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Use of ATP as Biological Indicator in Experimental Ecosystems Undergoing Natural and Stress Conditions.

by

M. L. MacKinnon
Great Lakes Biolimnology Laboratory
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November, 1975

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"The marine ecologist needs to observe his subjects in a controlled environment. The open sea is too big; the laboratory beaker is too small".

(New Scientist - February, 1967)

INTRODUCTION

Since the late 1950's experimental ecosystems have been widely used in Europe and North America for a variety of specific purposes by different scientists. Some were interested in doing experiments under natural conditions to study plankton ecology and improve productivity measurements (Thomas, 1958, '59, '60 and '61; Strickland, 1960; Goldman, 1962; McConnell, 1962; Antia et al. 1963; Strickland et al. 1969, Kemmerer, 1969); to study sedimentation processes (Charlton, 1975),and to understand the phosphorus cycle (Lean et al., 1975). Others wanted to verify whether artificial ecosystems could or could not be used to test the effects of toxicants or other "stress substances" on bodies of water and to see if the information provided could be extrapolated to natural ecosystems (Gatelier et al., 1973; Metcalf et al., 1971, 1973; Cepex Project, to be published). In October, 1974, the Great Lakes Biolimnology Laboratory initiated a series of studies on four Lake Column Simulators (LCS). Three experiments have now been run in order to become familiar with the Lake Column Simulators and to observe the effects of dredged spoils on aquatic biota.

The choice of studying dredged spoils, among the different sources of lake pollution, has been done because of the little information actually available on dredged spoils. The economically vital communication system represented by the Great Lakes is threatened by excessive accumulation of

sediments in some areas. In order to keep this waterway navigable, bottom sediments from the main harbours have been dredged for several decades. The actual annual volume of dredged spoils (approximately 15 million cubic metres) is not expected to change during the coming years. Regarding the disposal, it is recommended to continue open lake disposal of unpolluted dredged spoils because of the relatively high cost of confined disposal (Raphael et al., 1974). Yet the effects of dredged spoils on the fauna and flora living in the open lake waters are not well established and there is no "criteria for determining the elegibility of dredged spoils for open lake disposal".

This report deals exclusively with the ATP data[1] collected during the three experiments. It is written to clarify the effectiveness of ATP as an environmental parameter in artificial ecosystems undergoing natural and stress conditions and to indicate some limits and possibilities of Lake Column Simulators for predictive environmental purposes.

[1] The team of G.L.B.L. who participated on this project intends to publish all the results in a more comprehensive report in the near future.

DESCRIPTION

1. Lake Column Simulators

The four stainless steel Lake Column Simulators used are 4.5 metres high, 1 metre in diameter and contain approximately 3500 litres. The temperature is controlled at 22°C in the epilimnion and at 12°C in the hypolimnion (the thermocline is established between 2.25 and 2.50 m using a submersible pump which mixes the epilimnetic water). An automated lighting system with a 15 hour light period simulates dawn, high light and dusk (the maximum light intensity is about 2000 $ergs.cm^{-2}.s^{-1}$). All the specifications are detailed in an "operational manual for lake column simulators" issued by the manufacturer (Techwest, 1970).

2. Experiments

The first experiment started on October 1, 1974 and terminated on December 5, 1974. Column 1 could not be used because of technical difficulties. Columns 2, 3 and 4 were filled with raw hypolimnetic Lake Ontario water collected 1/2 mile offshore of the pumping station of the Hamilton filtration plant. Only one treatment (A) was applied (Table 1). It consisted of an addition of inorganic nutrients (No. 10 Chu medium, 1942, Appendix 1) 3 times a week. Column 2 received 33.6 mg P/week, Column 3 received 16.8 mg P/week and Column 4 received 8.4 mg P/week). Simultaneously algae (Stephanodiscus binderanus, Ankistrodesmus falcatus, and Scenedesmus quadricauda) were innoculated.

The second experiment started on December 10, 1974 and terminated on April 10, 1975. The four columns were filled with raw hypolimnetic Lake Ontario water collected 1/2 mile offshore of the pumping station of

Hamilton filtration plant. Three different treatments (A, B and C) were consecutively applied (Table 1). Treatment A consisted of an addition of autoclaved sand (48.7 kg wet weight per column), an addition of inorganic nutrients 3 times a week (No. 10 Chu medium, each column received 16.8 mg P/week), scraping of the wall three times a week to avoid periphyton growth, algal inoculation (Ankistrodesmus falcatus, Scenedesmus quadricauda and Chlorella pyrenoidosa) and fish addition (Notropis atherinoides). Treatment B started on January 20, 1975. As well as Treatment A, yeast extract was added to the four columns, 5 times a week (from 15.7 g/week at the beginning it was gradually reduced to .25 g/week on February 19, and re-increased to 1.96 g/week on February 21 until the end of the experiment). Treatment C was applied from March 25 to April 10. As well as Treatments A and B, dredged spoils collected on the bottom of Port Stanley harbour, Ontario, were added 5 times a week in column 3 (354 g/day) and column 4 (35.4 g/day); columns 1 and 2 did not receive any dredged spoils.

	Experiment I	Experiment II	Experiment III
Treatment A	Inorganic Nutrients Algae	Inorganic Nutrients Sand Algae Fish	none
Treatment B	none	Treatment A + Yeast Extract	Inorganic Nutrient Sand Algae Fish Yeast Extract
Treatment C	none	Treatment B + Dredged Spoils	Treatment B + Dredged Spoils

Table 1: Summary of experiments run in LCS.

The third experiment started on April 21, 1975 and terminated on August 8th, 1975. The columns were filled with raw hypolimnetic Lake Ontario water collected 1/2 mile offshore of the pumping station of the Hamilton filtration plant. Only two treatments (B and C) were applied (Table 1). Treatment B consisted of an addition of autoclaved sand (25 kg wet weight/column), an addition of inorganic nutrients 3 times a week (No. 10 Chu medium, each column received 16.8 mg P.week), an addition of yeast extract (1.96 g/week), scraping of the walls, algal inoculation (Ankistrodesmus falcatus, Scenedesmus quadricauda and Chlorella vulgaris) and fish addition (Notropis atherinoides). Treatment C was applied from June 11 to August 8, 1975. As well as Treatment B, dredged spoils collected on the bottom of Port Stanley harbour, Ontario, were added 5 times a week. Column 1 received 33 g/day, Column 2 received .33 g/day and Column 3 received 3.3 g/day; Column 4 did not receive any dredged spoils.

3. Sampling

In the epilimnion, continuous samples were taken with a 2 metre long tube. In the hypolimnion, composite samples were taken at approximately 30 cm from the inside wall with a needle syringe through 4 different sampling pores.

4. Data Pool

Temperature, light extinction and dissolved oxygen were measured daily. Adenosine triphosphate (ATP), chlorophyll a, particulate organic carbon and nitrogen were analyzed 2 to 3 times a week. Once a week, pH, soluble reactive phosphorus, ammonia, nitrites and nitrates, silica and alkalinity were measured; algal composition was determined. Metals (Co,

Cr, Cu, Cd, extractable Fe, dissolved Fe, Mn, Hg, Ni, Zn) were analyzed once a week during experiment III. Occasional measurements of zooplankton (number and grazing activity), of bacterial heterotrophs (number, type and activity) and of sedimentation rate have also been performed. Biomagnification of Cu, Cd, Pb, Zn, Se, As and Hg in algae, invertebrates and fish was examined.

5. ATP Analysis

The precedure is similar to that of Holm-Hansen and Booth (1966). 25 to 50 ml of water were filtered on Reeve Angel glass fibre filters (984H).[1] The ATP was extracted immediately after filtration by immersing the filter for 5 minutes in 5 ml of boiling Tris buffer (.02 M) or distilled water passed through a Milli-Q purification system.[2] The ATP content was measured on a Lab Line ATP Photometer which integrates the light decay due to the reaction of ATP on a luciferin-luciferase mixture (DuPont). A standard curve using different ATP standard solutions was used to convert counts per minute to $mg.m^{-3}$. All ATP measurements done in the LCS were replicated 2 or 3 times. For each replication, the range of values seldom exceeded $\pm$ 15 percent of the arithmetic mean.

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1. A comparison between 3 types of glass fibre filters (GF/C; Reeve Angel 934AH and Reeve Angel 984 H) showed the superiority, regarding reproductibility, of the last one as the coefficient of variation between 8 replicates was 19%, 23% and 8% respectively.

2. No significant difference was observed when aliquots of water were extracted in Tris buffer or sterily filtered bi-distilled water as the variation coefficient on 7 replicates was 9% and 6% respectively.

RESULTS

Before presenting the information gathered through ATP measurements, it is worth emphasizing that ATP represents the biomass of all living organisms (bacteria, phytoplankton, zooplankton) without considering their physiological state, degree of activity, etc. This gross parameter gives an image of all living matter which exists due to a combination of specific physical, chemical and biological conditions. The advantages of ATP over other biomass parameters are due to the fact that it corresponds to living material only (ATP disappears immediately after death) and its measurement is faster, more sensitive and more reproducible than other techniques used to measure biomass. Yet ATP has not been widely used for lake studies and its methodology is not completely standardized.

Table 2 summarizes all the ATP data collected since October, 1974. The numbers are the arithmetic mean of all of the ATP concentrations in the four LCS, measured during each series of experiments.

		Epilimnion				Hypolimnion			
Experiment	Treatment	Col.1	Col.2	Col.3	Col.4	Col.1	Col.2	Col.3	Col.4
I	A	not used	0.585	0.660	0.470	not used	0.125	0.120	0.120
II	A	0.310	0.380	0.380	0.630	0.190	0.140	0.210	0.210
	B	1.670	1.590	0.980	1.840	0.810	0.530	0.360	0.780
	C	control	control	0.440	3.240	control	control	0.250	3.200
III	B	0.995	1.215	1.820	1.520	0.285	0.300	0.245	0.720
	C	0.900	0.900	1.970	control	1.075	0.920	0.830	control

Table 2: Arithmetic Mean of ATP concentrations ($mg.m^{-3}$) measured in the LCS under different experimental conditions.

We observe in Table 2 that the epilimnetic ATP content was very low (.30-.70 $mg.m^{-3}$) during the first two experiments when treatment A was applied. It was higher (1.0-2.0 $mg.m^{-3}$) when treatment B was applied (Experiments II and III). Under treatment C the means varied inconsistently. The epilimnetic ATP was much higher and showed larger variations than the hypolimnetic ATP, except during treatment C.

A more detailed presentation of ATP data collected during the three experiments in the epilimnion of the four LCS is recorded graphically in Figures 1, 2, 3 and 4 where ATP concentration is plotted against time in days. The horizontal line corresponds to the mean ATP content during each treatment.

During the first experiment the ATP concentration gradually decreased with time in columns 2, 3 and 4 (column 1 was not used). Very crudely, the graphs look very much like typical time-decay curves. Overall, ATP concentration tended to decrease from about 1.40 $mg.m^{-3}$ on day 1 to 0.20 $mg.m^{-3}$ at the end of the experiment (the Spearman rank correlation coefficients were significant at the 1% level, Appendix 2). It is noteworthy that this trend tended to be reversed three weeks after the beginning of the experiment but only for a period of 15 to 20 days.

During treatment A of experiment II only one observation can be made about the relation between ATP and time. In all four columns the ATP followed the same pattern: after 35-40 days of experimentation the ATP content was very low (0.20 $mg.m^{-3}$).

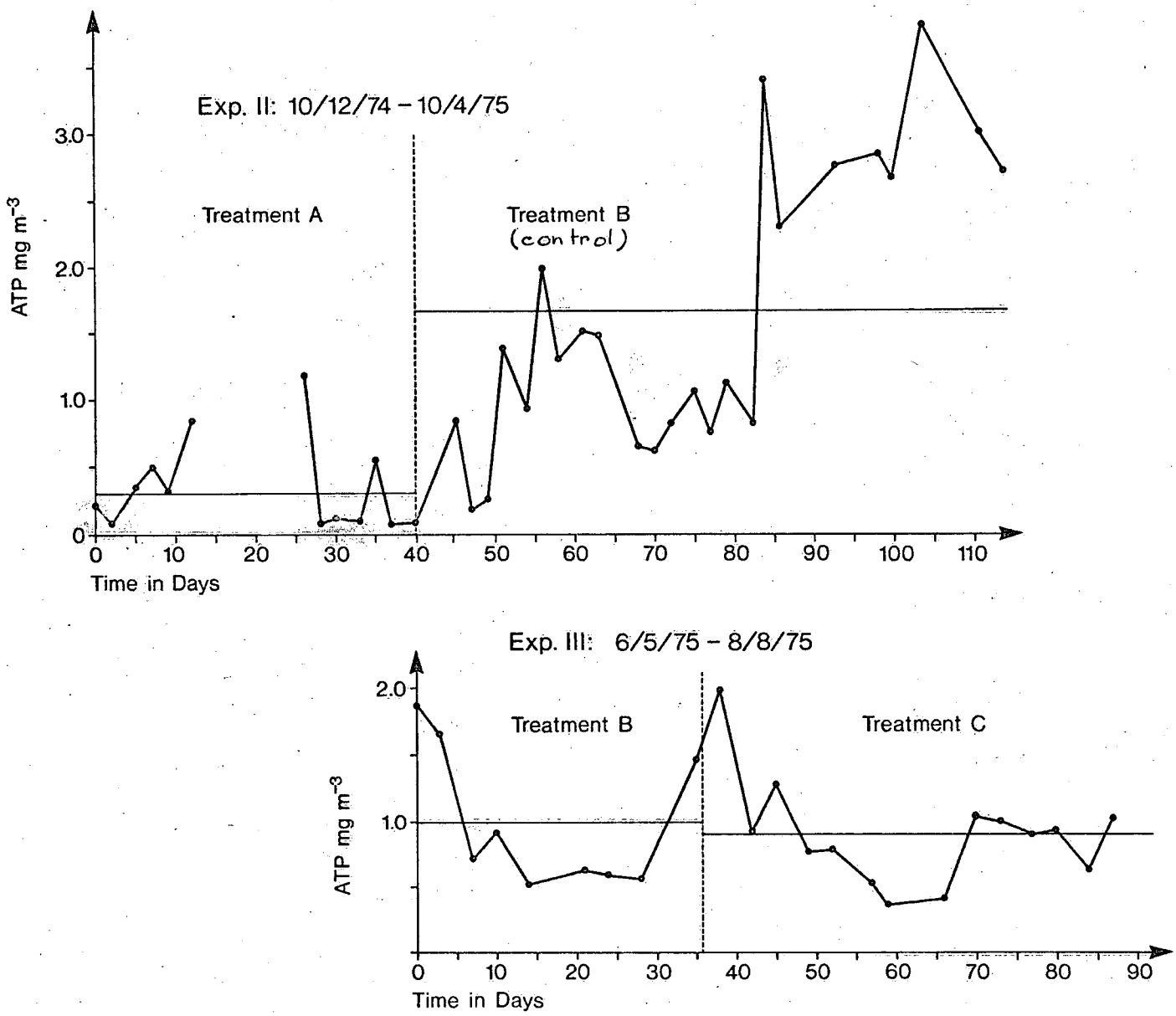

Fig.1 Epilimnetic ATP Concentrations Measured During Two Experiments Run in Lake Column Simulator No. 1

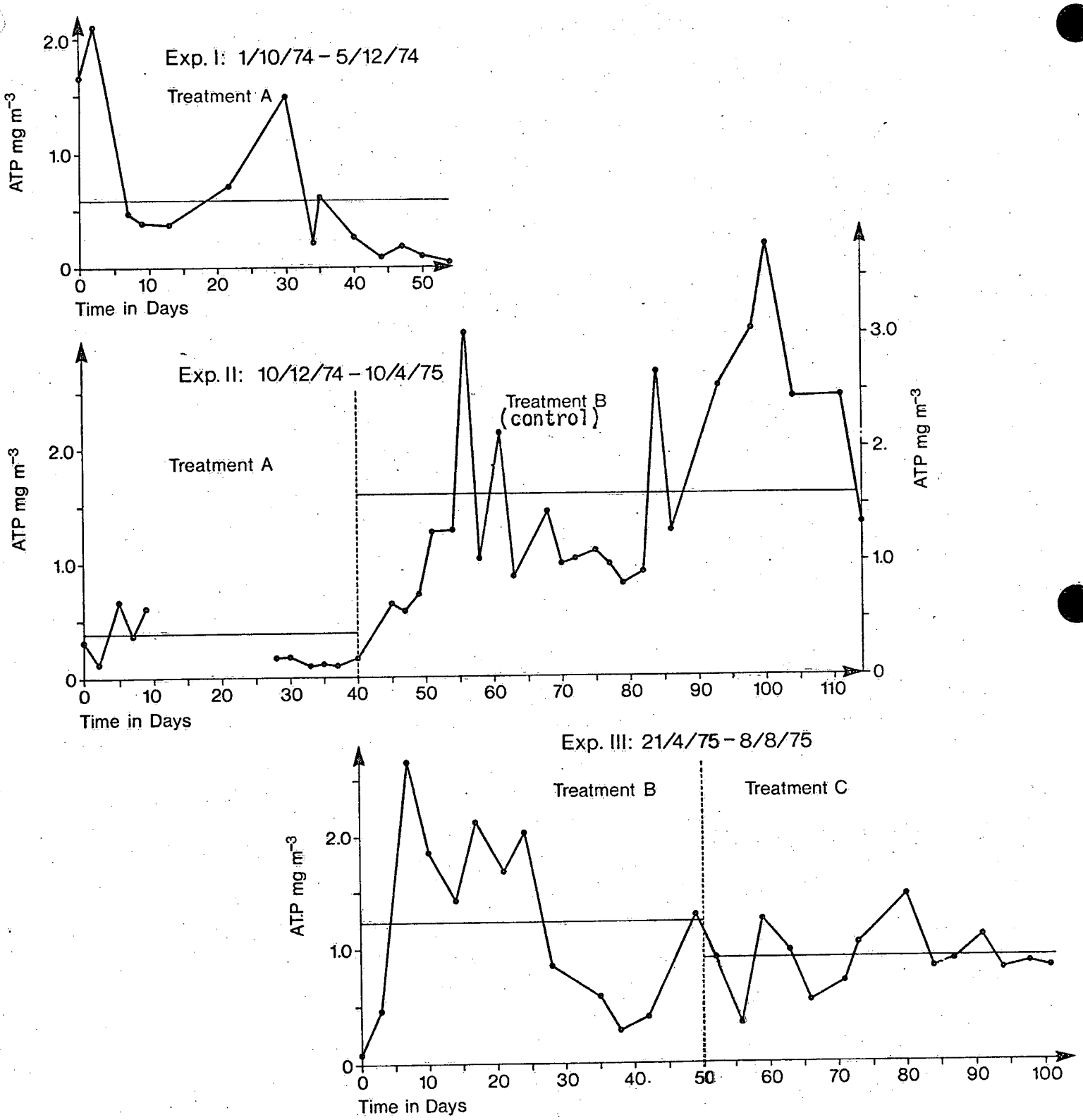

Fig. 2 Epilimnetic ATP Concentrations Measured During Three Experiments Run in Lake Column Simulator No. 2

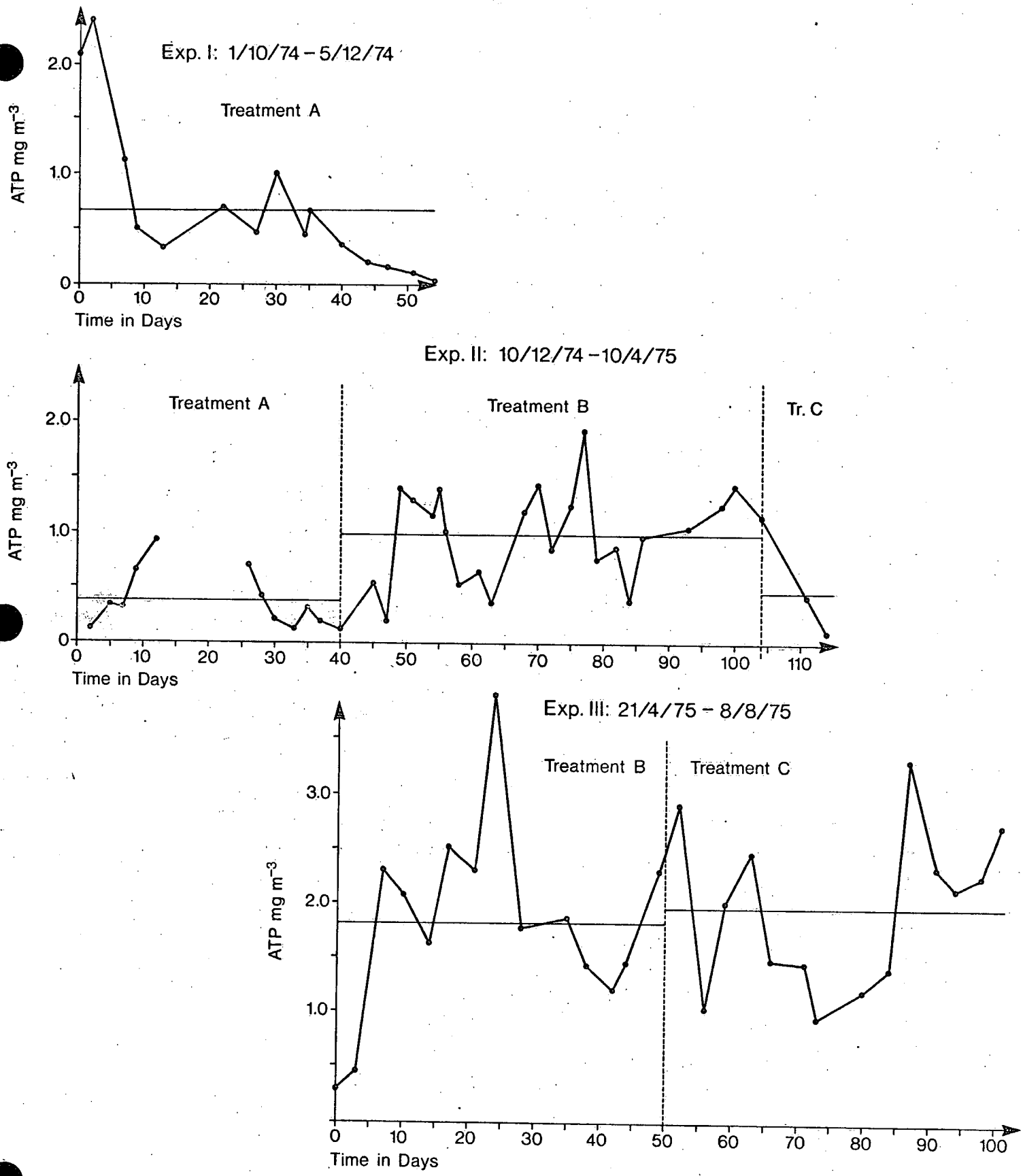

Fig. 3 Epilimnetic ATP Concentrations Measured During Three Experiments Run in Lake Column Simulator No. 3

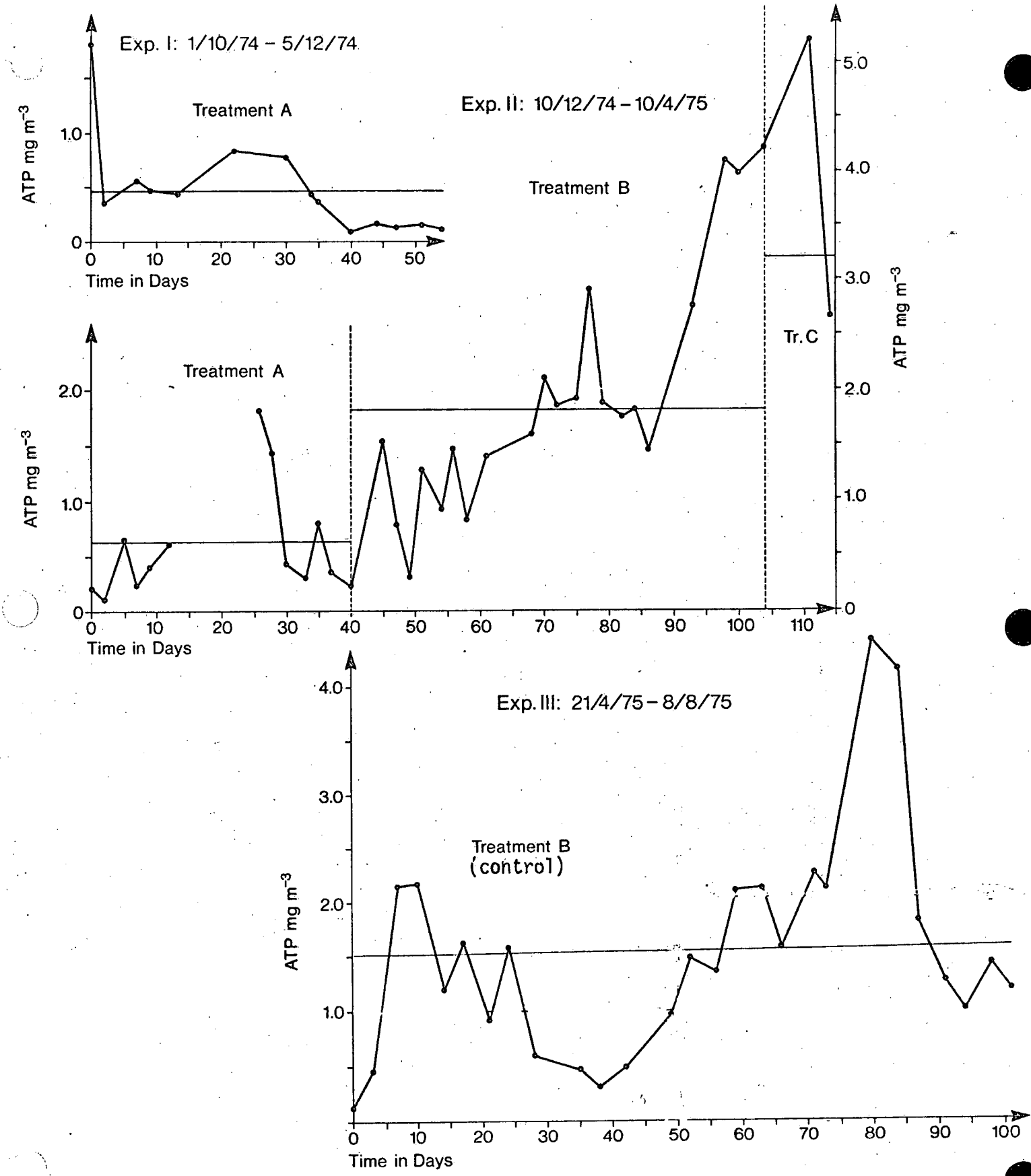

Fig. 4 Epilimnetic ATP Concentrations Measured During Three Experiments Run in Lake Column Simulator No. 4

During treatment B of experiment II ATP concentration increased in all four columns. The Spearman rank correlation coefficients were significant at the 1% level (Appendix 2). To compare the ATP mean before and after yeast addition, a t-test was calculated. It shows that the ATP increased significantly at the 1% level (Appendix 3).

In columns 1, 2 and 4, the ATP concentration went from 0.20 $mg.m^{-3}$ on the 40th day (1st day of yeast addition) to over 3.50 $mg.m^{-3}$ on the 100th day of experiment (60th day of yeast addition). As much as 40 to 50 days of yeast addition were necessary to increase the biomass to over 2.0 $mg.m^{-3}$. While increasing, the ATP concentration seems to follow a cycle. In column 1 and 2, a first increase followed by a decrease occurred between the 40th and 80th days (1st and 40th days of yeast addition); in column 4, it appeared between the 60th and 80th days (20th to 40th days of yeast addition). On the 85th day (45 days after yeast addition started) a second and more important increase occurred and lasted 15 to 25 days in columns 1, 2 and 4. In column 3, the ATP concentration was very different from that of the other columns. ATP did not increase sharply on the 85th day and it varied with oscillation around a mean content of 0.90 $mg.m^{-3}$.

During treatment C of experiment II the ATP concentration, in the control columns (1 and 2) and in the columns which received dredged spoils (3 and 4), started to decrease. This corresponds to the 100th and 110th day of experiment (60-70 days of yeast addition).

During treatment B of experiment III the relation between ATP concentration and time was the same in columns 2 and 4[1]. There was an increase of ATP 5 days after the experiment started and it was followed by a decrease on the 40th day. After 50 days, a second and more important increase occurred in the control column (No. 4). From the 70th to 90th day, there was more than 2.0 $mg.m^{-3}$ of ATP. Then the ATP concentration declined very sharply. It occurred 90 days after the yeast addition started. This pattern of increasing and decreasing was very similar to that of columns 1, 2 and 4 during experiment II.

During treatment C of experiment III dredged spoils were added to column 1 (33 g/day), column 2 (.33 g/day) and column 3 (3.3 g/day). The biomass distribution in column 1 and 2 remained fairly constant around a low mean content of 0.80 $mg.m^{-3}$ of ATP, whereas in column 3 the ATP concentration varied with oscillation around a mean content of about 1.80 $mg.m^{-3}$.

DISCUSSION

The results presented in Table 2 and in Figures 1 to 4 indicate that before one can explain the effect of dredged spoils, two questions regarding the preliminary experiments have to be answered. One concerns the possible heterogeneity of the four LCS. The second concerns the relative importance of time and of treatments (A and B) on the ATP concentration and whether or not these are independent of one another.

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[1]The relation between ATP concentration and time in column 1 is very different and this could be due to the fact that column 1 was filled with a water one week older than that of columns 2 and 4.

-1- Homogeneity of the four LCS

It has already been seen that after addition of yeast and dredged spoils (i.e. since mid-January, 1975), the biomass in column 3 did not react as that of the other columns. A t-test has been run to compare the mean biomass of one column with another under identical environmental conditions. The results presented in Table 3 show that the mean ATP content is similar in columns 1, 2 and 4 but is significantly different than the mean ATP concentration in column 3. During experiment II the epilimnetic ATP in column 3 is significantly lower than that of the other columns and during experiment III it is higher than that of the other columns.

	Comparison	Degree of Freedom	Significant Level at 5%	Calculated T	Observations
Exp. II treatment B	E1 E2	48	2.0	0.30	NS[1]
	E1 E3	45	2.0	3.08	S[2]
	E1 E4	44	2.0	0.58	NS
	E2 E3	45	2.0	3.05	S
	E2 E4	44	2.0	0.91	NS
	E3 E4	41	2.0	4.37	S
Exp. III treatment B	E1 E2	20	2.09	0.67	NS
	E1 E3	21	2.09	2.38	S
	E1 E4	20	2.09	0.04	NS
	E2 E3	25	2.06	1.75	NS
	E2 E4	24	2.07	0.68	NS
	E3 E4	25	2.06	2.53	S

Table 3: Test of reproductibility within columns

Not only the mean but the distribution of ATP (Fig. 3) also is different from that of the other columns. It seems therefore that column 3, for some unexplained reason, behaves differently than the other 3 columns, at least since the start of yeast addition in experiments II and III. It would be very interesting to know if, through analysis of other parameters, this

[1] Non Significant; [2] Significant

apparently aberrant behavior of column 3 is supported by measurements other than that of ATP. If it is, until it is explained, column 3 should be used very prudently, if at all.

The following comments will consider especially columns 1, 2 and 4 which constitute one homogeneous system.

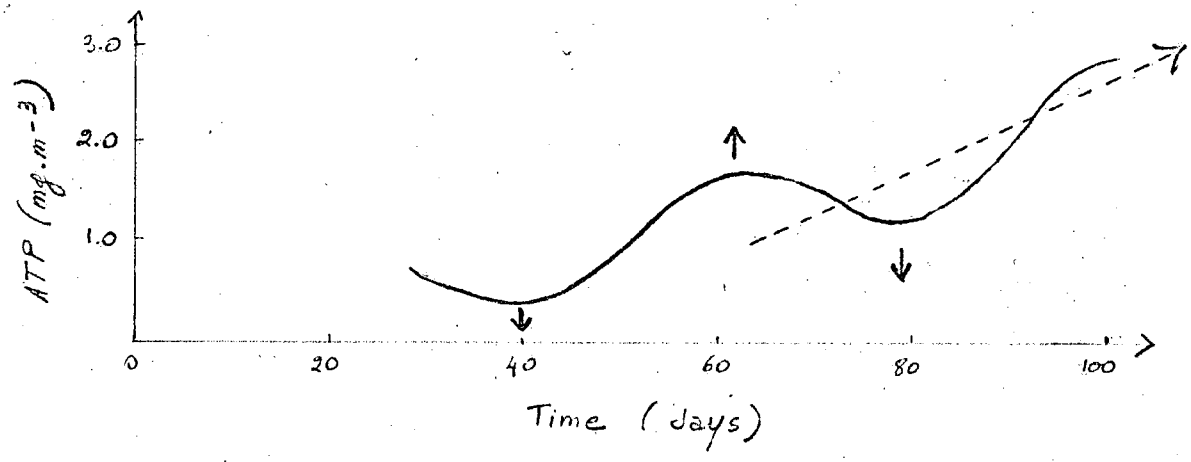
-2- Relevance of nutrients enrichment and time

The addition of inorganic nutrients seems inadequate to maintain a substantial biomass in the columns. Likely, the water is depleted in essential nutrients but as the enrichment of inorganic nutrients never exceeded 50 days, it is not known whether this depletion is temporary or definigive. It is noteworthy that the difference in the loading rates of Phosphorus during experiment I (33.6, 16.8 and 8.4 mg.P/week in columns 2, 3 and 4 respectively) does not appear on the total microbial biomass. This means that 8.4 as well as 33.6 mg.P/week affect similarly the biomass and that inorganic nutrients are not predominant in the establishment of organisms. The addition of inorganic nutrients and yeast extract together clearly provokes an increase of biomass. The mean ATP content, after enrichment, is approximately 4 times higher than the mean before yeast addition. This increase does not follow a straight line but seems to follow a cycle of increases and decreases. The ATP content increases immediately after enrichment and decreases around the 40th day of enrichment. A second and more important increase occurs on the 60th (experiment II) and 80th (experiment III) days. It is followed by a decrease around the 80th (experiment II) and 90th (experiment III) days. So a 50 day period of nutrient enrichment leads to the establishment of a large total microbial

biomass but it does not prevent a decrease of population from time to time. As this occurs either after 40 or 70-90 days of nutrient enrichment it seems that the cycle of increase and decrease in independent of yeast and inorganic nutrient enrichment. Among the feasible reasons for these low values, a temporary depletion of essential nutrients or a "column effect" could be mentioned.

Although identical in all the columns at the beginning of each experiment, the initial ATP concentration varies from 1.40 $mg.m^{-3}$ in experiment I to 0.25 $mg.m^{-3}$ in experiment II and to 1.0 $mg.m^{-3}$ in experiment III[1]. This variation in the initial microbial biomass content reflects the inconsistency of the physical, chemical and biological characteristics of the water used to fill the columns. During the first 40 to 50 days the ATP distribution is similar within columns but very different from one experiment to another. This 40 to 50 day period could correspond to an adaptation period for the living organisms to recover from the "column shock". It is only after 40 to 50 days that the biomass varies more consistently within experiments no matter when the water used to fill the columns was sampled in Lake Ontario.

The combination of the effects of time and nutrient enrichment gives the following profile:



This cycle of increase and decrease remains to be explained. The total microbial biomass composition is essential at this point to see the relative importance of phytoplankton, zooplankton and bacteria. In the LCS, is there every 40 days a situation similar to that of lakes in winter time although the environmental conditions (temperature, light, nutrient concentration, etc.) remain constant? Is it a LCS effect? In spite of these unclarified points, this observation is very meaningful and optimistic with regards to the potentials of artificial ecosystems. If it is verified by other data analysis and if it is reproducible during another experiment this means that the lake column simulators, enriched with inorganic nutrients and yeast, represent "healthy"artificial ecosystems able to support a large total microbial biomass with a cycle of 40 to 50 days. They represent, thus, suitable tools for testing the effect of toxicants.

-3- Effect of Dredged Spoils

The discussion is limited to column 1, 2 and 4 during experiment III[1].

The comparison between the mean epilimnetic ATP content before and after dredged spoils addition, shows no noticeable difference. The ATP concentration goes from 0.995 to 0.900 $mg.m^{-3}$ in column 1 and from 1.215 to 0.900 $mg.m^{-3}$ in column 2. Thus the dredged spoils do not modify the

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[1] The reason for not considering column 3 has been given on page 17. The second experiment is not really interesting because it is too short in terms of time and too excessive in terms of loading rates.

biomass within each column. However, the observation of Figs. 1 and 2 shows that after dredged spoils addition, the ATP distribution tends to stabilize. So dredged spoils regulate the variation of ATP with time.

The comparison between the mean epilimnetic ATP content in the control column (column 4) and that of the columns which received dredged spoils, shows that the biomass is significantly lower in the polluted columns than in the control column. It is possible that the effect of dredged spoils is indirect as a large biomass developed in the control column. They limit the development of a large algal biomass in reducing the light (shading effect). In the presence of dredged spoils the availability of essential nutrients may also be reduced due to adsorption and biological uptake. It is noteworthy that after 50 days of dredged spoils addition, the biomass was the same in the control and in the polluted columns. This means that a 50 day old biomass tends to decrease whether or not in the presence of dredged spoils and the possible existence of a cycle independent treatment reappears.

It has already been observed (Table 2) that under natural environmental conditions, the epilimnetic ATP is always higher than the hypolimnetic ATP and the ratio between hypolimnetic and epilimnetic ATP is comprised between 0.20 and 0.60. In the presence of dredged spoils, the difference is not as accentuated. A t-test shows (Table 4) that after dredged spoils addition, the difference between epilimnetic and hypolimnetic ATP is not significant at the 1% level (except in column 3).

	Comparison	Degree of Freedom	Significant Level at 1%	Calculated t
Before Dredged Spoils	E1/H1	15	2.95	3.48
	E2/H2	23	2.81	3.62
	E3/H3	24	2.81	5.79
	E4/H4	23	2.81	2.54
After Dredged Spoils	E1/H1	25	2.79	1.18
	E2/H2	25	2.79	0.12
	E3/H3	25	2.79	4.08
	E4/H4	25	2.79	1.53

Table 4: T-test to compare epilimnetic and hypolimnetic ATP means (Exp. III).

The trend of the vertical distribution of ATP towards homogeneity is not exclusively due to dredged spoils addition as it also appears in the column which received no addition. In order to elucidate this question, the third experiment has been divided into 4 periods of approximately three weeks each. The mean epilimnetic and hypolimnetic ATP contents during these 4 periods, have been calculated (Table 5).

Column Number	Days of Experiment	Epilimnetic ATP ($mg.m^{-3}$)	Hypolimnetic ATP ($mg.m^{-3}$)	Hypolimnetic ATP / Epilimnetic ATP
1[1]	1-35 (ϕ 1)	0.995	0.285	0.29
	38-66 (ϕ 3)	0.885	1.020	1.15
	70-91 (ϕ 4)	0.880	1.165	1.32
2	1-21 (ϕ 1)	1.475	0.220	0.15
	24-49 (ϕ 2)	0.915	0.410	0.45
	52-80 (ϕ 3)	0.910	1.185	1.30
	84-105(ϕ 4)	0.835	0.640	0.77
4	1-21 (ϕ1)	1.235	0.250	0.20
	24-49 (ϕ2)	0.735	0.440	0.60
	52-73 (ϕ3)	1.865	0.670	0.36
	80-105(ϕ4)	1.955	1.745	0.89

Table 5: Mean ATP content in Lake Column Simulators during 4 periods of Experiment III.

[1] There is no phase 2 in column 1 because technical difficulties delayed the experiments by two weeks and shortened the period before dredged spoils addition.

The graphs of the vertical distribution of ATP during the first phase ($\phi 1$)[1] and during the last phase ($\phi 4$)[2] of the experiment are drawn on Figure 5.

On the left side of Figure 5, representing the control column (No. 4) the effect of time alone is shown on the vertical profile of ATP. During the last period a real increase in ATP occurs, because under natural conditions, the LCS enriched in inorganic nutrients and yeast, provide an adequate environment for algae to develop and even bloom. The large production of algae in the surface layer is likely followed by a move down due to lack of space in the euphotic zone. Although not having the best conditions to be highly productive, the algae could still survive and even reproduce in the hypolimnion. As a result of this increase of ATP along the whole column, the vertical ATP profile approaches homogeneity (Hypolimnetic over epilimnetic ATP is equal to 0.90).

On the middle part of Figure 5, we see the vertical distribution of ATP in column 2 which received 0.3 g/day of dredged spoils. During the first period, the profile and mean of ATP content are very similar to that of the control column; there is an intermediary amount of ATP in the epilimnion and a low amount in the hypolimnion and the hypolimnetic over epilimnetic ATP ratio is very low (0.15). During the last period, the

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1. Phase 1 corresponds to a system which is 3 weeks old and which establishes itself without external environmental stress.

2. Phase 4 corresponds to a system which is almost 3 months old and which (for column 1 and 2) underwent three to six weeks of dredged spoils addition.

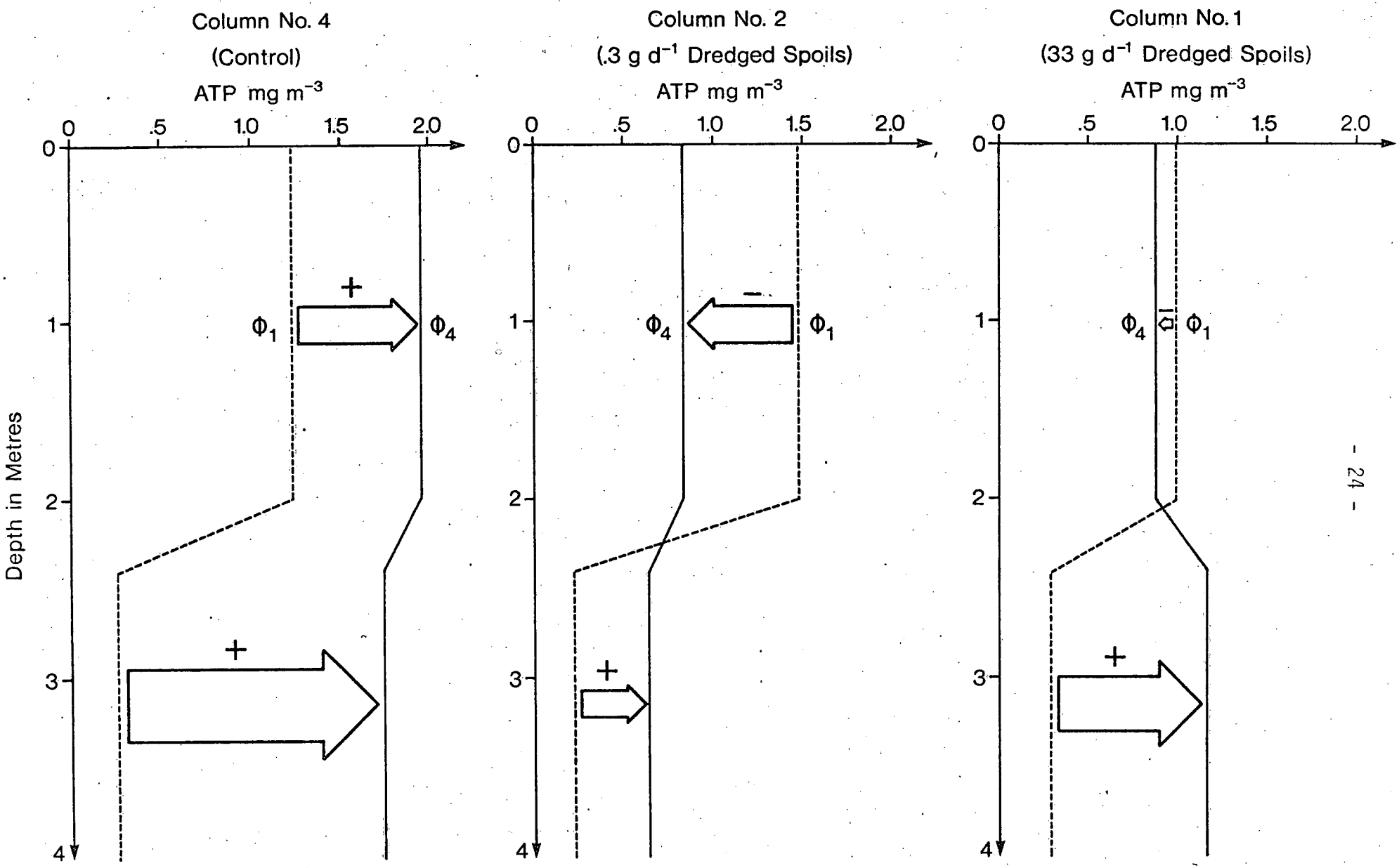

Fig. 5 Vertical Distribution of the Mean ATP Content During Experiment III Before (Φ_1) and After (Φ_4) Dredged Spoils Addition

vertical profile of ATP is as homogeneous as that of the control column (the hypolimnetic over epilimnetic ATP ratio is equal to 0.80); the only noticeable difference being the presence of a much smaller microbial biomass in the whole column.

On the right side of Figure 5, we see the vertical distribution of ATP in column 1 which received 33 g/day of dredged spoils. During the first period, the ATP vertical profile and the ATP content are similar to that of columns 2 and 4. The hypolimnetic over epilimnetic ratio is equal to .30. During the last period, there is for the first time, a larger amount of ATP in the hypolimnion than in the epilimnion. The hypolimnetic over epilimnetic ATP ratio is equal to 1.30. The epilimnetic content does not change but ATP increases significantly from the first to the fourth period in the hypolimnion.

We conclude that 0.3 or 33 g/day of dredged spoils addition circumvents the nutrient enrichment in inhibiting the expected increase in total microbial biomass. If they do not change the biomass in the epilimnion, they significantly provoke an increase of biomass in the hypolimnion. This effect is particularly evident with the largest amount of dredged spoils addition (33 g/day). The reason for this high hypolimnetic ATP content, in the absence of a high epilimnetic content, is likely due to a physical vertical transport of living organisms attached on the particles of dredged spoils.

In the LCS undergoing natural conditions, the epilimnetic ATP concentration is a good indicator of the trophic state: for example, when it is smaller than 1.0 $mg.m^{-3}$, the waters are considered oligotrophic

and when it is larger than 3.0 $mg.m^{-3}$, they are considered eutrophic. When dredged spoils are added, the epilimnetic ATP along does not provide enough information as its content tends to stabilize. Most variations occur in the bottom layer. When the ratio between hypolimnetic and epilimnetic ATP is superior to 1, the LCS could be considered polluted by dredged spoils.

CONCLUSIONS

It was not easy to describe the biological situation and to try to understand the metabolism of unknown artificial ecosystems using ATP, a fairly new aquatic biomass parameter. Nevertheless, our work has shown that ATP constitutes a sensitive environmental parameter in Lake Column Simulators undergoing both natural and stress conditions.

We had difficulties in establishing and maintaining in the open water, a population representative of the food chain organisms found in lakes under natural summer conditions (1 to 2 $mg.m^{-3}$ of ATP). As expected, initially, a large periphyton growth took place which is now limited by scraping. The problem of creating and maintaining a reasonably large biomass was somewhat resolved by inorganic nutrients and yeast extract addition.

It appears that the biomass followed a cycle of increases and decreases with approximately a 40 day period but more assays are needed to support this hypothesis.

When conditions in the Lake Column Simulators simulate natural oligotrophic lake conditions (1.0 $mg.m^{-3}$ of ATP), the ATP vertical distribution is heterogeneous because the epilimnetic biomass is much larger

than the hypolimnetic biomass. When dredged spoils are added the total microbial biomass remains constant in the epilimnion (there is a possibility that dredged spoils inhibit algal growth by shading effect) but increases in the hypolimnion inversing the natural vertical distribution of organisms. The Lake Column Simulators could be considered polluted by dredged spoils, when the hypolimnetic over epilimnetic ATP ratio is greater than 1.

Column 3 does not seem to react as the other three columns and its reliability for comparative studies is questionable.

Generally, the Lake Column Simulators represent a good opportunity to study lake equilibria and lake problems because they represent simple and controllable environments. When the metabolism of the Lake Column Simulators is better understood and controlled, information can be obtained which may allow a prediction of the situation within real lake systems.

RECOMMENDATIONS

The most striking point of this report is represented by the possible existence of a cycle of the biomass living in the Lake Column Simulators. As there are still uncertainties on this cycle we ought to re-observe it by repeating an experiment of approximately 150 days with inorganic nutrients and yeast extract addition (confer treatments A and B) before embarking into toxicology experiments.

As long as the reliability of column 3 remains questionable, it is as hazardous to use it. With only three devices (one of which has to be a blank) no comparative work can be validly made. So we ought to understand the particular behaviour of column 3 and find a way to normalize it.

For future toxicology experiments, we ought to let the biomass recover from the "column shock" and adapt itself in the columns enriched in organic nutrients and yeast, for a certain period until the biomass is identical and sufficiently large in all devices (approximately 60 days). The duration of toxicant addition should last at least 50 days (to see the effect on the cycle) and should not exceed 60 days (as we do not know what happens in a 120-day old system).

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APPENDIX

	Exact Composition	Stock added to LCS
$Ca(NO_3)_2$	0.0040%	126.0 g/l
$K_2 HPO_4$	0.0010%	31.5
$MgSO_4.7H_2O$	0.0025%	78.75
Na_2CO_3	0.0020%	63.0
Na_2SiO_3	0.0025%	78.75
$FeCl_3$	0.00008%	2.52

1. No. 10 CHU Medium

	1E	2E	3E	4E
Experiment I treatment A	not used	- .842	- .857	- .793
Experiment II treatment A	- 2.36	- .580	- .258	+ .236
treatment B	+ .680	+ .555	+ .202	+ .786
treatment C	control	control	-1.000	- .800
Experiment III treatment B	- .533	- .181	- .123	+ .320
treatment C	- .125	- .024	+ .371	control

2. Spearman rank correlation coefficients (underlined are the coefficients significant at the 1% level).

	Degree of Freedom	Significant level at 1%	Calculated T
Col. 1	36	2.57	5.69
Col. 2	35	2.57	5.70
Col. 3	33	2.57	5.17
Col. 4	32	2.57	4.16

3. Effect of Yeast addition (t-test to compare epilimnetic ATP means before and after yeast extract addition).

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