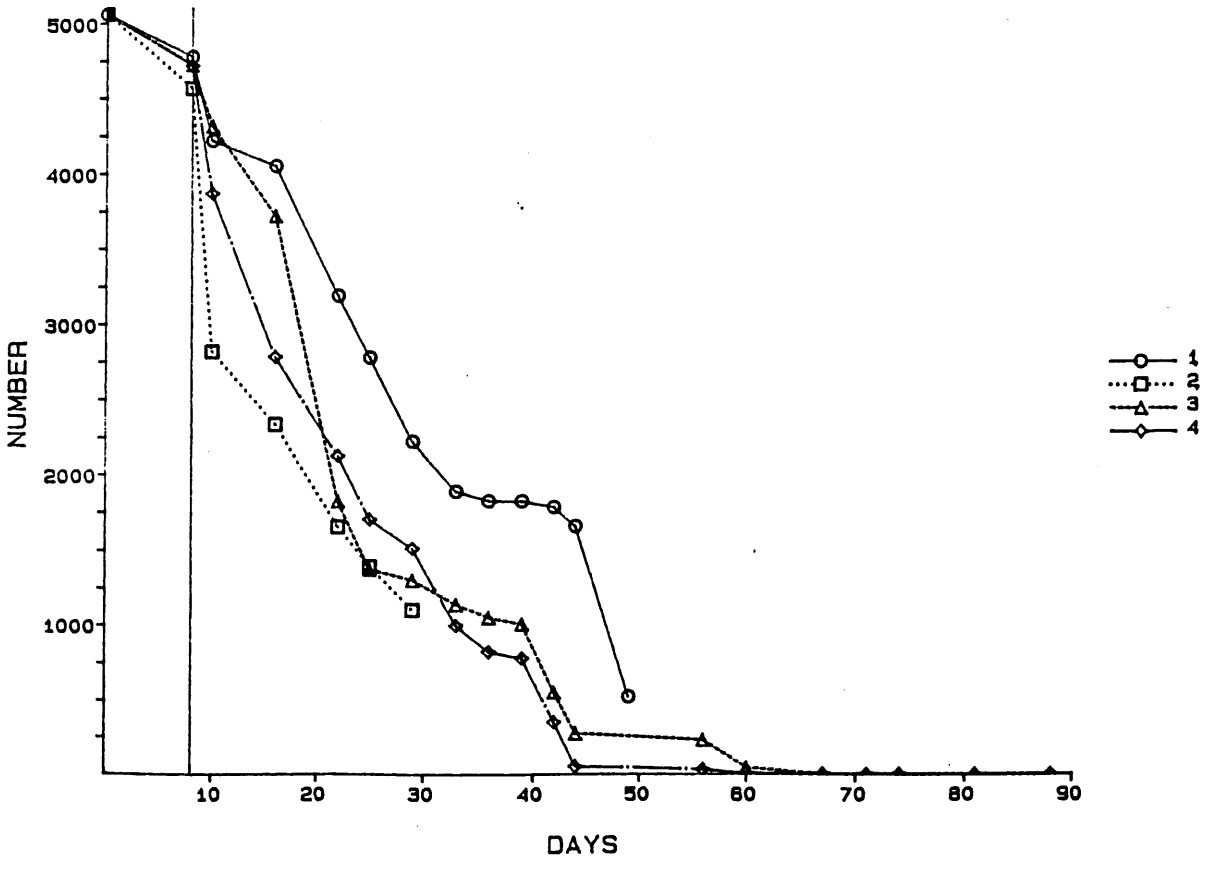

Fig. 17: Estimated survival in stagnant water columns with an inoculum of microflagellates.

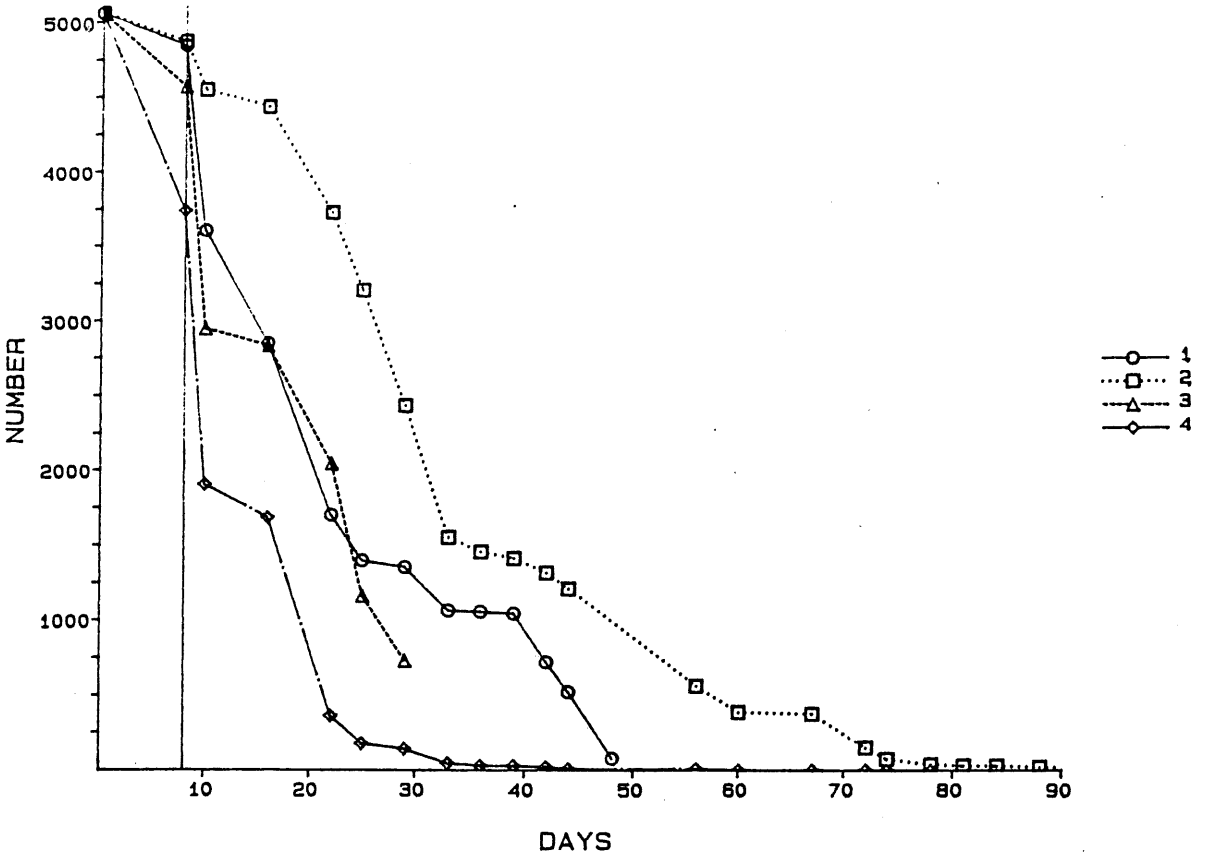

Fig. 18: Estimated survival in water columns with slowly circulating water.

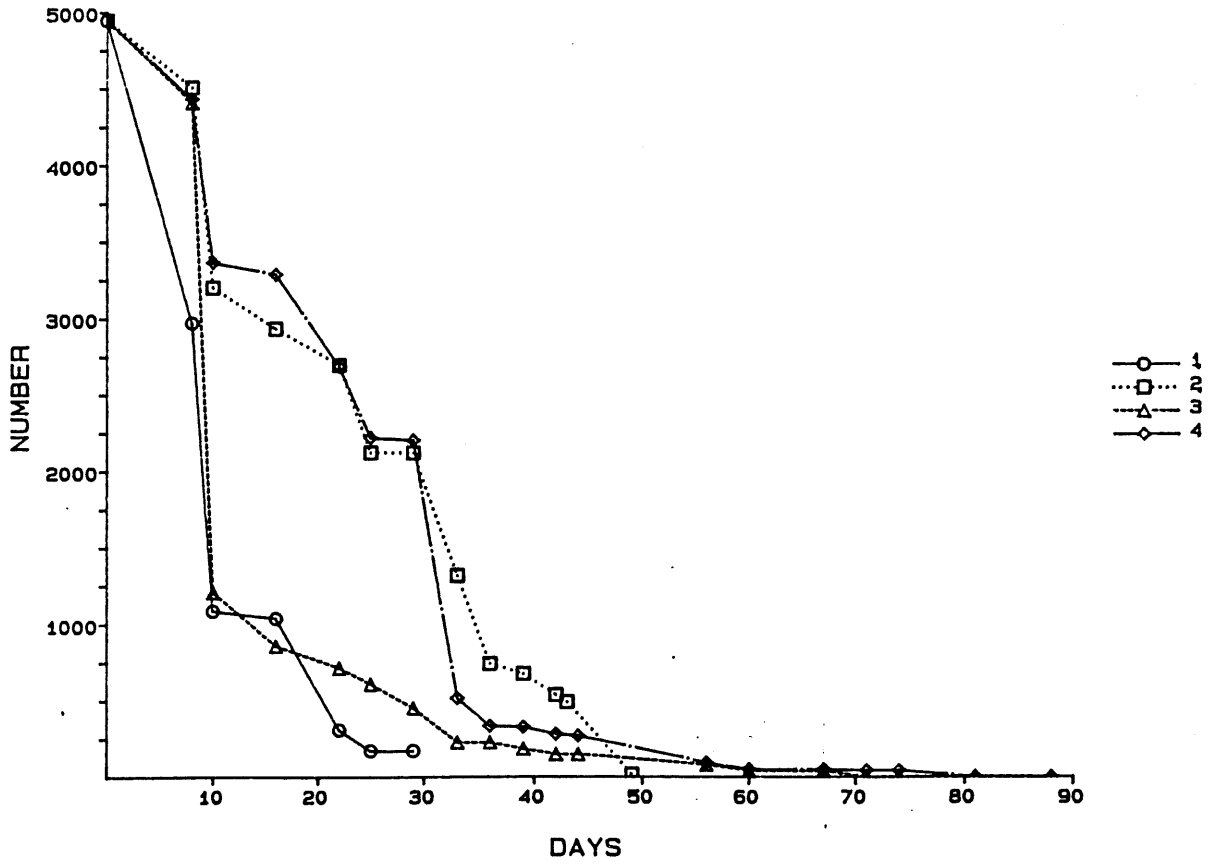

Fig. 19: Estimated survival in water columns exposed to daylight.

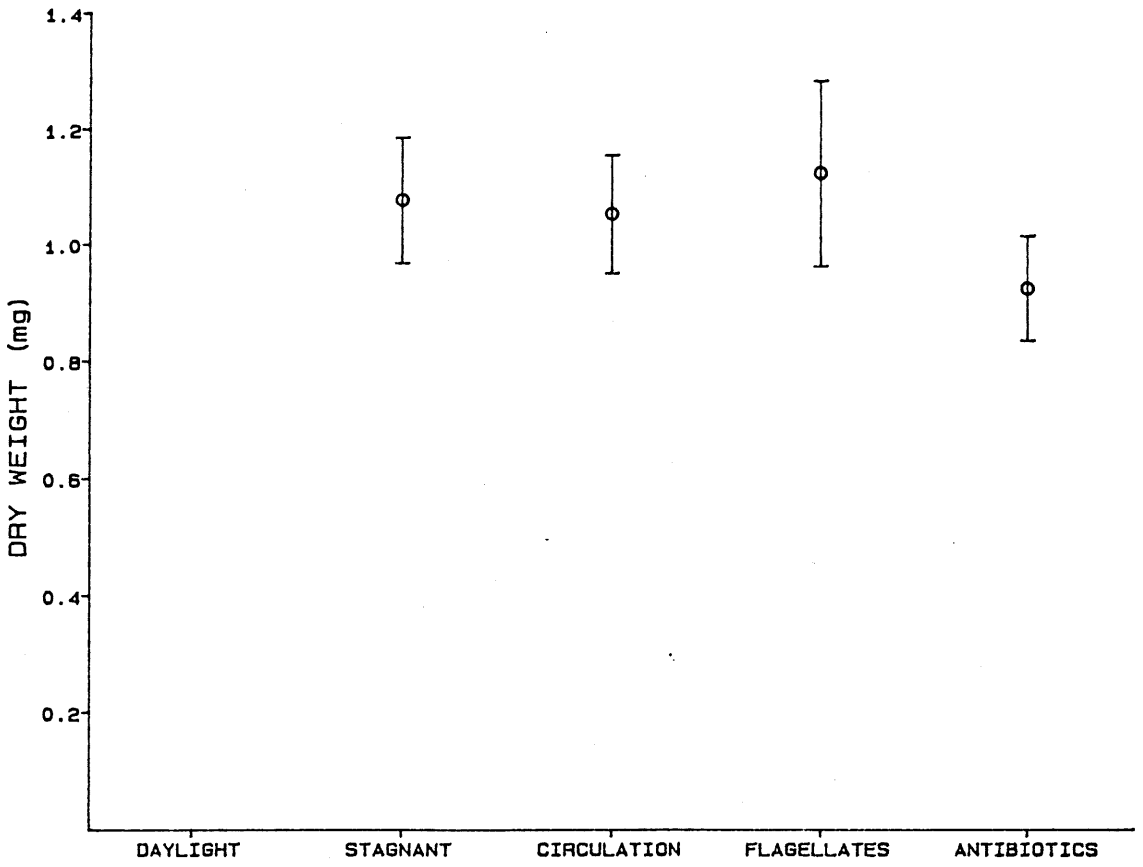

Fig. 20: Mean dry weights of yolk sac larvae at day 31 posthatching.

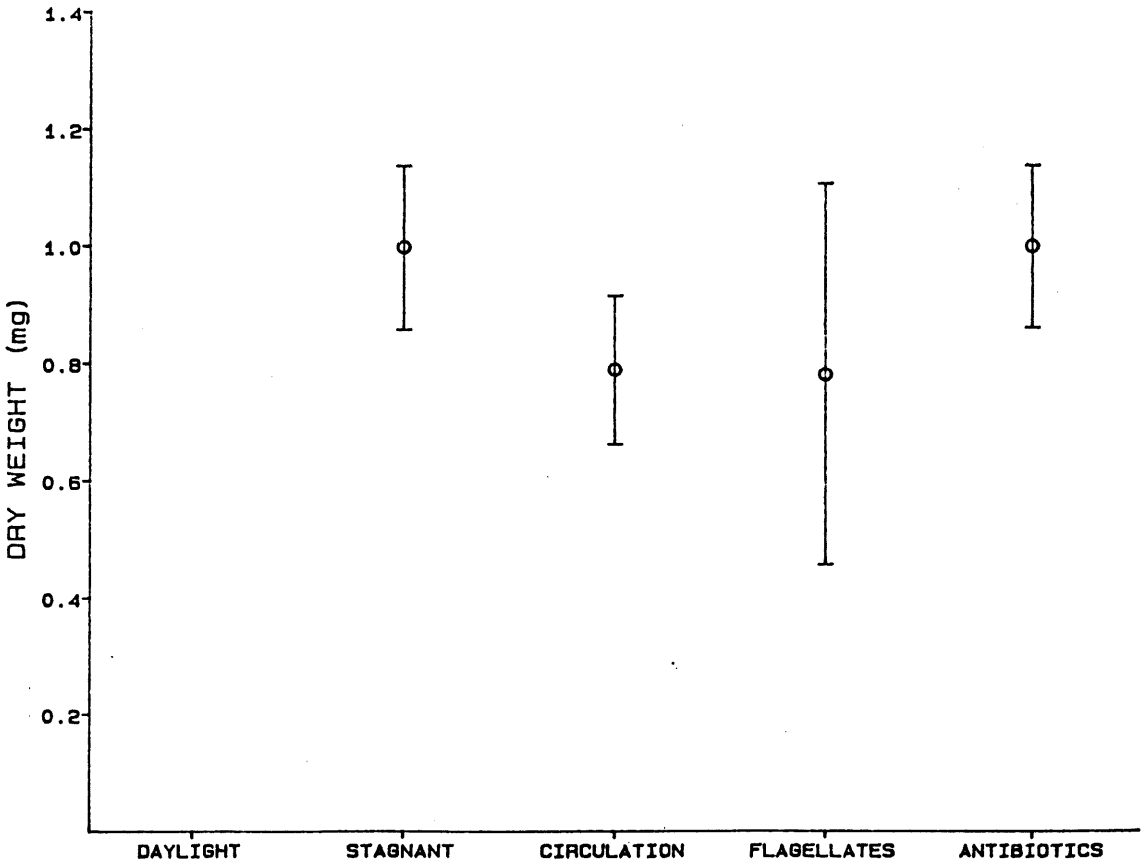

Fig. 21: Mean dry weights of yolk sac larvae at day 49 posthatching.

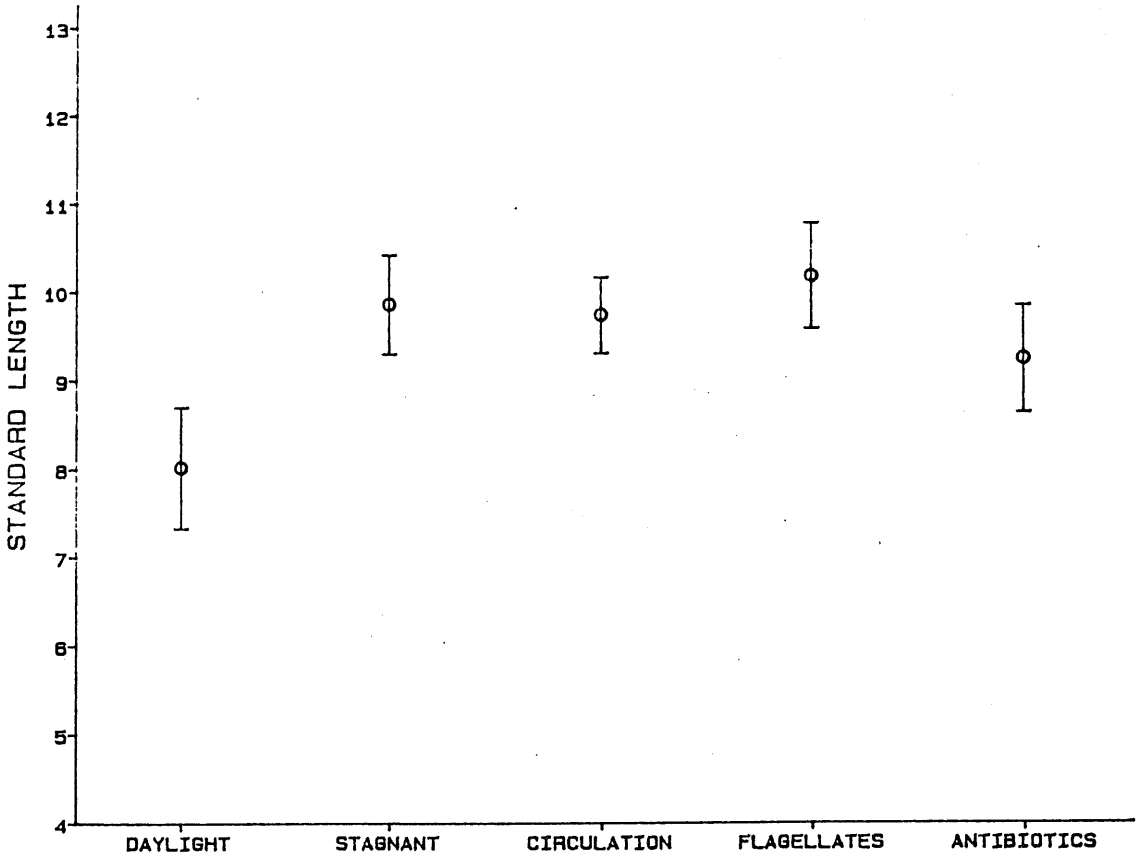

Fig. 22: Mean standard lengths of yolk sac larvae at day 31 posthatching.

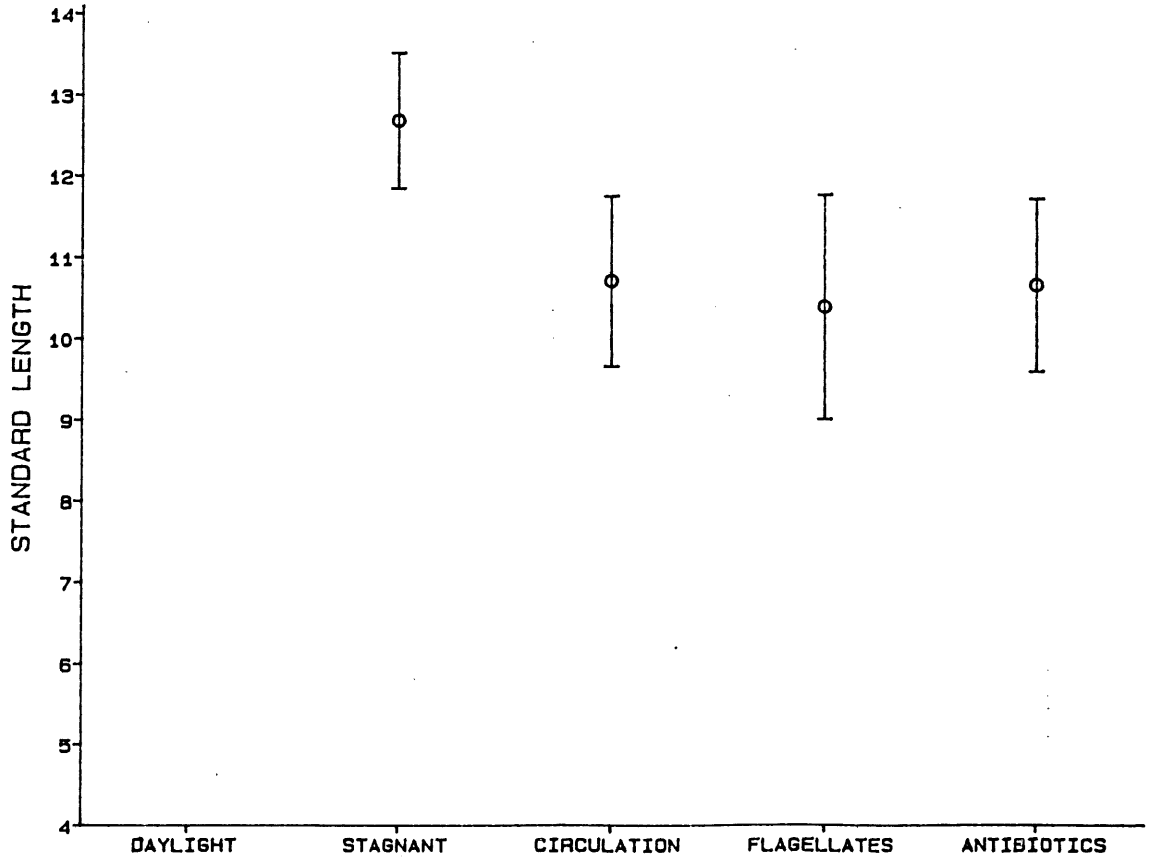

Fig. 23: Mean standard lengths of yolk sac larvae at day 49 posthatching.

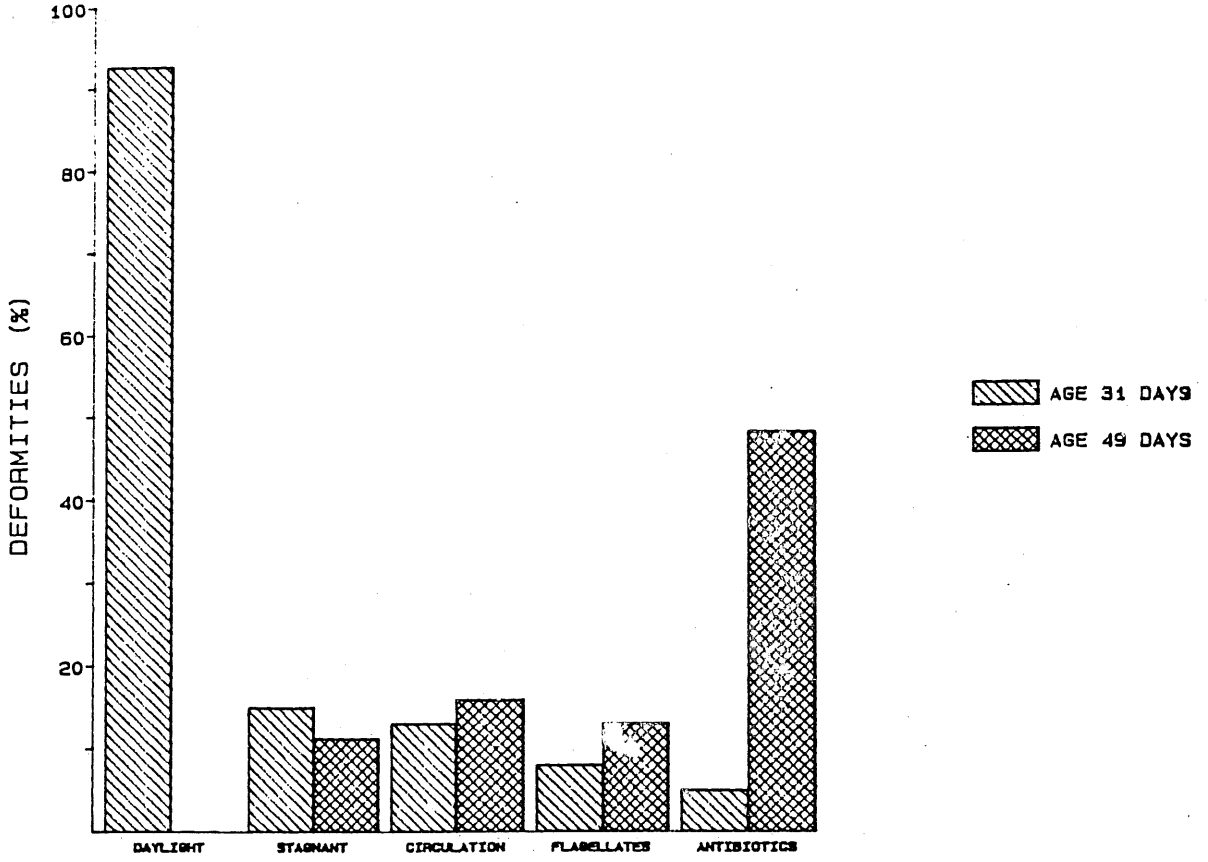

Fig. 24: Fractions of abnormal larvae.

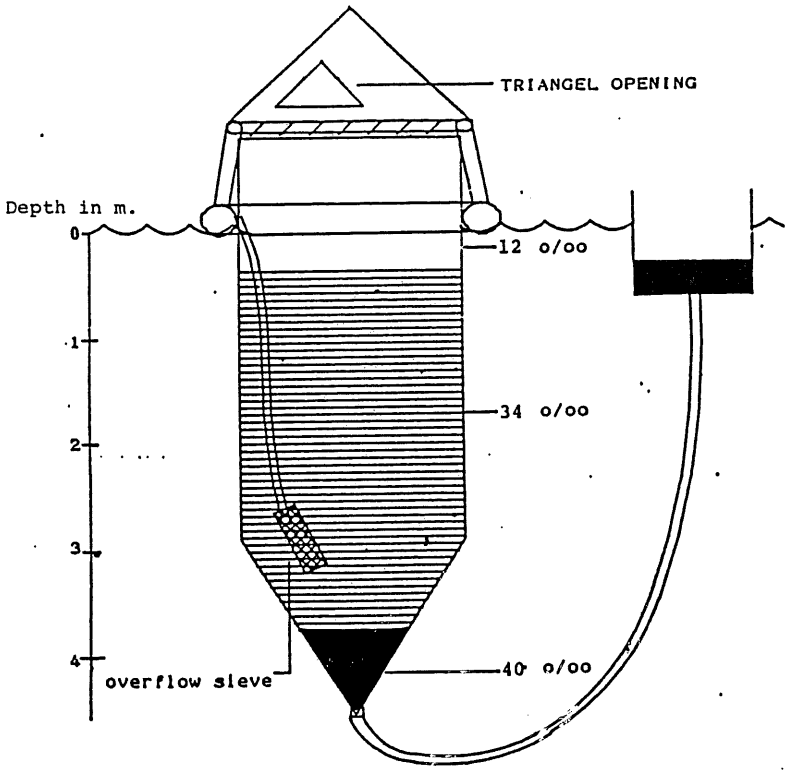

Fig. 25: Experimental unit with roof and draining hose.