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Design, experiences and selected results of meso- and microcosm experiments in shallow coastal waters 1981/95

Herrn Professor Dr. Ernst Albert Arndt zum 70. Geburtstag gewidmet.

Abstract

Since 1981 we have used a combination of field studies, field mesocosms, lab microcosms and lab experiments to investigate of tideless shallow brackish waters. The design and handling of pelagic and shallow water microcosms, and two types of lab microcosms is described.

The importance of such approaches is documented by selected results, e. g.

- the ammonium rhythm
- influence of the parallel induction of nutrient and light limitation on the microbial food web
- regulation of phytoplankton periodicity by nutrients and water temperature
- fate of introduced bacteria biomass
- stochasm versus determinism: long term behaviour of microbial food webs.

The results show clearly that such experimental approaches are indispensable for causal analysis of microbial food webs in aquatic ecosystems.

Introduction

Biologists of the Department of Biology at the Rostock University have been involved in ecosystem research in the Darss-Zingst Bodden chain since 1968 (Schiewer 1990). The ecosystem is a tideless eutrophic estuary with a small outlet to the Baltic Sea at the eastern end. It consists of 4 different shallow basins connected to each other by more or less smaller water bodies (Fig. 1).

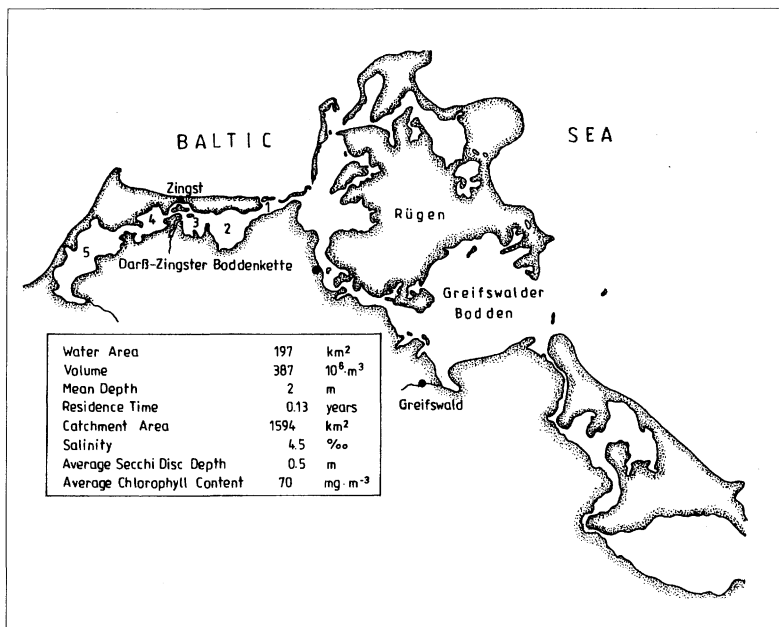


Fig. 1: The Darß-Zingst Bodden Chain (DZBC). 1 = outlet; 2 - 5 basins (Grabow, Barther Bodden, Bodstedter Bodden, Saaler Bodden).

Our research strategy involves studying all ecological levels to obtain a better causal interpretation. Figure 2 shows this strategy in simplified form. Mesocosm and microcosm experiments play an important role. Their importance has increased since 1981, when we discovered that microbial food webs might be important. Subsequently we showed that around 90% of the carbon flux is mediated through the microbial food webs caused by the ongoing eutrophication (Fig. 3).

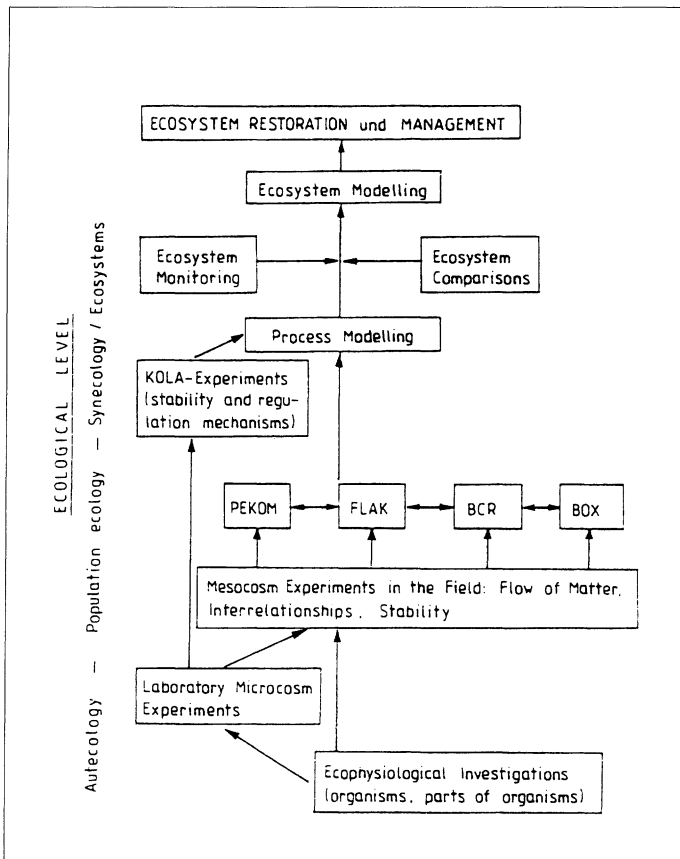


Fig. 2: Simplified review of the research strategy of the Coastal Research Group of the Department of Biology. PEKOM = Pelagic community enclosures; FLAK = shallow water community enclosures; BCR = benthic community respiration; BOX = agricultural waste water loaded enclosures; KOLA = complex laboratory experiments (microcosms) with natural communities.

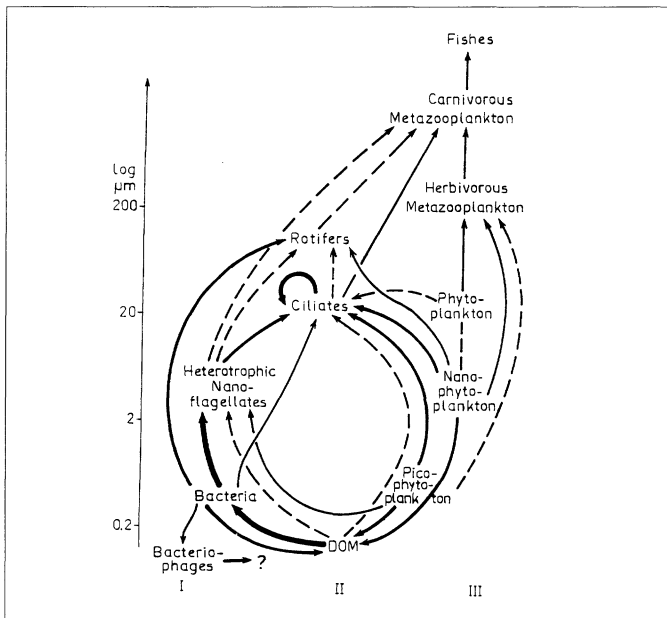


Fig. 3: Microbial food web.
 DZBC, late spring/early summer situation.
 I = side chain through bacteriophages (proven abundances of virus sized particles: $10^8 \times \text{ml}^{-1}$).
 II = microbial food web. Main pathway for carbon turnover, mainly by pico- and nanoplankton. Internal loop in the ciliate community (arrow).
 III = "classical" pelagic food web. The typical components are net plankton and fishes. Minor role only.
 DOM = Dissolved Organic Matter.

Design and handling of meso-/microcosms

We use two different mesocosm systems (Fig. 4 A, B):

- pelagic 1 m^3 polyethylen bags (PEKOM)
- shallow water $1.5 - 2 \text{ m}^3$ polyethylen bags with a metal ring (FLAK).

The PEKOM system is a floating device containing 4 polyethylen bags (Fig. 4 A) moored in a small harbour near the laboratory at Zingst. The bags are open at the top end without rain protection. This system can be used up to 6 weeks if the inside wall growth is prevented. That can be done by regular and thorough mechanical mixing twice a day.

We have used this system for various purposes, e. g. to test the influence of eutrophication, saprobisation, exclusion of organism groups and the UV-B radiation.

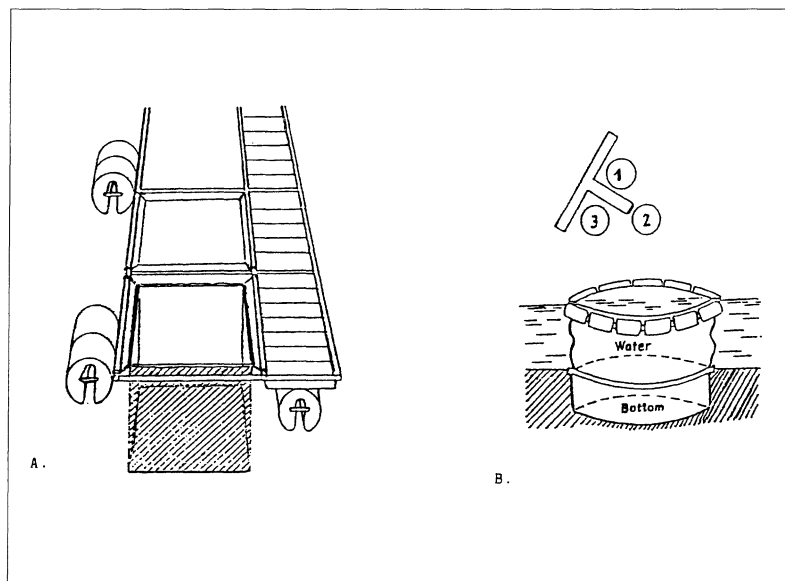


Fig. 4: Mesocosms.

- A. PEKOM-Mesocosm: 1 m³ volum, 4 polyethylen bags (1 x 1 x 1.4 m) open at the top end.
- B. FLAK-Mesocosm: Single mesocosm with 45 cm high metal ring driven 35 - 40 cm into the sediment (open to the bottom), polyethylen bag (height 1m, diameter 2 m, volume 1.5 - 2.4 m³) with floats and open to the ai. Installation of 3 mesocosms behind the breakwater.

The FLAK system was set up in the Kirr bay, a shallow experimental bay around 800 m from the laboratory (Fig. 4 B). It consists of 3 polyethylen bags with a height of 1 m and a diameter of 2 m. Mesocosm of this size are easy to handle and allow water volumes of between 1.5 and 1.9 m³ to be isolated. The polyethylen foil was attached to a metal ring at one end and floats at the other. The metal ring was driven 40 cm into the bottom to anchor the bags and to prevent water exchange near the sediment surface. The floats regulate the vertical position of the bags and prevent water input by wave-splash. The system is protected by a breakwater on the main windward side. The bags are open to the bottom and to the air. Such systems have been used far to 12 weeks. This is possible if the more intensive inside wall growth than in the PEKOM experiments is prevented by thoroughly brushing the polyethylen walls twice a week.

The FLAK device was used to test the influences of nutrients and climate on shallow water communities.

The design of our microcosms was simpler. We used 6 l glass bottles or 100 l polyethylen bottles. The main physical factors temperature and light were kept constant. Air was supplied by regular mixing or air bubbling. To prevent wall growth, the microcosms were regularly brushed.

The small microcosms were used for limitation experiments lasting up to 6 days and to study interactions in populations in experiments lasting up to 12 days. The larger mesocosms were used for studies into the influence of temperature, eutrophication, saprobisation and to problems of long term selfregulation.

All these meso- and microcosm experiments are efficient only if they are immediately backed up by appropriate laboratory investigations.

Selected results

Selected results will be presented for each type of meso- and microcosm to demonstrate the importance and necessity of such approaches.

AMMONIUM RHYTHM

During nutrient loading experiments 1981 we were surprised by a rhythmic variation in ammonium concentrations (SCHIEWER & BAADER 1982) in the ammonium loaded mesocosms (Fig. 5). It seems to be related to the dominating cyanobacteria community. Experiments with axenic *Microcystis firma* cultures showed that we could mimic this behaviour under controlled laboratory conditions. It takes place under growth limitation only, e. g. by phosphorus or CO₂. The rhythm (ABARZUA & SCHIEWER 1989 and ABARZUA & NIETZ 1990) was temperature sensitive, and its magnitude depended on the light intensity. It could be strengthened by adding KNO₃ and is somehow linked to photosynthesis (Fig. 6). No internal NH₄ pool was detected. The main purpose of the rhythm appears to be to protect the photosynthetic apparatus of cyanobacteria from excessive light. It was driven partly by reduction of NO₃ to NH₄ under growth limited conditions and partly by an unknown mechanism linked directly to NH₄ (ABARZUA et al. 1993).

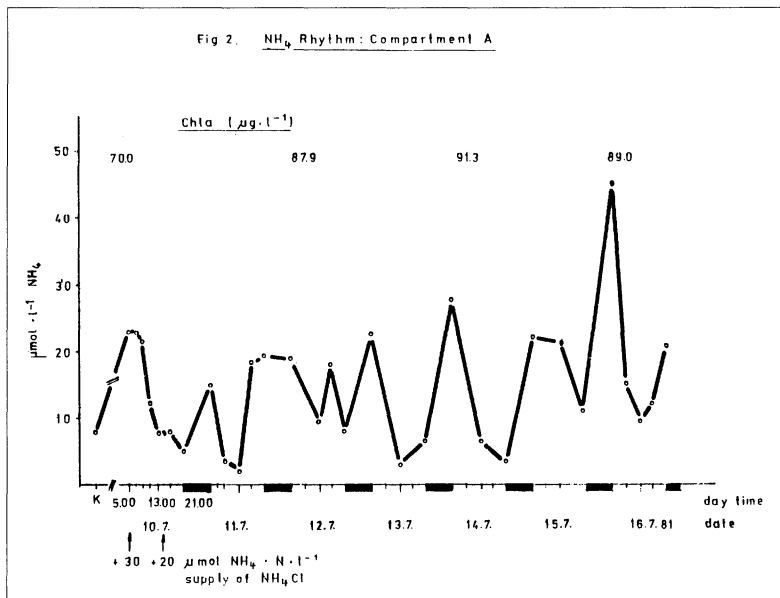


Fig. 5: Ammonium rhythm.
Results of the field mesocosm experiments PEKOM 81 (1981).
Chla = chlorophyll a -concentration. Arrows: times of NH₄-loads.
Dark blocks: night-time

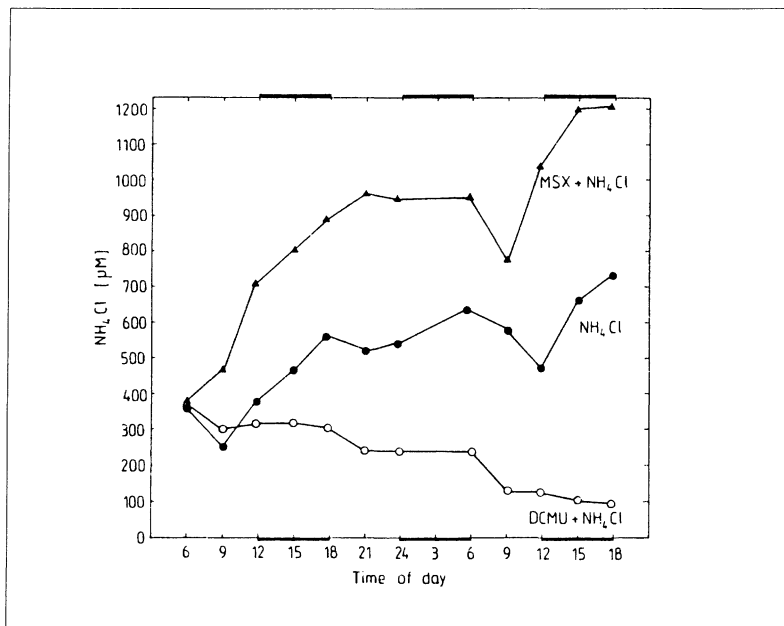


Fig. 6: Effect of DCMU and MSX on NH_4 rhythm in cultures of *Microcystis firma* in KHO_3 -containing medium. Inhibitor concentration: DCMU = 0,01 mM; MSX = 0,5 mM. Light/dark alternation = 6/6 h. (ABARZUA and NIETZ 1990)

PARALLEL INDUCTION OF NUTRIENT AND LIGHT LIMITATION

Increased eutrophication and the decline of submersal macrophytes led to a change from nutrient to light limitation of phytoplankton during spring and summer. We induced nutrient and light limited situations in mesocosms to compare the behaviour of the microbial food web under both conditions. Under light limitation we found:

- much more picophytoplankton
- increased protozooplankton
- strong reduction in mesozooplankton
- higher bacterial productivity.

These results are comparable with the overall development in the Darss-Zingster Bodden chain during the last 5 years (SCHIEWER, in press).

REGULATION OF PHYTOPLANKTON PERIODICITY

The typical annual phytoplankton periodicity between 1981/86 is characterized (BÖRNER 1984) by peaks of flagellates in winter, green algae in spring and autumn and cyanobacteria in summer (Fig. 7 A).

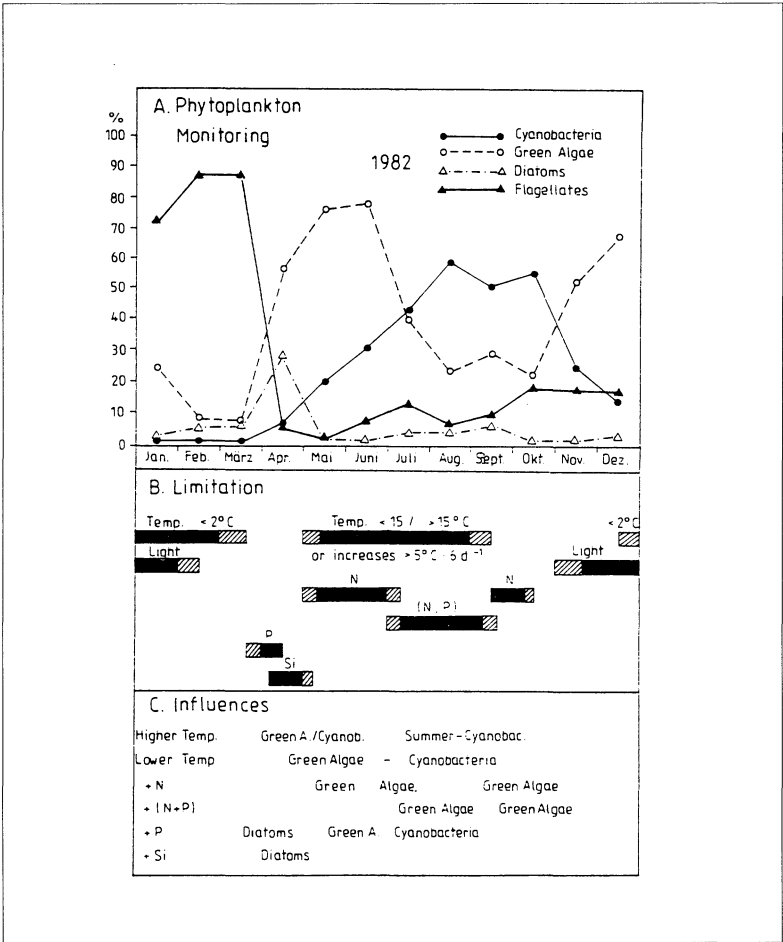


Fig. 7: Phytoplankton periodicity in the Zingster Strom, DZBC.

- A. Relative frequency of phytoplankton groups in percent calculated on biomass level and their seasonal distribution in 1982 (BÖRNER 1984).
- B. Generalized scheme of the main limiting physico-chemical factors. Temp. $> 15^{\circ}\text{C}$ promotes development of summer cyanobacteria; temperature increase by $> 5^{\circ}\text{C} \times 6 \text{ d}^{-1}$ induces a breakdown of existing phytoplankton population. N = nitrogen; P = phosphate; (N - P) = alternative N- or P-limitation; Si = silicon.
- C. Influences of temperature and nutrient loads on phytoplankton growth (promotion of groups)

By using small microcosm experiments, PEKOM and FLAK mesocosms we obtained a detailed picture how the limiting factors change throughout the year (Fig. 7 B) and how varying conditions cause the changes (Fig. 7 C). Most important was the discovery of the short term influence of increasing temperatures during spring and summer by FLAK experiments in 1986. A temperature increase of $> 5^{\circ}\text{C} \times (6 \text{ days})^{-1}$ induces a break-down of the existing phytoplankton community followed by structural and functional changes in the whole pelagic microbial community. This behaviour could be simulated in 100 l microcosms in the laboratory. Such climatic events occur 1 - 4 times in the course of a year. They are typical stochastic events, causing unexpected changes during the normal annual periodicity.

THE FATE OF MASSIVE RELEASE OF BACTERIAL BIOMASS

We used pelagic microbial communities from the Darss-Zingster Bodden Chain as test communities. The microbial food web (phytoplankton - bacteria - protozoons - metazooplankton) predominates in this tideless, highly eutrophic brackish pelagic system. Our attention focused on the impact of microorganisms released on a large scale into the estuarine ecosystem. Great care was taken to avoid contamination of the ecosystem with the bacterial cultivation medium. For this experiments we used 100 l microcosm under defined light and temperature conditions. Apart from the physico-chemical status of the water (e.g. temperature, light, nutrient availability), we monitored the principal structural and functional parameters of the microbial biocenosis and the reactions of the whole system.

The fate

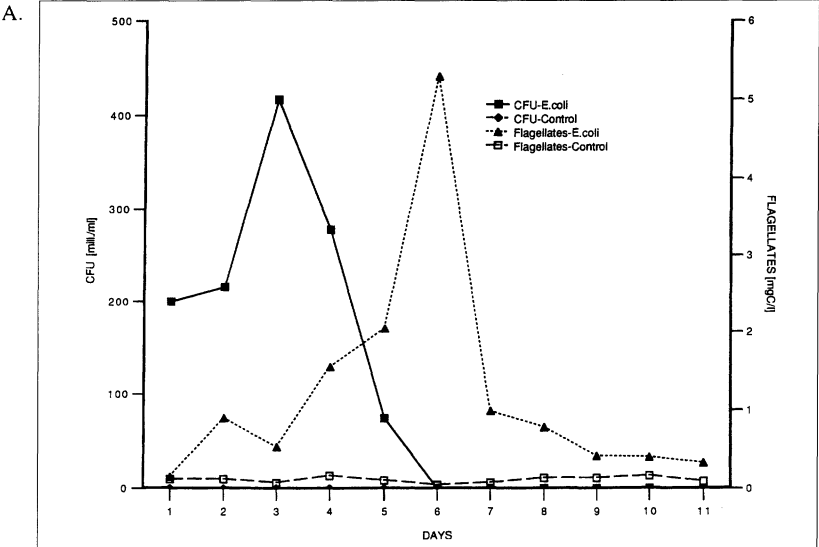
- of the released *Escherichia coli* bacteria,
 - their effects on the structure and functions of the various system components,
 - interactions between system components, and
 - effects on overall pelagic ecosystem responses
- were investigated.

The control mechanism for eliminating the perturbation caused by a massive *E. coli* input acts through a top-down control system. Heterotrophic flagellates (HNF) grazed on *E. coli* after a delay of 2 days (Fig. 8a,b) and increased their biomass as a result. This biomass is used in turn by ciliates in the classical way shown by an increase in ciliate biomasses (Fig. 8 c). The ciliates themselves are controlled partly, but to an insignificant extend, by metazooplankton (Fig. 8 d). An internal control

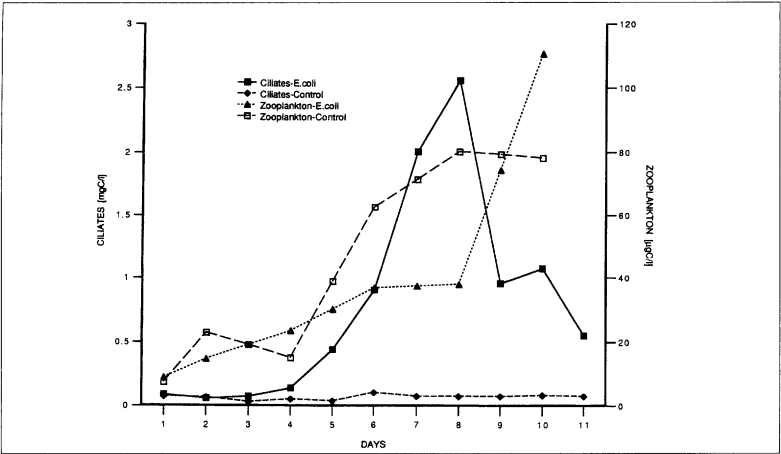
mechanism in the ciliate community plays a larger role. When the experiment started, the ratio of phytoplankton biomass to bacterial biomass was about 1:40. The quality of the phytoplankton serving as a food source for the metazooplankton is obviously reduced by the large bacterial fraction. The phytoplankton/ bacteria-ratio had been reduced to 1:3 by the 6th day. This ratio is obviously tolerated by the copepods.

The stability of the pelagic biocoenosis we studied was impressive. The additional bacteria were largely eliminated within 5 days despite the massive input. Although the bacterioplankton and, later, the phytoplankton fractions increased in size and remained stable, neither the HNF nor the ciliates succeeding them were able to sustain high biomasses for any appreciable length of time. High grazing rates eliminate the biomasses of both groups to low levels. However, signs of the massive bacterial biomass input were still present in the higher nitrogen and phosphorus concentrations (Fig. 8 e) and the increase in chlorophyll (Fig. 8 f) during the investigation period. Light limitation of phytoplankton prevented a more pronounced nutrient reduction. The bacterial biomass input can be transmitted to higher trophic levels only via the releases of nutrients and enhanced phytoplankton growth. Some of the phytoplankton will then be incorporated by metazooplankton.

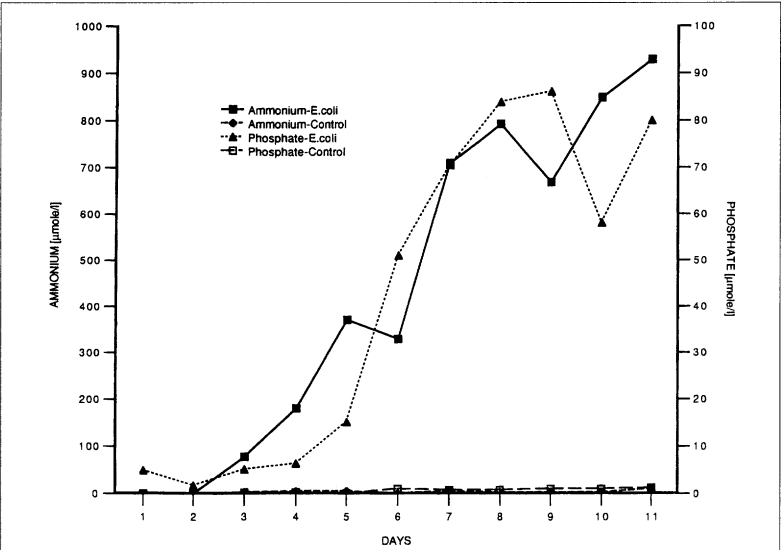
In order to survive, released bacteria must be able to grow, compete with autochthonous bacterial populations for substrates (MASON et al. 1983, MEZRIOUI et al. 1995), and above all withstand the grazing pressure (BrETTAR & HÖFLE 1992). The last of this factors minimized their competition with the natural bacterial population, but did not prevent their survival in aquatic ecosystems.



B.



C.



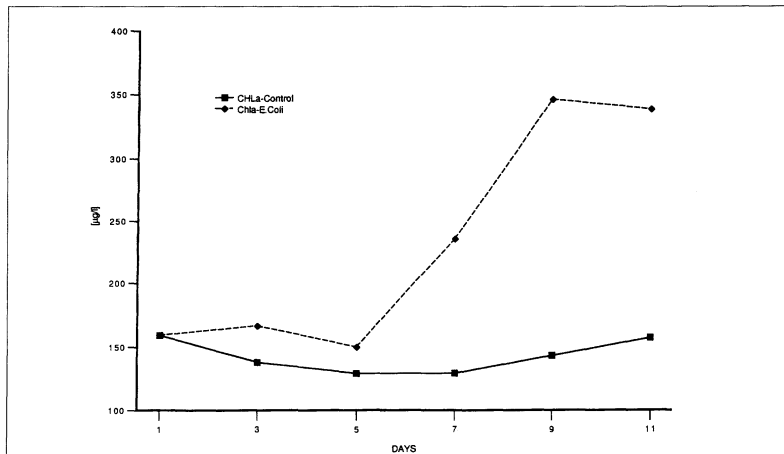


Fig. 8: Fate of massive released *Escherichia coli*.
 Microcosm experiments 1990: control and with *E. coli*.
 A. Bacteria-CFU ($10^6 \times \text{ml}^{-1}$) and heterotrophic flagellates ($\text{mg C} \times \text{l}^{-1}$), CFU: *E. coli* abundances evaluated by indirect enumeration of Colony Forming Units on selective medium.
 B. Ciliates ($\text{mg C} \times \text{l}^{-1}$) and metazooplankton ($\mu\text{g C} \times \text{l}^{-1}$).
 C. NH_4 and phosphate ($\mu\text{mol} \times \text{l}^{-1}$).
 D. Chlorophyll a ($\mu\text{g} \times \text{l}^{-1}$).

STOCHASM VERSUS DETERMINISM IN THE SELFREGULATION OF ECOSYSTEMS

Variations in species abundances in natural communities are governed by external factors and species interactions. The dynamics that arise can be deterministic or stochastic (chaotic). In a microcosm system in which external conditions can be held constant or varied in a controlled fashion (HEERKLOSS 1992), any stochastic fluctuations in biomass can be caused only by endogenous factors. Figure 9 shows the corresponding long-term behaviour of zooplankton, microphytoplankton and bacteria. Heerkloss found considerable variations in population size. The same picture was revealed by a more detailed analysis of the proto- and metazooplankton. The differences are caused by purely biotic interactions between the planktic components. The sequence in which peaks occur is stochastic even if external conditions remain constant. The system exhibits those non-linear dynamic characteristics to which mathematical models based on chaos theory can be applied.

Since the generation times of the populations involved are relatively short, an experimental analysis of the sequence of the population peaks is practicable. Future investigations will identify the fractal dimension and other chaos-theoretical parameters of the system.

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