INVESTIGATIONS ON PHYTOPLANKTON COMMUNITIES IN MICROCOSMS EXPOSED TO THE ENDOCRINE DISRUPTOR 17α-ETHINYLESTRADIOL

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Introduction

A number of xenobiotica were shown to have endocrine activity in laboratory tests with single species. However, fewer studies deal with endocrine activities on ecosystem level, e.g. in microcosm tests. Results of such studies are difficult to interpret as it is difficult to separate endocrine causes of a detected effect from other possible toxic properties of the tested chemical. Therefore, a microcosm test with 17α-ethinylestradiol (EE), a xenoestrogene with a similar structure as the natural hormone estradiol was conducted. EE is used e.g. as a contraceptive and found in surface waters in Germany due to its persistence, e.g. near sewage plants [1,2]. In this study, EE served as a model estrogen to estimate whether the effects of nonylphenol (NP) on aquatic biocenosis, which were detected in a former study[3,4], could be attributed to endocrine activities. To estimate the effect of endocrine disruptors on the entire ecosystem, all parts of it have to be considered comprehensively, including possible (indirect) effects on primary production. In this study, results of the investigation on phytoplankton is presented.

Methods and material

Cylindrical stainless steel microcosms (80 cm high, 60 cm wide) were filled with water (230 L) and sediment (10 cm) of an oligo-mesotrophic littoral area of a natural lake (Ammersee, Bavaria). To meet natural conditions, EE was applied continuously by controlled release with semi-permeable LPDE tubes. EE (Sigma-Aldrich) was dispersed in the tubes with triolein. Different EE concentrations in the microcosms were achieved by different lengths of the tubes, which were placed in the microcosms EE1 - EE5 for 39 days. To achieve higher EE concentrations in three microcosms EE was applied directly by adding an aqueous stock solution once (EE6, EE7) or twice (EE8). For details of the test design see Severin et al.[5]. Various physico-chemical parameters were measured. Phytoplankton samples were taken before, during and, to investigate the recovery, for three weeks after the removal of the tubes. Samples were fixed with about 12 drops of Lugol per 100 ml and sedimented in a plankton chamber according to the Utermoehl method. Algae cells were counted in an inverse microscope. Diversity indices H' and Evenness indices E following Shannon/Weaver were analysed. The biovolume was calculated following Hoehn et al. after identifying the species using Ettl et al. [6,7]. To detect changes in the community structure, the Principal Response Curve Method (PRC)) with the computer program CANOCO was used.

Results and discussion

EE concentrations in the microcosms with the controlled release system increased during the first 2 weeks. Maximum concentration reached was almost 700 ng/L in EE5. However, concentration courses decreased during the next week, showing a second peak in most microcosms (except EE3) before the tubes were removed. EE concentrations sank below detection threshold rapidly after the application end. By direct application maximum concentrations between about 50 µg/L (EE6) and 540 µg/L (EE8) were measured, showing a rapid decline. For time course of the concentrations see Severin et al.[5]. None of the measured abiotic parameters showed any correlation with the EE concentration.

Tab. 1 presents sum of the species number of different phytoplankton algae classes identified in all microcosms during the complete test period, revealing a considerable diversity. Number of species in each microcosm (about 15-30/microcosm at each sampling day) did not decrease until the end of the study, thus the phytoplankton community in the artificial test systems did not indicate any degradation.

Tab. 1: Number species of different of algae classes in the phytoplankton community

	Number of species
Bacillariophyceae	48
Chrysophyceae	8
Chlorophyceae	39
Conjugatophyceae	25
Cryptophyceae	15
Cyanophyceae	22
Dinophyceae	5
Euglenophycea	4
Oedogoniophyceae	1
Xantophyceae	2
Unidentified species	14

Evenness E did not show unambiguous EE dependent shifts (Fig 1). During the application period evenness of the treated microcosms varied over a wider range than the controls, but no correlation with EE exposure occurred, neither with concentration nor with toxodose. The results for the diversity index H' were similar (not shown here).

The biovolume of phytoplankton also did not unveil clear EE dependent changes. (Fig 2.) Biovolumes of the treated microcosms tended to be lower during the application period. This tendency disappeared after the end application. However, again no EE exposure dependency could be detected, therefore it was no possible to assign a significant effect to it. Differentiating the biovolume to algal class level supports this result.

First results of the PRC also showed no clear exposure dependent changes of the community composition.

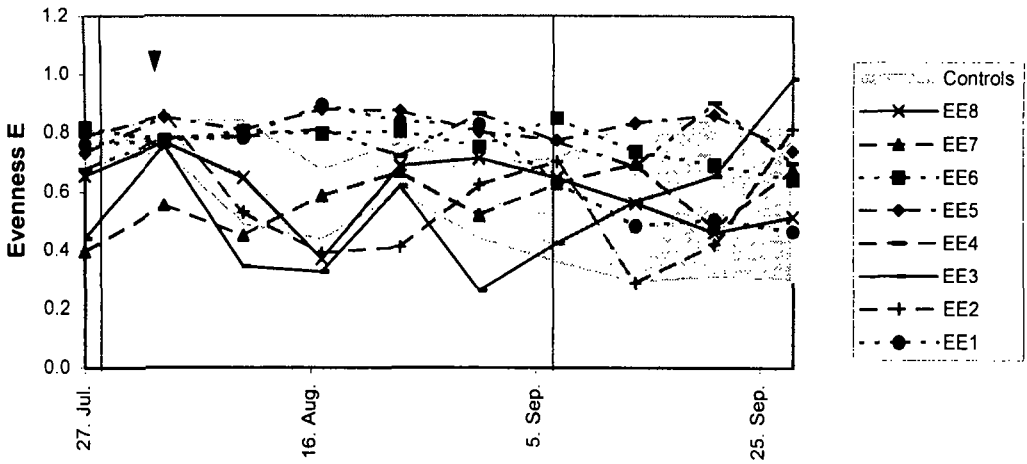

Fig. 1: Evenness E; vertical lines: beginning and end of controlled release application in EE1 - EE5; first direct application in EE6 - EE8 at the left vertical line, second direct application in EE8 is indicated by the arrow

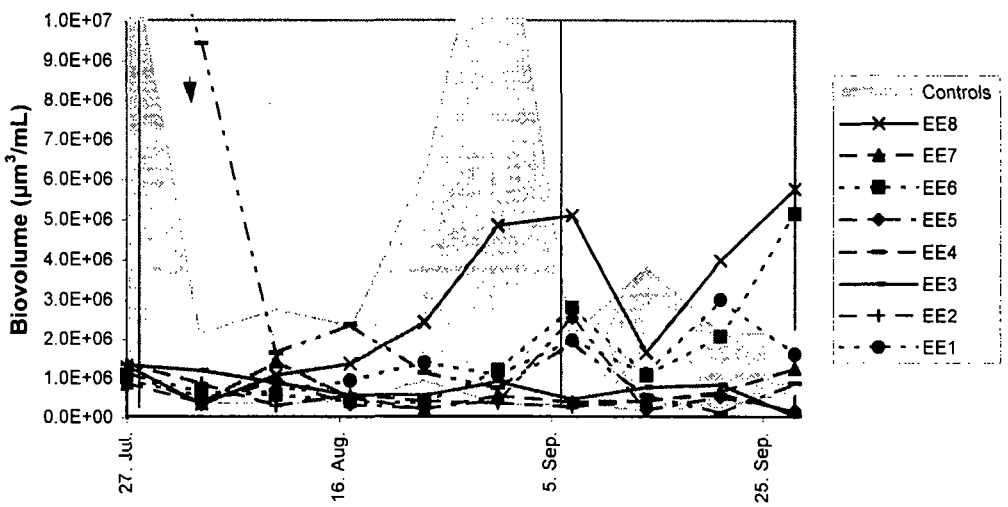

Fig. 2: Total biovolume of phytoplankton; vertical lines: beginning and end of controlled release application in EE1 - EE5; first direct application in EE6 - EE8 at the left vertical line, second direct application in EE8 is indicated by the arrow

The EE study presented here did not unveil clear EE exposure dependent effects on evenness, diversity, biomass and species composition of phytoplankton. Whereas in the NP study also no effects on evenness, diversity and total biovolume could be shown, there were indications for NP dependent changes on more detailed levels of the phytoplankton community, i.e. on biovolume of algal classes and community composition[3, 8]. (It should be kept in mind that it could not be said whether these effects to the phytoplankton community were direct or indirect, e.g. by primary affecting the zooplankton.) An explanation for this differences could be a different, non-endocrine mode of action of NP to the communities. However, there were some problems during the conduction of the EE study, e.g. a flood in Lake Ammersee, causing a muddy water with a possibly disturbed plankton community at the time water was taken for the microcosms. As there were considerable variations in the phytoplankton development during the pre-application period (not shown here), 50 L of the water of each microcosm was removed, mixed with water of the other microcosms and carefully replaced to get comparable biotic conditions at the beginning of the application. This mixing might have interfered with the development of more constant biocenosis, i.e. biocenosis not showing such a high variability of biotic parameters within the time course of each microcosm or between different microcosms as found in this study.

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