EFFECTS OF 17α-ETHINYLESTRADIOL ON THE ZOOPLANKTON OF AQUATIC MODEL ECOSYSTEMS

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Introduction

Various chemicals have been reported to affect the endocrine systems of a wide range of organisms within the aquatic environment. Many of these so called endocrine disruptors have estrogenic character which means that they have similar effects as the female sex hormone 17β-estradiol. Among these are industrial chemicals, pesticides heavy metals as well as synthetic hormones such as 17α-ethinylestradiol (EE), a compound of the contraceptive pill (30-50µg per pill). EE enters the aquatic environment through sewage treatment plant effluents and was found in natural rivers and streams in Germany in ng/L concentrations[1, 2]. Due to its persistence and the continuous discharge through oral contraceptives as well as a veterinary pharmaceutical, an increasing contamination of our surface waters with EE concentrations can be expected. We conducted a study to investigate the effect of EE on the zooplankton community of aquatic microcosms where the substance was continuously added to the water (controlled release) to mimic the discharge from sediments and sludges as it appears in the natural environment. A microcosm test with similar test conditions was conducted with the industrial chemical nonylphenol (NP)[3], a substance whose estrogenic property has been shown for vertebrates[4]. Since it was unclear whether the observed effects were due to the toxic or endocrine properties of NP, the experiment with EE served as a reference study.

Methods and Materials

Cylindrical containers (∅ 80 cm, height 60 cm) made from stainless steel were filled with a 10 cm layer of natural lake sediment, 230 L water and plankton organisms from an oligo-mesotrophic littoral area of Lake Ammersee (Bavaria, Germany) in June[3]. The microcosms were installed in an artificial outdoor pond to avoid extreme temperature fluctuations. An automatically operated transparent roof covered the model ecosystems in case of rainfall events to avoid water overflow. 17α-Ethinylestradiol (Sigma Aldrich) was applied in five different concentrations (EE1-EE5) using a controlled release system made from semi-permeable LDPE tubes[5] for a duration of six weeks (end of July – beginning of September). A dispersion of EE and Triolein was filled into tubes of different lengths which were then thermosealed. Due to the limited lengths of the LDPE-tubes a concentrated EE stock solution was directly added to three microcosms where µg/L-concentrations were achieved (EE6-EE8) once (EE6, EE7) respectively twice (EE8).

The EE-concentrations were measured in daily intervals shortly after beginning and end of dosing and weekly in August, using HPLC and UV detection. Five microcosms served as controls and remained untreated. In order to prevent that the plankton biocenosis within the single microcosms develops too individually before the treatment, 50 L of water was removed from all microcosms, mixed in a great container and redistributed into the microcosms carefully. This mixing was done twice in the pre-application period (end of June – end of July). From June to September the physical and chemical parameters of the microcosm water were measured and plankton samples were taken in weekly intervals.

Results and Discussion

The EE concentrations in the microcosms where the controlled release system was applied (EE1-EE5) are shown in Fig. 1. The concentrations increased shortly after the LDPE tubes were added to the microcosms but then dropped down in all microcosms. Three weeks after beginning of dosing, concentrations from 20-50 ng/L were measured. A few days before the end of application, the concentrations increased in all microcosms (except EE3) and decreased quickly after removal of the LDPE-tubes one week later. For the microcosms EE6-EE8 the time course of the concentrations was as expected: the highest EE concentrations were measured after the stock solutions were added and a rapid decrease of the substance could be observed (Fig. 2). The values for oxygen saturation, pH, conductivity as well as NO_3-N, PO_4-P and NH_4-N of the microcosms exposed were on the whole within the range of the controls.

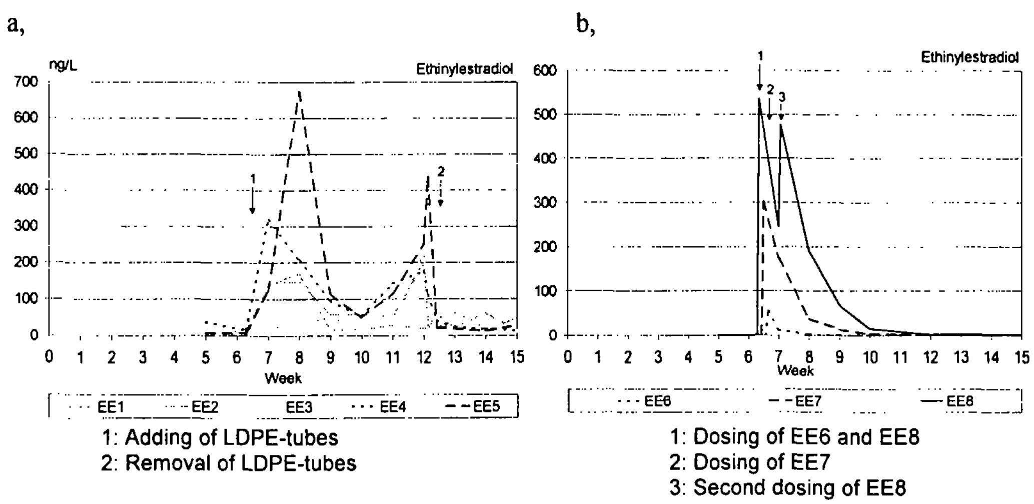

Fig. 2: *Concentration of EE in water of 230 L microcosms: a, Controlled Release, b, Direct dosing with stock solutions*

The comparison of the population dynamics and the abundances of the rotifers, cladocerans and copepods in the treated microcosms where on the whole within the range of the controls so that no clear effect of EE or a dose-response relationship could be identified (Fig. 3).

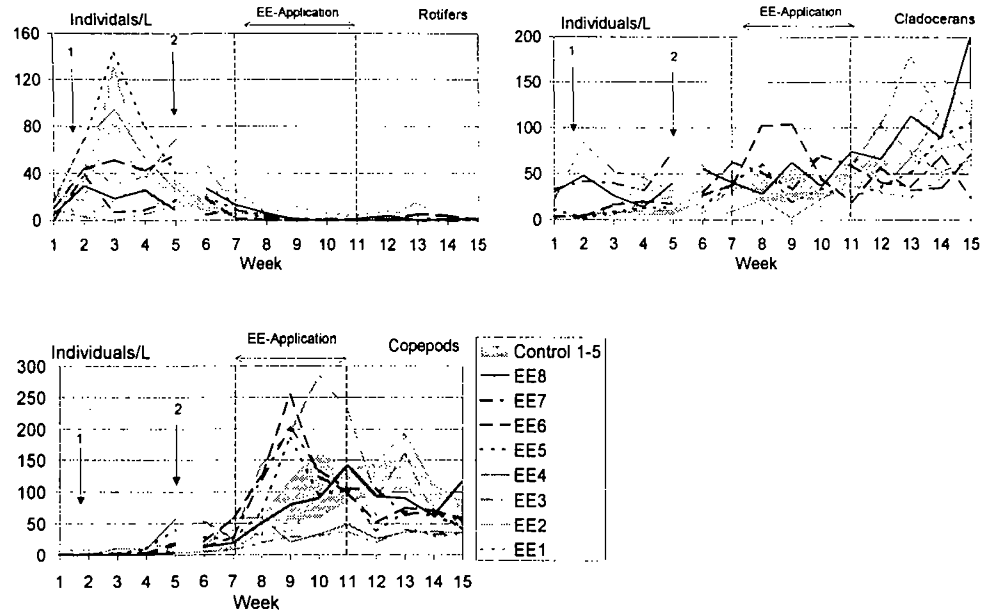

Fig. 3: Population dynamics of the rotifers, cladocerans and copepods in the microcosms

Conclusions:

Multivariate statistical methods (Principal Response Curves) showed that the EE-microcosms varied more from the controls in the pre-application period than from the beginning of the application on. It can thus be concluded that some stress on the population, e.g. the mixing of the water, had a greater effect on the zooplankton than the following treatment with EE.

A study with EE can be regarded as a reference experiment to investigate the effects of a substance with estrogenic potency on the zooplankton community. Since the mechanical stress on the biocenosis might have disguised an endocrine disrupting effect, it seems inevitable to repeat the experiment without applying multiple stressors.

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