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Effects of Anthropogenic Estrogens Nonylphenol and 17α-Ethinylestradiol in Aquatic Model Ecosystems*

Microcosm tests were conducted to investigate the effects of the estrogenic substances nonylphenol (NP) and 17α-ethinylestradiol (EE) on aquatic ecosystems. Maximum concentrations of 9 to 120 μg L^{-1} NP resp. 49 to 724 ng L^{-1} EE were induced by controlled release. The controlled release method allows the establishment of a continuous concentration course. The microcosms proved to run robustly with abiotic conditions close to natural. They developed biocenosis with similar characteristics as in natural ecosystems and, considering their given level of complexity, they can be used to describe possible risks for the environment. Both tested chemicals unveiled the potential to affect the plankton communities in the tested concentration range. NP exposure caused a reduction of Cladocera and Copepoda abundances and disturbed the phytoplankton structure. A $NOEC_{community}$ of 30 μg L^{-1} was calculated. In the first EE study, a flood in the lake where the microcosm water was collected caused additional stress and thereby a high variability, both between the microcosms and in each microcosm over time. Probably therefore the only effect found was a reduction of Copepoda abundance. In a second EE study Cladocera and Copepoda abundances were reduced, from which the phytoplankton benefited. Although a final interpretation is difficult for results of microcosm tests, there are indications that the found effects of EE and perhaps also NP may be caused at least partially by endocrine disruptive activity.

Wirkungen der anthropogenen Östrogene Nonylphenol und 17α-Ethinylöstradiol in aquatischen Modellökosystemen

Es wurden Mikrokosmostests durchgeführt, um die Wirkung der Östrogene Nonylphenol (NP) und 17α-Ethinylöstradiol (EE) in aquatischen Ökosystemen zu untersuchen. Mit Hilfe der „Controlled Release"-Methode wurden dabei maximale Konzentrationen von 9 bis 120 μg L^{-1} NP bzw. 49 bis 724 ng L^{-1} EE erzielt. Die „Controlled Release"-Methode ermöglicht einen kontinuierlichen Konzentrationsverlauf. Es wurde gezeigt, dass in den Mikrokosmen naturnahe Verhältnisse herrschten. Die Biozönosen entwickelten sich mit ähnlichen Eigenschaften wie in natürlichen Ökosystemen und eigneten sich hinsichtlich ihrer Komplexität, um mögliche Umweltrisiken aufzudecken. Beide Testsubstanzen zeigten das Potential, in dem untersuchten Konzentrationsbereich die Planktongemeinschaften zu beeinträchtigen. NP-Exposition verursachte einen Rückgang der Cladoceren- und Copepodendichten und veränderte die Struktur des Phytoplanktons. Der $NOEC_{community}$-Wert lag bei 30 μg L^{-1}. Bei einem ersten EE-Versuch verursachte ein Hochwasser mit Eintrübung in dem See, aus dem das Wasser für die Mikrokosmen entnommen wurde, zusätzlichen Stress und eine hohe Variabilität sowohl zwischen den einzelnen Mikrokosmen als auch in jedem Mikrokosmos über der Zeit. Wohl deshalb konnte als einziger Effekt ein Rückgang der Copepodendichte gefunden werden. In einem zweiten Versuch nahmen Cladoceren- und Copepodendichte ab, wovon das Phytoplankton profitierte. Obwohl eine endgültige Interpretation von Mikrokosmenergebnissen schwierig ist, gibt es Anzeichen dafür, dass die gefundenen Effekte von EE und möglicherweise auch von NP zumindest teilweise über Störungen endokriner Systeme erfolgten.

Keywords: Zooplankton, Phytoplankton, Endocrine Disruptor, Microcosm, $NOEC_{Community}$

Schlagwörter: Zooplankton, Phytoplankton, Endokriner Disruptor, Mikrokosmos, $NOEC_{Community}$

* Paper presented in part at the Late Summer Workshop "Monitoring Toxic Effects in Aquatic Systems", Schloss Maurach, Lake Constance, September 2003.

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DOI 10.1002/aheh.200300552

Research Paper

1 Introduction

1.1 Endocrine disruption

Science focuses on substances occurring in the aquatic environment and interfering with the hormone system of a wide range of organisms since the beginning of the 90s. Many of these so-called xenobiotics exhibit estrogenic properties which means that they have similar effects as the female sex hormone 17β-estradiol. This was shown for vertebrates in numerous studies whereas the results for invertebrates are scarce. The potential of these so called endocrine disruptors to be hazardous can be investigated on various test scales. However, still very few studies deal with effects of substances with known endocrine disruptive activity on multispecies test systems, at least partly because it is difficult to decide whether found effects were caused by endocrine or other, non-endocrine toxic activity of the test chemical. Therefore, we conducted tests with selected endocrine disruptors in aquatic model ecosystems (microcosms) to investigate effects on non-vertebrate organisms. One of these (4-nonylphenol; NP) has also relevant non-endocrine toxicity [1], while as far as known until now 17α-ethinylestradiol (EE) at least mainly acts as an endocrine disruptor and thus is regarded as a positive reference.

1.2 Investigated chemicals

Technical NP is among the chemicals with the potential to affect hormone pathways [1]. It occurs in the environment as a biodegradation product of alkylphenol polyethoxylates and is found in surface waters as well as in sewage treatment effluents and sediments. NP concentrations up to 180 μg L^{-1} were found in freshwaters, but most were below 3 μg L^{-1} (see [2, 3]). The estrogenic property of NP was shown for vertebrates, especially for fish in earlier studies, the results for invertebrates are controversial [4–9]. Only few studies with invertebrates investigated whether found NP effects e.g., in chronic experiments were (partly) of endocrine disruptive origin. Some new publications indicate that NP indeed affects invertebrates via the endocrine pathway [10, 11]. Concentrations of 10 μg L^{-1} were reported to disrupt ecdysone controlled processes [10]. Effects of NP on the zooplankton community were shown in only one field study with littoral enclosures where NP was added directly to the medium [12]. Beside its endocrine activity NP exhibits other relevant toxic properties on various endpoints of different species [1]. The exact mode of action mostly is unknown, although the surfactant character of NP may play a role.

The EC_{50} values for Crustacea mostly range between 100 μg L^{-1} and 470 μg L^{-1} (lowest at 4.8 μg L^{-1}). The only published EC_{50} for Rotatoria (*Brachionus calyciflorus*) was at 600 μg L^{-1}. Generally, algae exhibit a lower sensitivity, with EC_{50} values mostly higher than 500 μg L^{-1}. A detailed overview is given in [1].

The synthetic hormone EE is a component of oral contraceptives and veterinary pharmaceuticals. It enters the environment through sewage treatment effluents and is found in ng/L-concentrations [13]. Due to its persistence and the continuous discharge, an increasing contamination of surface waters with EE concentrations can be assumed. In the sewage effluents, where EE is set free by bacterial splitting of the conjugates, maximum concentrations are mainly below 1 ng L^{-1}, but 14.2 ng L^{-1} was reported [14]. In surface water, maximum concentrations range from 0.2 to 4 ng L^{-1}. Regarding the concentrations found in the environment, EE has, as far as known, apart of its strong estrogenicity only a low toxic potential [15–20]. Single species tests show first effects on fish between 1 ng L^{-1} and 100 ng L^{-1}, whereas effects on Crustacea occurred in range of lower μg/L to mg/L concentrations. No effect on the rotifer *Brachionus calyciflorus* was found up to 201 μg L^{-1}. The only published investigation with algae presented an EC_{50} in the *Scenedesmus* growth inhibition test of 840 μg L^{-1}. For an overview see Hense et al. and Jaser et al. [14, 21].

1.3 Data treatment

Ecotoxicological testing results in the identification of ecologically acceptable concentrations (EAC), defined as the highest concentration of a xenobiotic that does not lead to ecologically significant effects. One suitable endpoint to describe it is a $NOEC_{Community}$ value, which measures the effect of a chemical on community (or biocenosis) level [22, 23]. Multivariate statistical ordination methods such as the principal response curve method can be applied to describe direct and indirect effects on the populations as well as for the recovery of the systems [24]. The result of this calculation can serve as a base for deriving a $NOEC_{Community}$ [3, 13]. This analysis allows a more integrated view on shifts of species composition of the whole community (biocenosis) than calculating NOECs separately for each species of a model ecosystem calculated by univariate statistical methods and subsequently aggregating them to a single $NOEC_{Community}$. There are different possible approaches in risk assessment that lead to EACs, including methods that consider the time course of the concentration of the test substance in the microcosms (e.g. toxodose) [25]. In addition to ecotoxicological testing on biocenosis level, statistical extrapolation models with data from laboratory single species testing can be applied to obtain an $NOEC_{Community}$ on base of HC_5 values (hazardous concentration of 5% of the species) [26].

1.4 Suitability of the model system

Major aspects concerning suitability of artificial aquatic ecosystems in ecotoxicological studies are stability and similarity of their biotic and abiotic conditions to natural ecosystems [27]. Microcosms may shift from normal conditions e.g., due to their small scale and usually restricted exchange with their environment [28]. This could result in decreasing biodiversity, extinction of higher trophic levels, decreasing evenness (blooming of one/a few species), shifts of O_2 concentration over time as a consequence of extreme shifts of the organisms or/and limited gas exchange etc. Unnatural changes may reduce the ecotoxicological validity of the study.

Microcosms inevitably differ from natural conditions with regard to their biotic and abiotic properties [27]. Furthermore, natural conditions themselves show variability [29]. Nevertheless, a high similarity with some general ecological features e.g., of species composition and seasonal changes as described by Sommer [29] is desirable [23]. Furthermore, the variations between (untreated) microcosms should not be to high to enable the detection of shifts caused by the test substance.

Thus, for an adequate conduction of ecotoxicological tests it is necessary not only to describe the deviation of the treated microcosms from the controls, but also to regard the development of the biological and physico-chemical properties in the controls.

Although endocrine disruptive activity would not directly affect primary producer or abiotic variables, they should be included in the analysis of a comprehensive microcosm study. This is necessary to detect potential non-endocrine disruptive toxic effects and indirect effects.

Here, we give a summary of our studies, focussing on some main aspects of the experimental conditions (suitability, data treatment) and a comparison of effects of both chemicals on the plankton. Object of this paper is not to calculate effect concentrations for all analysed variables, but to investigate the mode of changes in the system and calculate general effect thresholds. For a more detailed description of the methodical procedures, data presentation, and discussion of each study see the cited literature.

2 Method

Microcosms studies were conducted in subsequent years with NP (one experiment, [2, 3, 13]) and EE (two experiments: ethinylestradiol 1 [13, 21] and 2 [14]). The studies began in late spring (May/June) and ran until autumn (about September). The aim was to investigate responses of zoo- and phytoplankton. The microcosms did not contain vertebrates.

2.1 Chemical application

Guidance documents for the risk assessment of pesticides in aquatic environment and for evaluation of results from higher tier test systems for this risk assessment have been developed by expert panels [23, 30]. They propose direct, discontinuous application of the test substance. This application strategy leads to strong concentration variations, which might reflect the situation for agrochemicals. However, other substances are released more continuously into the environment. We therefore chose, deviating from these guidance documents, a method releasing the chemical by diffusion from a depot in semipermeable LDPE (low-density polyethylene) tubes into the test system [31] (named “controlled release method”). A regression design with usually 6 different concentrations, achieved by application with differently long LDPE tubes, was selected. Due to the selected method, no replicates were possible. The concentration ranged from those relevant in the environment to those where effects were detected in laboratory tests. Furthermore, control microcosms (mostly six) were run without treatment.

2.2 Test system

The sizes of multispecies systems that are applied in aquatic ecotoxicology range from small test vessels with to enclosures to natural ponds. The volumes of mesocosms start from 15 m^3, the smaller test systems are called microcosms [30]. Compared to laboratory experiments meso- and microcosms consider higher levels of complexity and biological organisation so results can be obtained which are comparable to the conditions in natural ecosystems [32].

The aquatic microcosms used in our studies were constructed of cylindrical, bottom-closed stainless steel containers (∅ 80 cm, height 60 cm) and filled with water, sediment, and the natural plankton biocenosis from an oligo-mesotrophic littoral area of Lake Ammersee (Upper Bavaria, Germany), including the natural plankton. The microcosms were placed into an artificial outdoor pond to stabilize temperature.

2.3 Test design

After pre-application periods of 4 to 5 weeks different concentrations of the chemicals were applied in microcosms by controlled release for 6 to 9 weeks, then the tubes were removed. Samples were taken usually weekly during pre-application, application and recovery period.

Various abiotic variables were measured (concentration of the test chemical, phosphate, nitrate, ammonium and oxygen concentration, pH, conductivity and temperature; for details see [13]).

Zooplankton was fixed in a 20 % sugar, 5 % formalin solution, phytoplankton with Lugol solution using the Utermöhl method [33]. Plankton species were identified to the lowest possible level, mainly species and genus level, and quantified using microscopes. For Copepoda, the different developmental stages were counted separately. Number of species per microcosm, abundances, diversity index and evenness (Shannon-Wiener), and phytoplankton biomass were calculated. For methodical details see Hense et al. [2] and Severin et al. [3].

2.4 Numerical analysis

To analyse the difference in composition and abundance of taxa between controls and exposed assemblages, principal response curves (PRC), an ordination method based on redundancy analysis (RDA), were calculated using CANOCO 4.0 [24]. All calculations were performed using $\log(x+1)$ transformed relative abundances. A linear combination of variables (changes of species abundance) was calculated to determine the deviation of each exposed assemblage from the mean of control assemblages at each sampling day expressed as canonical coefficients (c_{dt}). Principal response curves were derived by plotting the c_{dt} against time. Thus, the *x*-axis represents the mean of the controls and the c_{dt}s of the treated microcosms their deviation from the controls for each sampling date. Species scores indicate increased or decreased abundances of taxa in exposed assemblages.

The c_{dt} values before application begin can be used to calculate a 95 % confidence interval for unaffected microcosms. By comparing this interval weekly with c_{dt} of microcosms treated with different concentrations a $NOEC_{Community}$ can be derived [3, 13].

3 Results and discussion

An overview of the main results is given below, specifying some data as examples. For other details see [2, 3, 13, 14, 21, 31].

3.1 Controlled release

Figure 1 shows the concentration courses. In the NP study the concentration increased rapidly after beginning of the treatment and remained more constant after three weeks. Maximum concentrations ranged between 9 $\mu g\ L^{-1}$ and 120 $\mu g\ L^{-1}$ NP. In two EE studies concentrations of 49 to 724 ng L^{-1} were reached. The time course of the concentration was variable in both studies, with peaks at the beginning and the end of the application period and a minimum in between. In the second EE study the last peak arose after a renewing of the tubes, however, it also occurred in the first EE study without a renewing.

After removal of the tubes the concentrations of NP and EE declined rapidly, reaching the detection level within approximately two weeks.

Controlled release application appeared to be a suitable method to simulate exposure situations in the real environment. Direct application produces suddenly arising, short time concentration pulses. Repeated direct application creates a series of peaks, which usually do not reflect the environmental situation of most xenobiotics and may itself influence the reaction of the ecosystem. The intermittent exposure leads to considerable short-time concentration variations (e.g., a decrease of about 50...75 % by application of NP every second day [34]). The frequent concentration analysis needed by such time-courses often is hardly practicable, scarcer analysis interferes with an estimation of effect concentrations, as the real exposure is unknown. In contrast, the concentration course by controlled release is more consistent and better suitable to detect chronic effects. Measuring the real concentration enables to derive threshold values (e.g., NOEC, toxodose). However, the time course of the concentration is not always predictable and has to be taken into account when the results were interpreted.

3.2 Suitability of the microcosm test system

The microcosms proved to be robust during the investigations. The physico-chemical parameters in the control microcosms were in reasonable ranges [13, 21], enabling natural biocenosis to develop [29]. Seasonal shifts were detectable, e.g., increase of oxygen saturation in the beginning, probable due to increased algae and macrophyta growth followed by a decrease towards the end in autumn, when plant activity declined. None of the abiotic parameters were completely different from those measured in natural ecosystems.

The plankton communities also proved to be satisfyingly stable over the test period. As an example, results for phytoplankton in the second EE study are shown. Phytoplankton plays a key role a primary producer in most natural lenthic ecosystems and usually is present with a number of species, thus it is appropriated to give general information about the ecosystem [29, 35]. The data only include algae larger than 5 μm in at least one dimension and with abundance higher

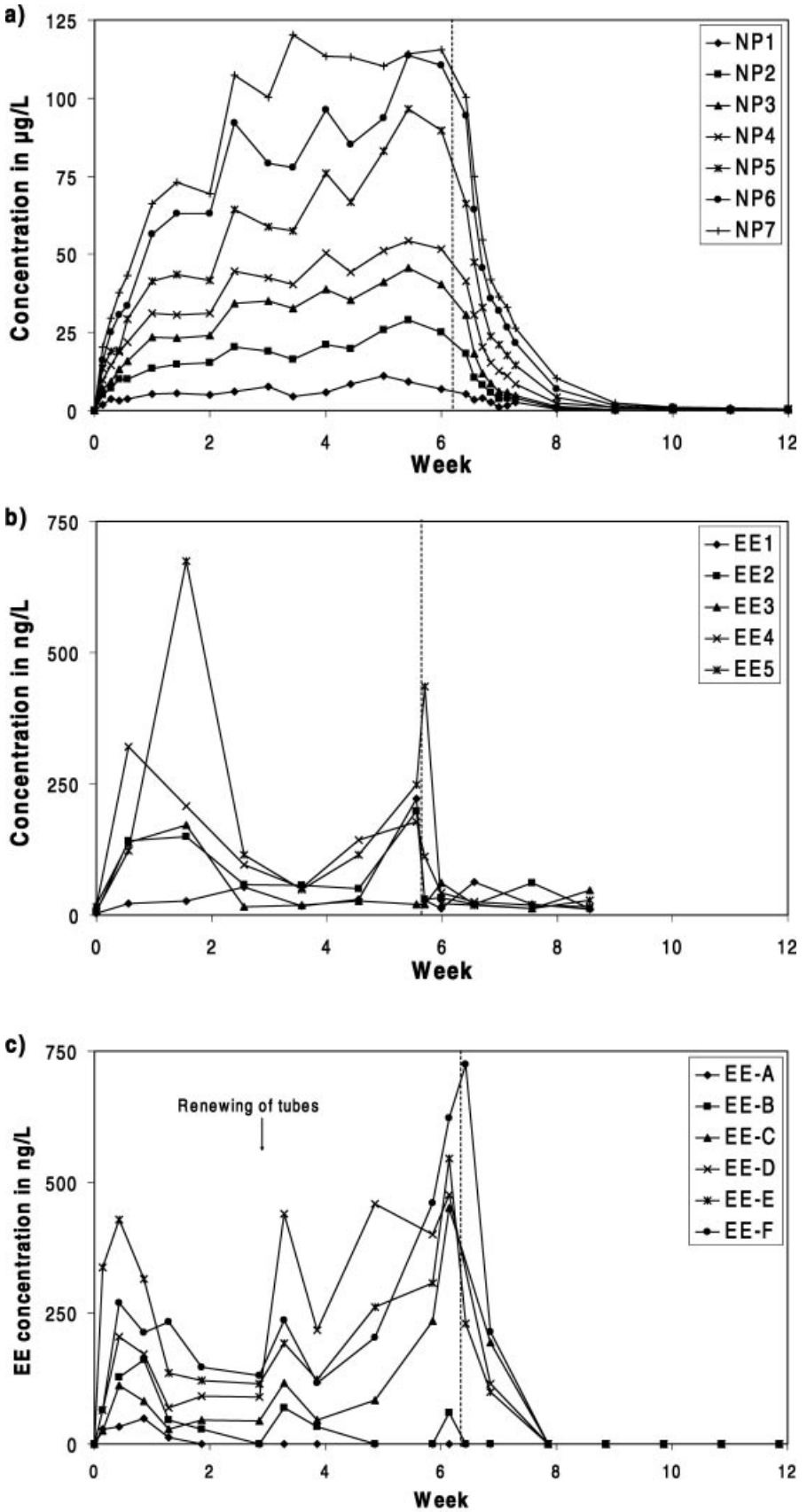

Fig. 1: Concentration courses after beginning of dosing; dotted line: end of dosing; a) NP (adapted from [26]), b) first EE experiment (adapted from [19]), c) second EE study [12].

Konzentrationsverläufe nach dem Beginn der Applikation; gestrichelte Linie: Ende der Applikationsphase; a) NP (angepasst aus [26]), b) erster EE-Versuch (angepasst aus [19]), c) zweiter EE-Versuch [12].

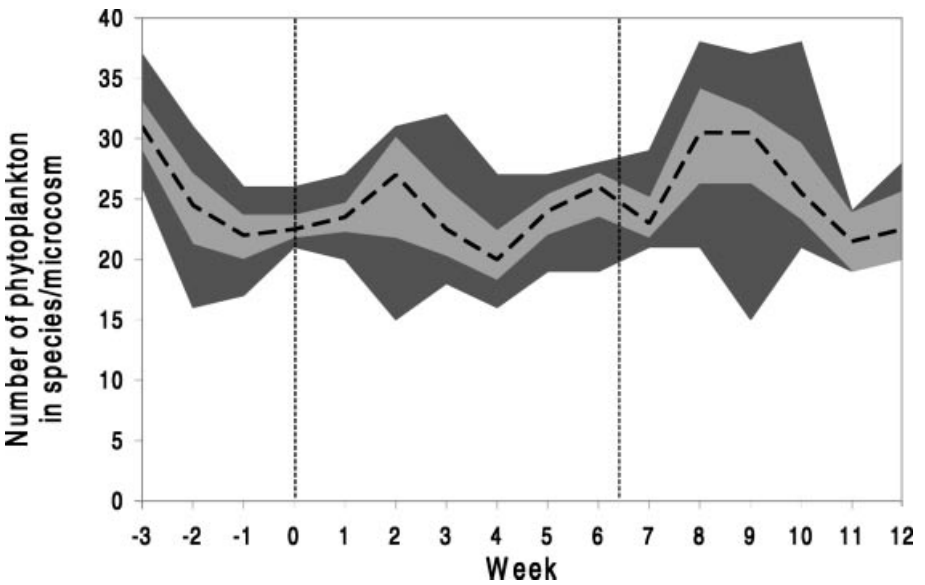

Fig. 2: Number of phytoplankton taxa per microcosm in five control microcosms in the second EE experiment; dotted line: median; light gray: 25...75 percentile range; dark gray: 0...25 percentile respectively 75...100 percentile range; vertical lines: beginning and end of treatment period.

Anzahl der Phytoplanktontaxa je Mikrokosmos in den fünf Kontroll-Mikrokosmen im zweiten EE-Versuch; gestrichelte Linie: Median; hellgrau: Bereich des 25...75-Perzentils; dunkelgrau: Bereich des 0...25-Perzentils bzw. des 75...100-Perzentils; vertikale Linien: Beginn und Ende der Applikationsphase.

than the determination limit of our counting procedure (1.3 individuals per millilitre, corresponding to at least 4 counted individuals per sample). The number of phytoplankton taxa in the control microcosms fluctuates as described from natural ecosystems [29, 35], but the mean in the controls stayed almost constant after a slight decline in the pre-application period (Fig. 2). The found numbers (mean 20...31) were well within the range reported from various natural lenthic ecosystems of this latitude [36, Burkhard Hense, pers. com.]. Diversity index H' and evenness index E (Shannon-Wiener) were nearly identical at the beginning (week 0) and the end (week 12) of the testing period (Table 1). In the middle experiment (week 7) number of species, H' and E showed a maximum. Their values (2.0 to 2.5 for H', 0.60 to 0.80 for E) also range well within those reported from natural ecosystems (e.g. [37]). The data indicate that the microcosms ran stable, i.e., that a relevant, unnatural species impoverishment during the test period due to the artificial conditions did not occur. The biodiversities support a vital ecosystem.

Although the microcosms showed some fluctuation, the variation between them was not too high during the test period. After a higher variation during the pre-application period, the standard deviation of number of taxa, H' and E was about 10...20% of the mean. A high variation of the controls would have interfered with the detection of chemical effects. The behaviour of the zooplankton corresponds with these conclusions [3, 13, 14, 21].

The results of the NP study were similar.

Table 1: Number of taxa per microcosm, diversity index H', and evenness index E (Shannon-Wiener) in the control microcosms for phytoplankton in the second EE experiment (mean ± SD). Week 0 and week 7: Beginning and end of treatment in the treated microcosms.

Anzahl der Taxa je Mikrokosmos, Diversitätsindex H' und Evennessindex E (Shannon-Wiener) des Phytoplanktons in den Kontroll-Mikrokosmen im zweiten EE-Versuch (Mittelwert ± Standardabweichung). Woche 0 und 7: Anfang und Ende der Applikationsphase in den behandelten Mikrokosmen.

	Number of taxa	H'	E
Week −3	31.3 ± 4.6	2.0 ± 1.1	0.60 ± 0.31
Week 0	23.0 ± 2.2	2.1 ± 0.2	0.66 ± 0.08
Week 7	30.0 ± 7.2	2.5 ± 0.4	0.79 ± 0.09
Week 12	23.2 ± 3.9	2.0 ± 0.3	0.64 ± 0.10

In the first EE experiment the fluctuation (i.e., the variation between the controls) was higher. The standard deviation of number of taxa, H' and E in the controls for each sampling week ranged mostly between 20% and 40% of the mean, but up to 60%. However, after an initial period of settling, the mean values remained almost constant. Adverse environmental conditions, i.e., a flood with murky water

stressed the ecosystem in the lake before water and sediment for the microcosms was collected. This may have caused increased variability sustaining during the whole study, which makes detection of effects difficult.

In the microcosms a succession occurred, similar to the seasonal succession often described for natural waters [29, 37]. At the beginning of the studies, zooplankton was mostly dominated by Rotatoria, which were subsequently replaced by Crustacea. In phytoplankton usually high numbers of diatoms and blooms of *Dinobryon* spp. (Chrysophyceae) were observed at the beginning of the test, followed by a period of low phytoplankton density reflecting the clear water phases in lakes. Subsequently the number of Desmidiaceae (Conjugatophyceae) and/or Dinophyceae increased. In autumn, the number of diatoms and partly *Dinobryon* spp. increased again. It cannot be decided, whether these parallel development follow the same ecosystematic causalities [28]. However it enables a nature-related test situation. The biotic variables often showed some variability in the pre-application period (e.g., blooms of algae species in some microcosms), but had converged until beginning of the treatment enabling comparable starting conditions.

3.3 Effects of nonylphenol

The abiotic variables showed no significant correlation with the NP treatment. According to the PRC of the zooplankton, all treatments differed from the controls, but this was most

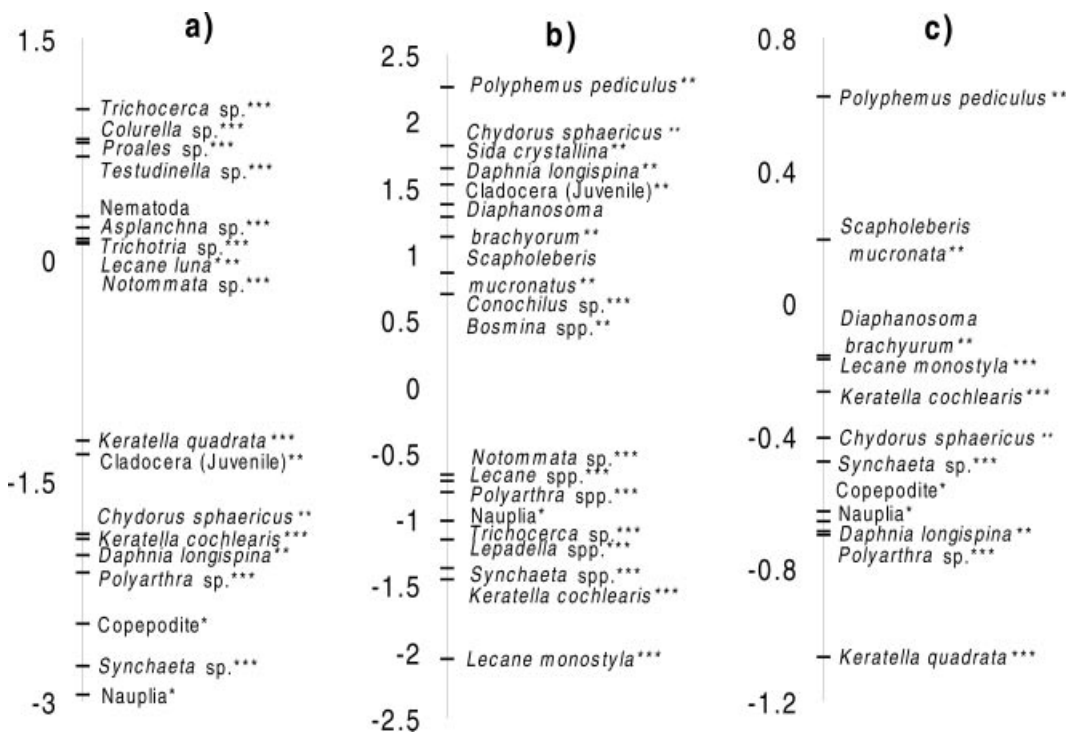

Fig. 3: Highest positive and negative species scores of zooplankton principal response curve (PRC) calculation; a) NP experiment (total number of analysed taxa: $n = 51$), b) first EE experiment ($n = 34$), c) second EE experiment ($n = 41$). Generally, in a) und c) positive values indicate an increase, negative a decrease of abundance during treatment. In the first EE experiment the abundances in the treated microcosms varied without connection to the treatment, thus the species scores only represent the connection of the species with the main variation component. *: Copepoda; **: Cladocera; ***: Rotatoria.

Höchste positive und negative Species-Score-Werte, die mittels der Principal-Response-Component (PRC) -Methode für das Zooplankton errechnet wurden; a) NP-Versuch (Gesamtzahl der analysierten Taxa: $n = 51$), b) erster EE-Versuch ($n = 34$), c) zweiter EE-Versuch ($n = 41$). Allgemein zeigen in a) und c) positive Werte eine Zunahme und negative Werte eine Abnahme der Dichten während der Applikationsphase im Vergleich zu den Kontroll-Mikrokosmen an. Im ersten EE-Versuch variierten die Dichten in den behandelten Mikrokosmen ohne Bezug zur Applikation, daher zeigen die Species-Scores nur die Stärke der Verbindung mit der Hauptvariationskomponente an. *: Copepoda; **: Cladocera; ***: Rotatoria.

pronounced in NP5 to NP7 [3]. The deviation appeared directly after begin of treatment and maintained until its end. Species scores of the PRC showed that abundances of Copepoda and Cladocera decreased in the treated microcosms, as indicated by negative species scores in Figure 3a. This especially applied to their juveniles (nauplia, copepodite, and, lesser, juvenile Cladocera). The Rotatoria species reacted less uniformly. Most of the species tended to decrease, but some taxa increased. Numbers of zooplankton taxa per microcosm were smaller at the end of treatment. The main effect on phytoplankton was a shift of the algae class dominating the biomass from Conjugatophyceae to Dinophyceae [2]. The PRC also showed shifts of phytoplankton structure in the highest treated microcosms. These shifts seem to be closer connected with the edibility of the algae than with their taxonomical relations [2]. Changes of phytoplankton appeared with a time lag compared to those of zooplankton.

During the five weeks of post-treatment period, the plankton communities only partly recovered.

3.4 Effects of 17α-ethinylestradiol

In none of the two EE studies a correlation of any abiotic variable with EE concentration was found.

First EE study. The only significant effect on plankton was a reduction of the copepods. However, some of the other biotic variables showed distinctive features, e.g. increased variabilities. The PRC did not unveil unambiguous shifts of zoo- and phytoplankton structure connected with the treatment [21]. The species scores showed no similarity with those of the NP experiment (Fig. 3b), indicating the absence of similar shifts of species composition in the treated microcosms of both experiments.

As mentioned above, a flood with markedly limited translucence occurred in Lake Ammersee before and during the collection of the water for the microcosms. This may be the reason for the variability of most biotic parameters, which was higher than during our other studies, both between microcosms and over time. The high background noise made the detection of EE dependent effects difficult. Therefore the study was repeated in the following year.

Second EE study. Almost all investigated biotic variables showed significant deviations after beginning of the treatment [14]. Both zoo- and phytoplankton species composition shifted compared to the controls (PRC). Zooplankton composition shift appeared almost directly after begin of treatment and only partly recovered after the end. Generally the abundances of the Crustacea decreased, although two cladocers showed inverse behaviour (Fig. 3c). Again juvenile Crustacea belonged to the most affected. Zooplankton species with most negative species scores (indicating strong decrease) were almost identical with those of the NP experiment. With the exception of the evenness *E* all phytoplankton variables increased. As in the NP study, changes of phytoplankton appeared with a time lag compared to those of zooplankton.

During the five weeks of the post-treatment period, the plankton communities only partly recovered.

3.5 Comparison of nonylphenol and 17α-ethinylestradiol effects

Assuming that the absence of detectable effects in the first EE study can to a high degree be contributed to problems resulting from the environmental conditions, EE and NP exposure showed some similarity regarding the effects on plankton. Both reduced Copepoda and Cladocera abundances, zoo- and phytoplankton species structure changed. Regarding the species scores of the PRC calculation allows us to assess the degree of shifts of single taxa groups. Zooplankton and, to a lesser degree, phytoplankton taxa responded similar with regard to the main affected species. Furthermore, time courses of the deviations of biotic variables showed parallels, e.g., changes in phytoplankton occurred later than in zooplankton. However the impact of the tested concentrations seemed to be stronger for EE. Function of an ecosystem is normally considered to be less sensitive than structure due to functional redundancy [38]. Therefore, Giddings et al. [23] stated that protection of functional endpoints is not sufficient. NP only changed the structure of the phytoplankton. In contrast, the increase of biomass of the primary producer phytoplankton indicates potential functional changes. However, shifts of abiotic parameters as reported elsewhere for chemicals affecting function of tests systems [39] were not found.

Both chemicals seemed to have affected zooplankton, primarily Crustacea and consequently, due to top-down control, indirectly the phytoplankton (Fig. 4). It is not clear, whether Rotatoria species were only indirectly affected, e.g., via resource competition with Crustacea and/or predator/prey interaction with Crustacea and phytoplankton. Possibly also a direct influence of the chemicals existed, superimposing with indirect changes. However, the nonuniform reaction of the Rotatoria species as well as their comparably high EC_{50} in single species tests – although not many data were published [1, 9, 21] – support at least a dominance of indirect caused changes. The same holds true for phytoplankton.

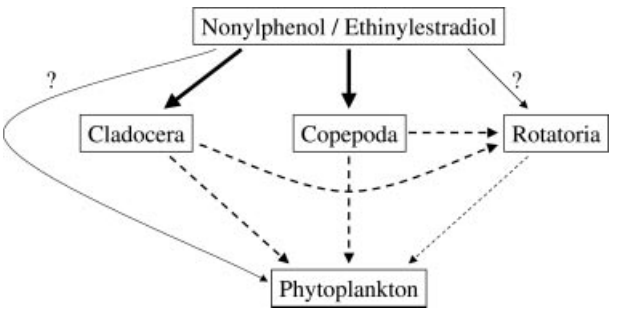

Fig. 4: Probable main effect pathways of NP and EE on phytoplankton in microcosms; solid line: direct effect; dotted lines: indirect effects (e.g., via trophic or competitive interaction); line widths indicate the strength of the relation.

Wahrscheinliche Haupteffektwege von NP und EE im Mikrokosmos; durchgehende Linie: direkte Effekte; gestrichelte Linie: indirekte Effekte (z. B. via trophische oder kompetetive Interaktion); die Liniendicke zeigt die Stärke der Verbindung an.

Furthermore, the time lag of phytoplankton changes supports the indirect cause of effects.

3.6 Ecologically acceptable concentrations

For NP a $NOEC_{Community}$ for zooplankton was calculated [3]. The mean no-observed-effect concentration of all treatment weeks was 30 μg L^{-1}, the lowest no-observed-effect concentration 19 μg L^{-1}. These concentrations were within the range of published environmental concentrations.

For the second EE study, variability of the EE concentration prevented a meaningful calculation of a $NOEC_{Community}$. First effects were found in microcosms with a maximum EE of 450 ng L^{-1} (mean during treatment period 63 ng L^{-1}), wheras no effects occurred in a microcosm treated with maximum 49 ng L^{-1} (mean 27 ng L^{-1}). The application of the toxodose may better deal with the concentration variability, as analysing toxodose diminishes fluctuations over time. Regarding the maximum of the c_{dt} derived from the pre-application period as an ecological acceptable limit comparable to EAC results in a threshold toxodose of about 3000 ng d L^{-1} in the second EE study. The toxodose concept, which was adapted for results of single species tests in ecotoxicology by Schramm et al. [25], may be helpful for the assessment of chronic risks from results of test systems with variable concentrations or time scales. However, the applicability of concept for results of multispecies test systems has to be verified.

3.7 Are the effects of endocrine disruptive origin?

Due to the, as far as known, high endocrine disruptive, but low non endocrine disruptive toxicity of EE, it can be assumed that the found effects may at least partly be caused by an interference with the steroid hormone system of zooplankton. This corresponds with the indirect impact on phytoplankton. For NP, which has a relevant non endocrine disruptive toxicity, a statement based on the results of microcosm studies is more difficult. The presented parallelisms of the effects of EE and NP would be in agreement with similarity of the mode of action, but further investigations are necessary to achieve a clearer assessment. Estrogen signaling has been shown to be present in invertebrates [40]. However, substances known to be disruptors of the estrogen system in vertebrates may also interact with the ecdyson pathway [10].

4 Conclusion

The test systems filled with sediment and water from an oligo-mesotrophic littoral area of Lake Ammersee (Bavaria, Germany) showed a high stability during the test period and abiotic as well as biotic characteristics, which are close to nature. They are therefore suitable for ecotoxicological testing. The reproducibility of a microcosm system depends critically on the test conditions. The ability of stressed biocenosis to unveil the toxic potential of chemicals is only limited. Controlled release application is an appropriated method to simulate exposure situations in the real environment. The regression test design is favourable because EAC values can be established.

NP and EE have the potential to affect the plankton community in the tested concentration ranges. EE influenced structure as well as function of the ecosystem. NP primarily affected the structure. However, the found decrease of Crustacea abundances may also be considered as a criterion for the ecosystem function, as it could inhibit the growth of higher consumers, e.g., fish, which were not included in this study.

For both tested chemicals, a partial recovery was observed after the end of treatment.

Although it cannot be ascertained unambiguously by such complex test systems whether found effects were of endocrine origin, the results give first indications that not only EE, but also NP may have endocrine disruptive activity in the microcosms.

Acknowledgements

We thank Dr. I. Jüttner, Dr. K. Koci, Dr. C. Klimm, G. Sigl, F. Stadler, A. Sommer, M. Hofmann, and J. Ruberg (Institute of Ecological Chemistry, GSF) for their assistance during the studies, and the Institute of Soil Ecology, GSF, for access to the artificial pond and support, especially Dr. A. Hartmann, Dr. M. Schloter, Dr. M. Jontofsohn and V. Barbosa.

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[Received: 15 December 2003; accepted: 5 August 2004]