National Environmental Research Institute
Danish Ministry of the Environment

Responses of marine plankton to pollutant stress

Integrated community studies of structure and function

PhD Thesis

Morten Hjorth

Roskilde University

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Abstract: The thesis analyses effects of pollutants on natural plankton communities on the basis of three independent mesocosm experiments and a series of laboratory experiments performed in Denmark and Greenland. The work focus on integrating functional and structural measures of community responses to reveal indirect effects and co-effects with the abiotic environment on three trophic levels, namely bacteria, phytoplankton and zooplankton. The role of mesocosms and community studies in risk assessment and their usefulness in integrating ecological knowledge into ecotoxicology is discussed with examples of work done on natural communities of phytoplankton and zooplankton. Abiotic conditions such as UV light and nutrient concentrations are shown to influence pollutant effects.

Keywords: functional and structural response, community, marine plankton, mesocosm, indirect effects, pollution.

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Contents

Preface

This thesis is written as part of the fulfilment of a Ph.D. from Roskilde University, Denmark. During the project I was based at the Department of Marine Ecology, National Environmental Research Institute, Roskilde, Denmark and registered under the Graduate School of Environmental Stress (GESS) as part of the Environmental Sciences Programme, Roskilde University.

When I began this project I came from a background in marine ecology and ecotoxicology, pollutants and everything that went with it was utterly new to me. Fortunately, I was taken under the wings of my patient supervisors, Ingela Dahllöf and Valery Forbes, who both have taught me some tricks of the trade. To Ingela, I am deeply grateful for all the effort, enthusiasm and fun she brought into the project. Without her, the field work at Søminen and in Greenland would probably not have turned out the way they did. Valery has always been prepared to give her opinion and advice to whatever us out by the fjord had come up with it.

My time among the good people at NERI has been great. I have been helped by a lot of people in many ways and I owe them thanks. Especially I would like to thank Dorthe G. Petersen, my fellow Ph.D. student in Ingela's group for all the support and good talks we have shared during the last three years. The rest of the gang in "sommerhuset" has also been great and thank you for feeding my fish!

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Finally, I would like to thank Zita for believing in me all the time and my family and friends for the support.

Morten Hjorth

Roskilde, October 2005.

List of manuscripts

Manuscript I: Hjorth M., Haller R., Dahllöf I. 2005. The use of ^{14}C tracer technique to assess the functional response of zooplankton community grazing to toxic impact. *Revised version submitted to Marine Environmental Research.*

Manuscript II: Hjorth M., Dahllöf I., Forbes V. E. 2005. Effects on the function of three trophic levels in marine plankton communities under stress from the antifouling compound Zinc Pyrithione. *Accepted for publication in Aquatic toxicology.*

Manuscript III: Hjorth M., Dahllöf I., Vester J., Henriksen P., Forbes V. E. 2005. Functional and structural responses of marine plankton food webs to pyrene contamination. *Submitted to Limnology and Oceanography*.

Manuscript IV: Hjorth M., Dahllöf I., Forbes V. E. 2005. Effects of pyrene on plankton communities under two different nutrient conditions. *To be submitted*.

Summary

The thesis analyses effects of pollutants on natural plankton communities on the basis of three independent mesocosm experiments and a series of laboratory experiments performed in Denmark and Greenland. The work focuses on integrating functional and structural measures of community responses to reveal indirect effects and interactions with the abiotic environment.

The mesocosm studies were conducted in Isefjord, Denmark during a three year period. In all the experiments function and structure of bacteria, phytoplankton and zooplankton was monitored in periods of 6-12 days. An array of endpoints was used including biomass, composition, growth and activity for all the three selected trophic groups. A new approach to study zooplankton community grazing activity as a functional response to pollutant stress, was developed as part of the thesis (manuscript I).

A risk assessment of an antifouling compound zinc pyrithione and its effects on plankton communities was the goal of the first mesocosm experiment (manuscript II). Direct effects of ZPT lead to cascading indirect effects throughout the community, eventually causing different developments of the communities, which was still evident at the end of the experiment.

The other two mesocosm experiments focused on the effects of a common polyaromatic hydrocarbon (PAH), pyrene. One experiment (manuscript III) was a prospective study of the nature and strength of pyrene effects on plankton communities. Direct effects of pyrene were evident in phytoplankton structure and function and after a lag-period of two days, also on bacteria. The integrated pelagic community function was affected at all exposure levels even on the last day of the experiment, suggesting that the present assessment criteria are not generally protective. The data implied that in contrast to earlier presumptions, PAHs might be an important stress factor for pelagic systems and not only benthic systems, since effects were found at concentrations as low as 2.5 nmol L^{-1}. Nutrient and substrate limitation hid the effects of pyrene on total community function, whereas effects on specific activities, abundance and algal community composition where more evident. The goal of the last mesocosm experiment (manuscript IV) was to test a hypothesis if biomass and nutrient availability would influence the effects of PAHs on pelagic communities. Effects were compared for two exposures of one concentration of pyrene on two pelagic communities that differed in initial biomass due to previous nutrient spiking. The results confirmed an assumption of a stronger and more widespread effect of pyrene on nutrient enriched phytoplankton communities with no dilution effect of pyrene. Diatoms were the least sensitive algae to pyrene exposure and indirect effects on zooplankton caused a lower abundance of zooplankton.

The role of mesocosms and community studies in risk assessment and their usefulness in integrating ecological knowledge into ecotoxi-

cology is discussed with examples of work done on natural communities of phytoplankton and zooplankton. Abiotic conditions such as UV light and nutrient concentrations are shown to influence pollutant effects.

Sammenfatning

Dette PhD projekt omhandler effektstudier af miljøfarlige stoffer på marine planktonsamfund. Studierne er baseret på tre uafhængige mesokosmos eksperimenter og en række laboratorie eksperimenter udført i Danmark og Grønland. Projektet fokuserer på at integrere funktionelle og strukturelle undersøgelser af samfundsrespons med henblik på at afdække indirekte effekter og kombinationseffekter med abiotiske faktorer.

Mesokosmos eksperimenterne varede i 6-12 dage og blev udført i Isefjorden henover en tre-års periode. Funktionelle og strukturelle parametre af bakterier, fytoplankton og zooplankton blev undersøgt i alle mesokosmos eksperimenterne. En række variable blev brugt til at beskrive de tre trofiske niveauer inklusiv biomasse, sammensætning, vækst og aktivitet. I forbindelse med metodevalgene blev der, som et led af PhD projektet udviklet en metode til at måle græsnings aktivitet af zooplanktonsamfund, således at det kunne bruges som et funktionelt respons på stress fra miljøfarlige stoffer (manuskript I).

Formålet med det første mesokosmos eksperiment (manuskript II) var at undersøge antibegroningsmidlet zinkpyrithion (ZPT) og stoffets effekter på planktonsamfund, således at data kunne indgå som et led i en risiko vurdering af ZPT. Direkte effekter af ZPT på fytoplankton og zooplankton førte til indirekte effekter igennem hele samfundet, der ultimativt førte til forskellige udviklinger af planktonsamfundene, som var signifikant forskellige fra ikke-eksponerede samfund ved afslutningen af eksperimentet.

De to øvrige mesokosmos eksperimenter var fokuseret på at studere effekterne af pyren, en almindeligt forekommende polyaromatisk kulhydrat (PAH). Et eksperiment (manuskript III) var en undersøgelse af på hvilken måde, og i hvor høj grad, pyren havde effekter på planktonsamfund. Der var tydelige direkte effekter af pyren på sammensætningen og fotosynteseaktiviteten af hele fytoplanktonsamfundet, og efter en lag-periode på to dage, var der også synlige effekter på bakterier. Den integrerede funktionelle aktivitet af planktonsamfundet var påvirket på alle eksponeringsniveauer ved slutningen af eksperimentet, hvilket antyder at de gældende vurderingskriterier ikke er generelt beskyttende for hele planktonsystemet. Data indikerer, i modsætning til tidligere antagelser, at PAH'er kan være en vigtig stress faktor i pelagiske systemer og ikke bare på bentiske systemer, da effekter var målbare ved meget lave koncentrationer (2.5 nmol L^{-1}). Næringsbegrænsning påvirkede effekter af pyren på funktionerne af hele samfundet, hvorimod effekter på specifikke aktiviteter, antal og sammensætning af fytoplanktonsamfundene var mere tydelige. Formålet med det sidste mesokosmos eksperiment (manuskript IV) var at teste hypotesen om at biomasse og tilgængelighed af næringsstof kan påvirke effekter af PAH'er på planktonsamfund. Effekter af en eksponeringskoncentration af pyren blev sammenlignet på to planktonsamfund med forskellig initial biomasse af fytoplankton som var frembragt ved forudgående næringsstof tilsætning. Resultaterne bekræftede en antagelse om en stærkere og mere vidtgå-

ende effekt af pyren på næringsberigede fytoplanktonsamfund. Kiselalger var de mindst følsomme alger overfor pyren eksponering og indirekte effekter på zooplankton forårsagede en lavere mængde af zooplankton.

Mesokosmos eksperimenters rolle i risikovurdering og deres anvendelse til at integrere økologisk viden ind i økotoksikologi, med det formål at opnå en bedre forståelse af effekter fra miljøfarlige stoffer på samfundsniveau er diskuteret. Ved hjælp af eksempler fra eksperimenter udført på naturlige samfund af fytoplankton og zooplankton vises det at abiotiske forhold som UV lysindstråling og koncentrationer af næringsstoffer påvirker effekter fra miljøfarlige stoffer.

1 Introduction

Purpose

The purpose of my work has been to emphasise the need of ecology within the field of ecotoxicology. The underlying working hypotheses have been 1) there is and has been a lack of ecology in ecotoxicology (Cairns 1986; Baird et al., 1996; Luoma 1996; Chapman 2002; Preston 2002) and 2) more ecology in ecotoxicology is beneficial to improve our understanding of the impact and consequences toxicant have on the environment. Firstly I will introduce ecotoxicology and risk assessment, then comment on the role of ecology in ecotoxicology, before I present the framework of my studies given by the communities investigated, the endpoints used and the experimental approach. I will strive to exemplify what is meant about ecology in the context of ecotoxicology and why it is necessary to have more ecology in ecotoxicology. The examples are based on the four manuscripts that comprise this thesis, and on additional results not yet published or submitted, but that also have been a part of my Ph.D. Finally, I will conclude on the extent the purpose of my thesis has been fulfilled and how.

Ecotoxicology

A definition or characterisation of the field of ecotoxicology is to study ecological and ecotoxicological effects of pollutants on populations, communities and ecosystems in conjunction with the fate of pollutants in the environment (Forbes and Forbes, 1994). An essential part of ecotoxicology is to provide data and knowledge for risk assessment of pollutants occurring in the environment (Newman, 1998). A vast range of tools, experimental approaches, test organisms and ecological systems are used to assess risk from pollutants to the environment. These range from simple single species test in dose-response experiments to larger scale ecosystem response studies.

Risk assessment

The aim of a risk assessment is to evaluate the risk of detrimental effects from contaminants on the environment. Data on pollutants and their effects are almost always incomplete because of the vast amounts of possible pollutants present and limited funding for thorough effect analyses. Therefore risk assessment is about extrapolating the limited available information to the entire environment. Data from standardised single species tests, population studies and more seldom from community studies are used to calculate effect levels. The effect levels range from effect concentrations, where variables are reduced by x % (EC_x) compared to unaffected controls, to threshold levels that are the highest concentrations at which no effects are observed (NOEC), or the lowest observed effect concentration (LOEC). If data on the pollutant in question are limited and only comprised of acute toxicity data on standard single species tests, usually an application factor is applied to account for effects on whole systems. Application factors ranges from 10 to 1000 and they are applied by dividing the lowest toxicity values with the relevant factor.

Single species tests are frequently used tools in risk assessment because they are fast and easy to use, economical and have a high repeatability. They can be standardised, they allow for effect comparison between different pollutants and the interpretation of data is straightforward. However, the value of a test is no better than the choice of test organism and endpoint as discussed by Cairns (1986).

If more data are available (e.g. NOECs for several species), they can be used for modelling species sensitivity distributions (SSD). This involves fitting NOECs to a statistical distribution and estimating the pollutant concentrations at which 95% or more of the species are protected. Several assumptions about the endpoints have to be fulfilled for an extrapolation to be valid. For example, the variability in sensitivity between the tested organisms and those present in the environment has to be similar. Additionally, the measured variables have to be representative of the ecosystem for the result to be protective, meaning the choice of variables must reflect what kind of system is investigated (Calow 1996).

There is a need for improvement in these extrapolations and thereby in risk assessments in general. The intrinsic uncertainties in extrapolating effects from limited studies to ecosystems in this way have been under scrutiny (Chapman et al., 1998; Forbes et al., 2001; Selck et al., 2002). Extrapolating data from single species tests to community level effects introduces much uncertainty (Preston 2002). The response of an individual organism to stress from pollutants may be very different in its natural surroundings compared to in standardised laboratory conditions such as those used in single species tests. No competition for space and/or resources within or among populations and between species is present in single species tests. Furthermore, changes in the abiotic environment or succession of community structure due to toxicant exposure are factors that can impact the state of the community both positively and negatively and such factors are greatly reduced, or not present under controlled laboratory conditions (Brock et al., 2004). It is clear that the ecological relevance of the study increases, with increasing complexity, however identifying clear cause-effect relationships may be more difficult (figure 1). Therefore extrapolation from single species tests to the community level may not be representative for the community response.

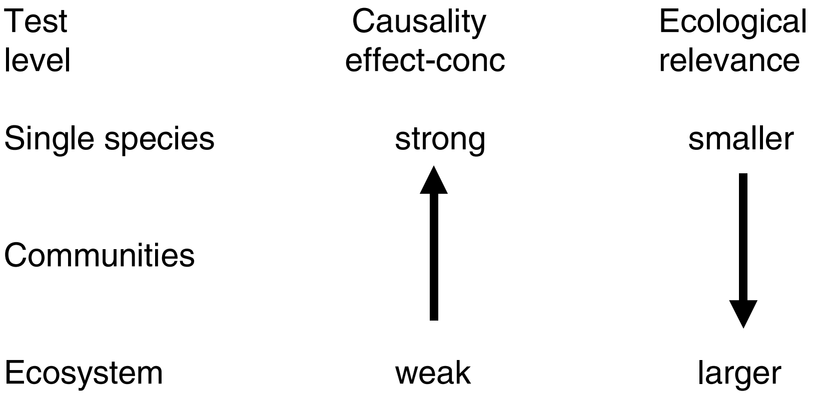

Figure 1. Relation between complexity of study and causal relationship and ecological relevance (Caquet 2000).

Ecology in ecotoxicology

Ecology can be characterised as the study of all interactions between organisms and their environment, and how these interactions fluctuate on both temporal and spatial scales, in turn determining the structure and function of an ecosystem (Begon et al., 1996; Polis and Strong, 1996; Levinton, 2001; Chapman, 2002). These interactions are fundamental in ecology, but not in ecotoxicology (Baird et al., 1996; Preston 2002). Although that discrepancy has been debated for some time, I believe it is still valid and relevant to integrate ecology and ecotoxicology for an improved understanding of contaminant effects on the environment. Ecological interactions where one or more participants are affected by a toxicant can lead to indirect effects (Wootton 1994; Preston 2002; Fleeger et al., 2003). Typical indirect effects are 'bottom-up', such as changed prey uptake by zooplankton due to changes in abundance or composition of their prey, namely phytoplankton, which are suffering from direct effects of a toxicant. In relation to ecotoxicology, pollutant impact must be thought of as a factor influencing the ecosystem and its interactions on an equal basis with other abiotic changes (Luoma 1996; Parker et al. 1999). Impacts should not necessarily be weighted more or less, but in combination with all other factors which influence an ecosystem. Such a point of view would enhance the integration of ecology and ecotoxicology and may lead to a stronger merge between the fields of ecology and ecotoxicology thus becoming a research field in stress ecology as advocated by Parker et al. (1999) and Van Straalen (2003). Food web models are central in order to evaluate direct and indirect effects simultaneously, so pollutant changes to the structure and function of the food web can be investigated (Koelmans et al., 2001).

2 Thesis framework

The following section sets the frames of the work I have done. The part of the marine ecosystem that has been my interest will briefly be introduced, and which communities in that ecosystem my focus has been directed towards. How these communities were investigated, in terms of the selected experimental approach and the array of variables I chose to study is the content of the next paragraph. The selected pollutants and the reasoning behind choosing them for my studies conclude the setting of the framework.

The marine planktonic ecosystem

The marine planktonic ecosystem has been the focus of this study. It is the foundation of almost all organic matter production in the sea, providing the energy basis for the higher trophic levels. The marine planktonic ecosystem is a huge and complex system to investigate and a system for which all mechanisms and dynamics are still not fully understood (Verity and Smetacek, 1996). A marine planktonic food web can be divided into organism size groups ranging from viruses to fish larvae. Most size groups consist of functional groups of autotrophic phytoplankton and heterotrophic zooplankton. At the base of the planktonic food web is the microbial loop, which consists of bacteria, heterotrophic nanoflagellates and ciliates, and which plays a major role in recycling of energy and substrate (Nielsen 2005).

Experimental approach

A natural way of integrating ecology into ecotoxicology is to use mesocosm designs in the experimental work (Cairns, 1988; Caquet et al., 2000). In a mesocosm experiment it is possible to estimate effects on parts of the community, and broadening the perspective and assessing the effects on the entire ecosystem simultaneously. As such, mesocosms have been used for many years both in ecological and ecotoxicological studies. The majority of my thesis builds on data obtained in mesocosm experiments and the advantages and disadvantages of this approach will be discussed later in this introduction.

As it is not possible to cover all known components of the plankton realm, throughout my studies I have focused on three important trophic levels, namely bacteria, phytoplankton and zooplankton communities. These communities have vital functions in marine food webs. Phytoplankton as primary producers, converts light energy into organic matter, and bacteria as part of the microbial loop which degrade organic matter and release nutrients from organic matter lost from growth and grazing. Finally zooplankton (>45 µm) are the heterotrophic community that represents grazing and the link upwards in the trophic hierarchy. This is a simple and limited view of the plankton ecosystem, but through integrated studies of structure and function of each trophic level, it has been possible to gain new knowledge on pollutant stress responses within these limits. To characterise the effects, I have chosen a variety of variables, which are summarised in table 1.

Table 1. Structural and functional variables of plankton communities analysed in the thesis work and the methods used.

Community	Measurements		Endpoints
	Structure	Function	
Bacteria	diversity		Molecular fingerprinting
	abundance		cell counts (flow cytometry)
		growth	^{14}C-Leucine incorporation
Phytoplankton	diversity		pigment composition
	abundance (biomass)		Chlorophyll a concentration
		primary production	^{14}C-HCO_3^- incorporation
Zooplankton	diversity abundance		taxonomic determination through microscopy
		prey uptake	uptake of ^{14}C labelled algal prey
Abiotic variables	temperature, salinity, nutrient and pollutant concentrations		

Pollutants in focus

In principle, any pollutant that is present in the marine environment could have been my choice in these studies. The pattern of responses in the pelagic food web would differ depending on the mode of action and the chemical and physical properties of the pollutant. The knowledge about the nature and quality of information, which can be deduced from community studies and method development, would to some extent be general, regardless of the particular chemical stressor. During my thesis, the focus has been on two different compounds (Figure 2). In the first part of my work (manuscript I & II), the stressor of choice was a recently introduced antifouling compound zinc pyrithione (ZPT). The actual reason for that choice was an acute need for a risk assessment of this compound, which is new to the marine environment. Our pelagic effect studies, together with parallel effect studies in sediments and fate analyses contributed to this risk assessment, which was published earlier this year (Dahllöf et al., 2005).

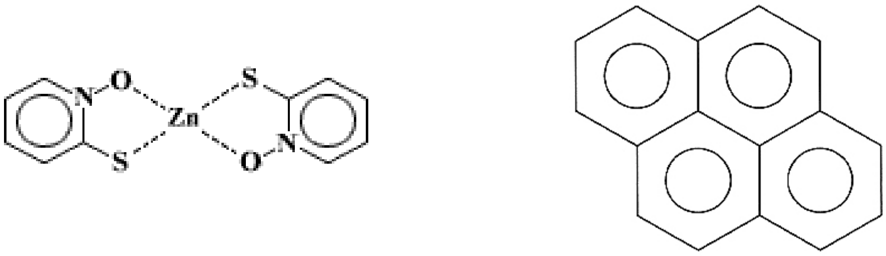

Figure 2. The two pollutants in focus in this thesis. To the left zinc pyrithione and to the right pyrene.

ZPT

The ban of tri-*n*-butyl tin (TBT) as an active compound in ship paints has led to the development of second and third generations of products supposedly less harmful to the marine environment, one of which is zinc pyrithione. ZPT is an organo-metal complex, in which the zinc ion is complex-bound to two pyrithione groups. Studies on the fate of ZPT in seawater have shown that approximately half of the nominal concentration added is transformed to copper pyrithione, while the other half is transformed to other unidentified complexes depending on the availability of other ligands in the water (Grunnet and Dahllöf, 2005). Only one attempt of measuring ZPT in the marine environment has been reported using a method with a detection limit of 20 ng/l, where no ZPT was detected (Thomas et al 1999). However the usefulness of this method has been questioned by the author himself (Thomas, personal communication). Pyrithione, the ligand in ZPT, has also been measured in one study (Mackie et al. 2004), where concentrations up to 105 ± 5 nM were detected in the Mercy River, UK, which corresponds to ~50 nM ZPT if all the pyrithione originated from ZPT. As a part of the previously mentioned risk assessment, a new analytical method was developed to estimate ZPT and the transchelation product CPT (Grunnet and Dahllöf, 2005b). Another new antifouling compound, DCOI, was chosen for the development of a method to asses effects on zooplankton grazing activity (manuscript I).

Pyrene

The remaining part of my thesis work (manuscript III & IV) has focused on the effects of pyrene, a polycyclic aromatic hydrocarbon (PAH). PAHs are some of the most toxic compounds found in the marine environment with carcinogenic and mutagenic capabilities (Yu 2002, Shaw et al. 1994). They originate mainly from oil spills (petrogenic) and combustion of fossil fuels and wood (pyrogenic). Oil products and waste from combustion contain a mixture of PAHs, determined by the nature of the source. Measurements of environmental concentrations are often reported as the sum of several PAHs. The choice of pyrene as a suitable model compound in effect studies of PAHs is rooted in several facts. It is a dominant PAH produced from incomplete combustion of oil and oil products (Verschueren 1983; Prahl et al. 1984), and the presence of pyrene in marine sediments is often used as an indicator of previous oil spills and PAH contamination (Rudnick and Chen 1998; Khan and Islam 2005). Pyrene may persist in the water column for long periods, as shown by Yamada et al. (2003), although PAHs are believed to be rapidly transferred into the sediments due to a high hydrophobicity and insolubility (Den Besten et al. 2003). Pyrene has recently been used as a model compound in modelling environmental fate of PAHs, where simulations found steady concentrations of up to 5 nmol L^{-1} pyrene in the water column within a five month time frame, under a given set of realistic conditions (Khan and Islam 2005). Additionally, the Oslo and Paris Commission (OSPAR) have suggested a predicted no effect concentration (PNEC) of 3.4 nM for 4-ringed PAHs (OSPAR 2004).

My studies on pyrene effects were more of a prospective kind than they were part of a risk assessment. The goal was to assess the type of effects pyrene would have on plankton communities as a proxy for PAHs in general,. The range of exposure concentrations was chosen on the basis of pilot studies on natural phytoplankton and bacterial communities and by following the EAC range advised by OSPAR (2004).

3 Results

As described earlier, the effect of a pollutant will depend on a large number of factors, including intrinsic features of the organism's life-history such as reproductive rate, generation time and mobility. The type and magnitude of a response is also dependent on abiotic conditions, like nutrient status, pH, light, salinity and temperature, which together with the nature of the exposure (magnitude, duration, frequency) can influence a response. Interactions between populations and with the surrounding environment during and after exposure to pollutants is what leads to indirect effects, where species may be affected indirectly through interactions with other species that are directly affected (Wootton 1994). The importance of performing more ecological toxicity studies to complement standardised toxicity tests is exemplified below with the different studies I have made during my PhD.

Single species vs. communities

An example of different responses in the environment and in the laboratory is a study I have made on the effects of the same pollutant (ZPT) on natural versus laboratory populations of copepods. It showed a difference in response between lab strains and natural organisms. In this case there was a considerable difference between the response observed in a natural community consisting of several species and a population of laboratory-reared *Acartia tonsa* to exposure from the same pollutant (Figure 3), where the latter was more tolerant. This is of course, only a single comparison, from which a general conclusion is difficult to make, but it illustrates the point that there can be major differences between the response of a natural community and a laboratory population to the same pollutant.

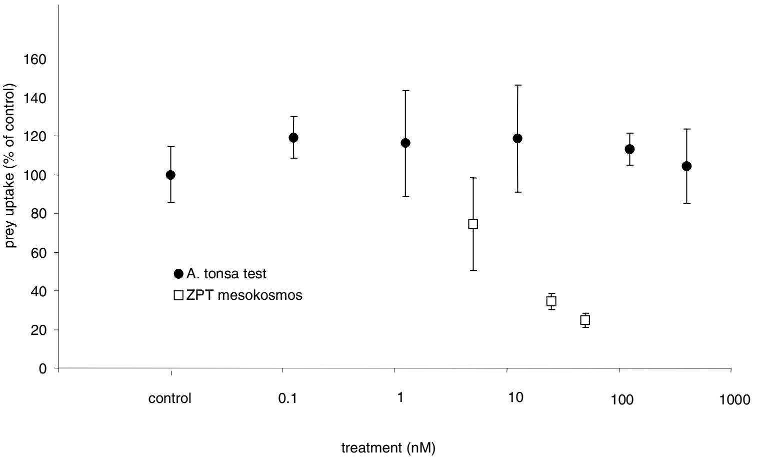

Figure 3. Comparison of effects from ZPT on zooplankton community grazing determined by radiolabelled prey uptake. Solid points are derived from a dose-response experiment on laboratory cultures of the copepod *Acartia tonsa*. Open squares represent data from a mesocosm experiment with natural communities (manuscript I). Error bars represent standard deviation.

The species sensitivity distribution approach (SSD) can be used with data on function and structure of communities (Fisher & Burton, 2003). Data obtained in the mesocosm study on effects of ZPT (manuscript II), have recently been applied in such a context, in which the SSD is part of a risk assessment of the compound (Dahllöf et al., 2005). Eight NOEC and LOEC values from several trophic levels obtained in community studies on ZPT were used to determine a species sensitivity distribution. The conclusion was a recommended 95% protection level of ZPT in the pelagic environment of 0.2 nM (Dahllöf et al., 2005). In a comparison of SSDs calculated from single species data and community studies of effects of ZPT, the 95% protection level was 4 times lower using the community data, than the level found when using the single species level (Dahllöf et al., 2003).

3.1 Interactions with the abiotic environment

The effects of pollutants on natural communities may also depend on abiotic conditions of the environment. Factors such as pH, nutrient status, amounts of biomass in the water column and levels of light irradiance, in particular UV-light, can interact with the pollutant or indirectly have an influence on the effects the pollutant may cause.

Nutrients

In relation to my work on effects of ZPT, there is a link between nutrients and the effect of the antifouling biocide. Studies have suggested a correlation between phosphate concentration in the water and sensitivity of phytoplankton towards ZPT. At low concentrations of available phosphate per cell, toxicity of ZPT increases (Maraldo and Dahllöf, 2004), and overall there seems to be indications of a reduction in phytoplankton sensitivity to ZPT toxicity with increasing phosphate concentration. The mechanism behind this relation is not fully understood, but several possibilities have been presented. If phosphate reaches a threshold level, zinc released from ZPT would complex forming zinc-phosphate complexes which are not toxic for algal cells (Maraldo and Dahllöf, 2004). The binding of zinc to polyphosphate bodies inside the cell may lower internal concentrations of the two and thereby cause phosphate deficiency. On the other hand the binding of zinc may also protect against elevated concentrations of zinc (Paulsson et al., 2002).

To investigate this linkage specifically in relation to a risk assessment of ZPT, I did a small study to investigate whether or not dissolved phosphate levels protects algal primary production against effects from ZPT. As an endpoint primary production of natural plankton communities was used under varying phosphate concentrations and algal densities. Water samples were collected from the inner parts of Roskilde fjord. Mesozooplankton were removed and the experiment was conducted under laboratory conditions.

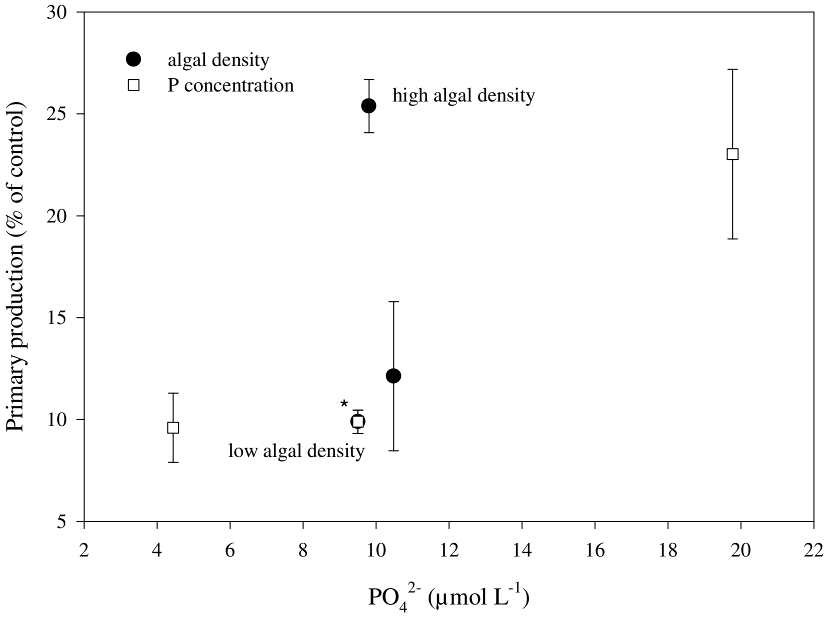

Figure 4. Relation between effect of ZPT on primary production per µg Chl a (measured as % of control) and concentrations of phosphate and chlorophyll a. The data with *in situ* concentrations of phosphate and chlorophyll a is marked with an asterisk.

There was a correlation between increasing concentrations of phosphate and decreasing sensitivity, when comparing primary production at increasing phosphate concentrations (figure 4). At the *in situ* concentration of phosphate and chlorophyll a the primary production is only 9.89% of the control. Reducing the amount of phosphate by half gave no effect and suggests that the remaining active phytoplankton were not influenced by the interactions of phosphate and ZPT. Conclusions drawn from this simple study are that the effect of short-term exposure to ZPT on primary production decreased with increasing phosphate, and supports the idea that enough phosphate both can support growth and complex-bind Zinc in and/or outside the cell. This can have implications for the effects of ZPT in relation to eutrophication, if effects can be expected to be more severe in less eutrophicated areas. On a general level, the example shows that abiotic interactions such as nutrient concentrations might influence the response of a community to a pollutant. Such an influence can be hard to predict and account for in simple dose-response studies if the relationship is unknown, but might show itself in more complex studies.

Density

The study of the interactions between the pollutant ZPT and phosphate illustrates, not only that dissolved nutrient concentrations can affect the impact of a pollutant, but that nutrient availability, which leads to a higher community density, also has a role to play. In this case high cell densities of phytoplankton caused a dilution effect of ZPT, where the overall effect was weakened through a lesser amount of ZPT per cell, but the dilution effect could also have been due to other things related to higher cell densities for example changing resistance to stress of individual cells or a higher excretion of organic matter that interfered with the ZPT complex.

In my study of the other pollutant pyrene, it became evident that nutrient availability and community density (biomass) have other effects on the magnitude of the ecotoxicological effect. The first pyrene mesocosm experiment was designed and carried out, as a prospective investigation of the effects pyrene would have on plankton communities (manuscript III). The plankton communities were nutrient and substrate limited at the time of the experiment, causing very low activity levels in both control and pyrene exposed communities. Less obvious effects of pyrene were found on total community function compared to the ZPT experiment, whereas effects on specific activities, abundance and algal community composition were more evident. If nutrient limitation indeed was the reason for the pattern of responses, it leads to the hypothesis that traits such as biomass and nutritional availability would have an influence on the effects of PAHs on pelagic communities. The aim of the second mesocosm study on pyrene was therefore to compare effects at one concentration of pyrene on two pelagic communities that differed in initial biomass due to previous nutrient availability. It turned out that effects of pyrene were more apparent in the nutrient enriched communities, thereby confirming the hypothesis from the first study. Furthermore, under the conditions of biomass and nutrient status investigated, there was no dilution effect due to the doubling in algal biomass (manuscript IV), as was the case for ZPT. The pyrene amount should probably be lower to observe a dilution effect, whereas in this experiment, pyrene was in excess in both biomass conditions.

Spatial and temporal variation

Sensitivity of communities in the marine pelagic environment may vary on a seasonal time scale as shown by Maraldo and Dahllöf (2004). They showed a highly seasonal variation in sensitivity of phytoplankton function towards the antifouling biocides ZPT and CPT. Likewise it seems logical also to assume a variation in sensitivity on larger spatial scales such as temperate areas versus polar areas. Such variability is illustrated in a comparative study I made on phytoplankton community function in relation to pyrene exposure in Denmark and Greenland coastal waters (figure 5). A short-term dose response experiment using a range of pyrene concentrations was conducted on phytoplankton communities from Roskilde fjord, Denmark in June 2004. The same experiment was performed one month later on phytoplankton communities sampled in Greenland waters on the west coast of Greenland. Two stations in Greenland were sampled, one in the open sea approximately 2 nautical miles west of the city of Sisimiut, and one in an open bay next to Sisimiut close to a harbour, an airport and an oil depot. The sensitivity of the Danish site was significantly lower than both the Greenland communities ($p<0.05$, Tukey's test). The difference is even clearer when calculated EC_{25} values are compared. The data on the Danish community yielded a value of 205 nM pyrene, where the primary production was reduced by 25% compared to the control community, whereas the open sea community and the bay community from Greenland had

EC_{25} values of 1.7 and 1.8 nM pyrene, respectively. The response at the highest concentration of pyrene (250 nM) may be influenced by the low solubility of pyrene. Roskilde fjord has both lower salinity and higher temperature than the study areas in Greenland, which increases the solubility of pyrene in Roskilde Fjord compared to Greenland. The response at the highest concentrations in Greenland may therefore be an artefact, as the communities were not exposed to 250 nM pyrene. The exact factor that determines the difference in sensitivity between the Danish and Greenland sites could be anything from the structure of the phytoplankton community, the conditions of the abiotic environment to the nature of the interactions with the rest of the plankton ecosystem. The study was not comprehensive enough to identify the factors causing the differences, but serves to stress the point of conducting risk assessment studies for the arctic environment in actual arctic surroundings. There was no apparent difference in sensitivity between the two Greenlandic sites as could have been expected from their likely difference in exposure levels of PAH. There are tidal movements in the area with a diurnal cycle and water level change of 3-4 m, which might contribute to the similarity of the site responses. Lately, the demand for data on effect studies on pollutants in the arctic has been highlighted (MacDonald et al., 2005; Chapman & Riddle, 2005; Riddle & Chapman, 2004), recognising that such data are needed to perform accurate risk assessments in polar regions. If existing data and knowledge of thresholds and effect concentrations from temperate areas are used in polar risk assessment, there is a danger of using assessment data, which are unsuitable to protect polar ecosystems.

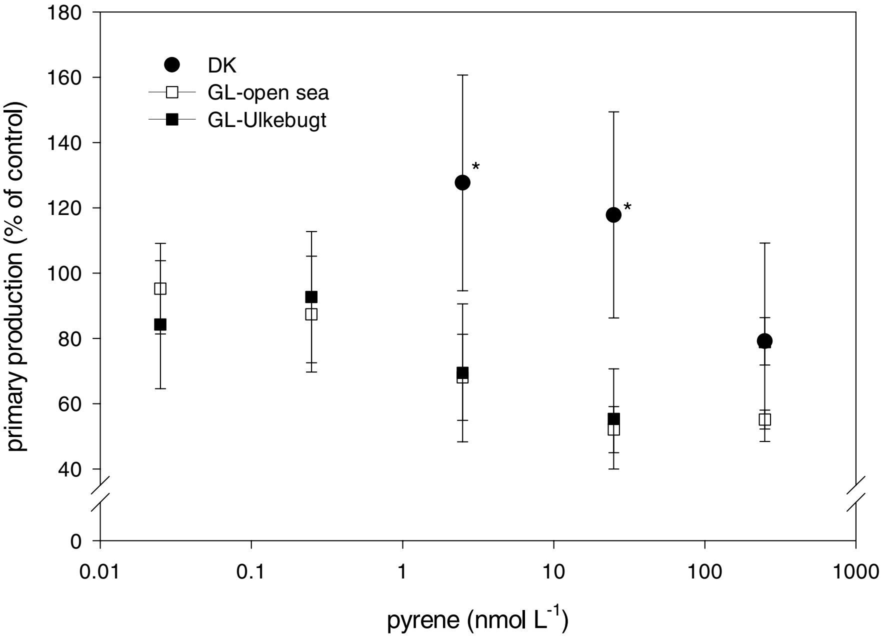

Figure 5. Response in primary production of phytoplankton communities from one Danish and two Greenlandic locations. Data are shown as primary production level in % of control with error bars indicating SD (n=5). Asterisks indicate a significant difference ($p<0.05$, Tukey's test) from the other sites.

UV light and pyrene

The last example of how abiotic conditions may influence the response of communities to pollutants is again taken from some of my studies in Greenland. The relative levels of harmful ultraviolet (UV) light are higher in Greenland and other polar areas. As a result polar ecosystems are more susceptible to damaging effects of UV light. Light, and UV light in particular, is absorbed by many PAHs that turn into reactive excited state species (radicals). These photoactivated radicals then have the potential to damage cells of organisms leading to inhibitory effects or increased mortality. The acute toxicity of these compounds increases up to 50 000 times when exposed to UV-light, which enhances the production of radicals (Boese et al. 1998; Lyons et al. 2002). Although this has been demonstrated in laboratory experiments its ecological importance *in situ* remains to be assessed (McDonald & Chapman 2002). In an experiment performed on the West Coast of Greenland I studied if there were any combination effects of UV light and pyrene exposure on the function of natural phytoplankton communities. Samples of natural phytoplankton communities were taken off the coast of Sisimiut, Greenland and large zooplankton (>45 µm) were removed. The phytoplankton samples were exposed outside to a range of pyrene concentrations for one hour, one set was shaded from UV light and another set was not. Replicate subsamples from both the UV-exposed and shaded communities, were then taken and incubated with ^{14}C labelled bicarbonate for four hours to estimate effects on primary production, while the remaining water were kept in the two light treatments for the same amount of time to measure effects on biomass. After the incubation period, the radiolabelled samples were fixed for later analysis and the biomass measured.

Some preliminary results are shown in figure 6, where biomass of phytoplankton decreased with increasing pyrene concentration. The study did reveal a much higher sensitivity of phytoplankton communities to pyrene when they were simultaneously exposed to UV light than when they were shaded from UV light. The community exposed to UV light had an EC_{50} value of 16 nM pyrene as opposed to an EC_{50} value of 117 nM pyrene for the community shielded from the UV light. It was however first at concentrations of pyrene between 2.5 and 25 nM that the effect was more severe in the UV light-exposed community and the simultaneous stress from UV light and pyrene exceeded the tolerance threshold of the algal community.

Pyrene seemed to have a stronger effect at the really low concentrations without UV light exposure than with UV light exposure. That might have to do with the location of radical formation. Radical formation in the UV light-exposed communities also takes place outside the cells where the radicals can react with all things present in the water such as dissolved organic matter, cell membranes and water itself. This process will lead to a lower concentration of the actual pyrene compound, and the biological interaction of radicals will be limited to cell surfaces. The dose of pyrene is thereby lower for the UV light-exposed communities than for the UV shaded community at the same nominal concentration. At higher concentrations the risk of exposure from pyrene itself increases in the UV light-exposed communities, and this is where the UV light effect becomes apparent. The UV light effect in this case occurs inside the algal cells where UV-induced radicals react directly with vital biological components resulting in a higher toxicity than observed for the non-UV induced effects in the shaded community (Arfsten et al., 1996; Diamond 2003). It should be kept in mind that this concentration range is around the lower OSPAR EAC value for pyrene. Besides the interesting fact that this study support the idea of an enhanced effect from PAHs and UV light together (Arfsten et al., 1996), although not as strongly as mentioned above, it also makes another important point. The experiment shows that any realistic and ecologically correct risk assessment studies concerning pyrene and other PAHs in high UV light-exposed areas have to include the possible co-effect from UV light, or there may be a risk of underestimating the possible effects from PAHs.

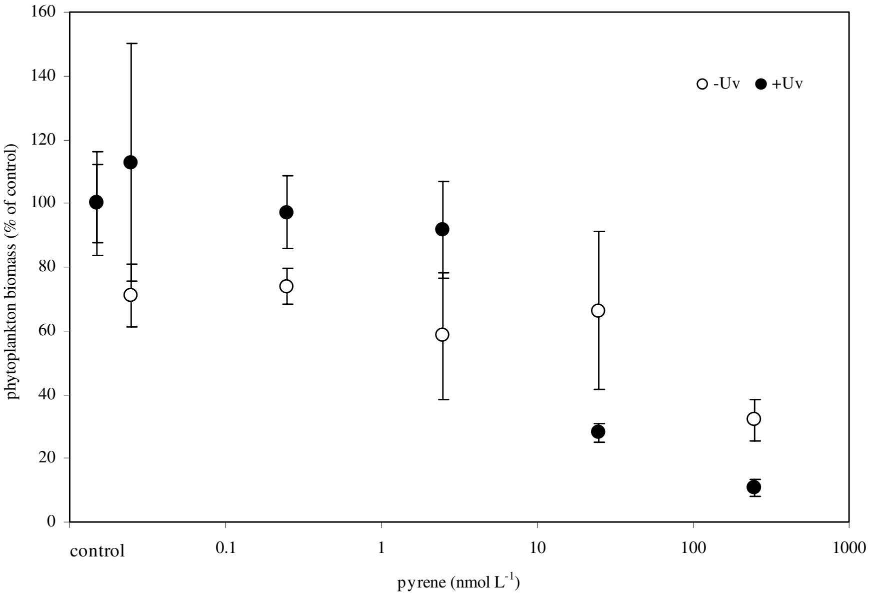

Figure 6. The effect of UV-light and short term exposure to pyrene on biomass of natural phytoplankton communities from Greenland. Data points are mean ± SD (n = 4).

3.2 Indirect effects

A great advantage of community studies is the possibility to investigate and assess indirect effects of stress from a pollutant. Such indirect effects have been shown in my work (II-IV), where exposure to zinc pyrithione (II) and pyrene (III & IV) had severe direct effects on phytoplankton, which in turn affected zooplankton grazing activity. Other indirect effects act inversely on the ecosystem and affect predators, in which case predation on lower trophic levels decreases such as grazing on phytoplankton. Abundance, growth rate and composition of phytoplankton are consequently affected. Generally in my studies, the indirect effects have the largest potential for changing function and structure of communities in a more permanent degree than the immediate direct effects (manuscripts II-IV). The persistence of indirect effects is also important in relation to the ability of communities to retain information from previous pollution impact events, known as the community conditioning hypothesis (Matthews et al., 1996), which states that even though it is not observable, almost all impacts or changes leave lasting effects on structure and function of communities.

4 Issues in mesocosms studies

Doubts have been raised concerning the reliability, relevance and the ability to predict effects in natural ecosystems from data obtained in closed community systems such as mesocosms (Calow 1996; Crane, 1997). Reliability and relevance must be acknowledged as important no matter the specific goal of the study and the lack of any of those can be an issue of concern when using mesocosm studies in risk assessment (Caquet et al., 2000). The requirement of a good predictability depends more on the design and goal of the experiment.

Reliability

A good relationship between cause and effects is vital for the reliability of results together with the confidence that data describes the desired function or structure. Problems may rise if replicate treatments are compared, which not only differ in pollutant exposure level, but also in their biotic and abiotic composition. The natural variability of a site might overshadow any difference between sites due to general ecotoxicological effects. In the case of the present mesocosm studies the preparation of mesocosms was carefully planned to ensure as little difference between treated and untreated mesocosms as possible. In all three mesocosms studies (manuscripts II, III & IV), the causality between exposure and effect was clear, despite the use of nominal concentrations.

Repeatability

Repeatability to the same degree as in standardised tests can only be ensured if exactly the same initial conditions are present each time in each experiment. This implies that that the entire ecosystem has to be reproduced, which is an impossible task. On the other hand, it can be argued that natural ecosystems are intrinsically dynamic systems and fluctuations in responses to pollutants must therefore be expected. However, by choosing reliable endpoints which at the same time simplify the investigated system and are representative of both the function and structure of the system, repeatability of response patterns can be expected (Brock et al., 2004). A good repeatability was achieved in the two pyrene mesocosms for those reasons, in that I found a similar pattern in response, although magnitude and severity of the indirect effects depended on the organisms present at the time. In the ZPT mesocosm the response pattern was somewhat different as there were also direct effects on zooplankton, but such a difference can be expected since it is a different type of compound.

Predictability

Predictability of the results in a mesocosm experiment relies much on the design of the experiment. From the first mesocosm with pyrene (manuscript III), we predicted a relation between effect and nutrient availability and performed a second mesocosm experiment similar in design to test the prediction. In that sense, the mesocosm approach was successful in predicting the outcome. Another vital aim of predictability in risk assessment concerns effect predictions in the environment at a given exposure level of a pollutant. This may prove difficult, especially in marine pelagic ecosystems, where underlying reasons for patterns and features are not fully understood (Verity et al., 2002), and hence not all possible indirect effects are known. The difficulty of prediction lies within these indirect effects, and because the fate of a compound will differ thereby giving different doses at the same added concentration. Perhaps it is the uncertainty in the predictability that is the strength of mesocosm studies, as it provides information on the magnitude of safety factors necessary for an adequate degree of protection in risk assessment

Performance

One approach that can aid in differentiating between direct and indirect effects in mesocosm studies is by studying the performance level of the communities (Chapman 2002; Preston 2002), and if performance is changing in response to pollutant exposure. Fitness of a population can be measured through endpoints such as population growth rate, survival of young individuals and other similar parameters. Another way of evaluating fitness, which involves functions of the particular population, could be zooplankton grazing potential (manuscript I). On a community level this could be used as a performance measure. The rationale is that the zooplankton community performance level corresponds to a given potential amount or rate of prey uptake. The method described in manuscript I, measures a potential prey uptake and has been used on natural communities after exposure to pollutants (manuscripts II & III) to assess the effects on zooplankton function and performance. The approach works well in this short term context, where it is easy to remove intra- and interspecific competition factors that might disturb the outcome, by removal of other grazers and a supply of excess prey. If the performance level should be estimated in a similar manner on bacteria and phytoplankton communities, it should also be through an endpoint that measures specific activity with excess resources and where competition is removed. These tests should be short-term to ensure that no adaptation of the community to the new beneficial circumstances occurs, but that the performance of the exposed community is accurately reflected. This is the case with the grazing method, which only takes one hour to perform in contrast to other functional endpoints as for example egg-production or hatching. The way of combining measurements of both function and structure, as applied in the mesocosm experiments, can also be seen as a measurement of the community performance level. Specific activity describes the fitness of the individuals and this can be compared to the performance level of the whole community (total activity). Furthermore, I was able to integrate all the functional responses in one variable (percent similarity of community functions to control) through multivariate analysis (manuscripts II & III), that can be interpreted as a parameter for the functional performance level of the whole community.

5 General conclusions

I believe that the overall purpose of the work presented in this thesis has been achieved successfully on several levels. A cornerstone concept in ecology is the interaction between species, populations and/or communities and their environment. Through the integration of structural and functional variables on several trophic levels, I have been able to analyse these interactions and the impact pollutants can have on communities through these interactions as indirect effects. Thereby I have applied ecology within ecotoxicology to gain a better understanding of the response patterns, which was one of my purposes. In the mesocosm studies it has been an emerging pattern that indirect effects are traceable in a community after a relatively long time after any direct effects have vanished.

Studies of such an integrated view of function and structure on several trophic levels are seldom reported. Most studies at the population and community level using mesocosms have mainly considered effects on community structure, such as abundance and diversity of selected organism groups, and have not as frequently taken effects on the functions of a community into consideration (Møhlenberg et al., 2001). Moreover, measurements of structure and function should be on the same level of specificity in order to elucidate combined effects as demonstrated.

I have shown through examples of my work that in studies of pollutant effects on natural communities, abiotic conditions such as nutrient concentrations, UV light and geographical differences can have an influence and therefore should be considered. I have shown that caution must be taken if effects of pollutants on communities are evaluated without including the conditions of their interactions and their environment. To include the natural variability of ecosystems in risk assessment through such studies is also helping to better understand effect responses in ecosystems.

An ideal risk assessment should consider the ecology of the species or community of interest, clarify the interactions and determine the impact of exposure on the studied interactions. That is in essence what has been attempted in this thesis, where community responses are analysed by comparing exposed community responses with control communities. Furthermore, risk assessment should be done not only on single species, but also on communities or populations in ecosystems, which could participate in probabilistic methods such as sensitivity distributions. Some of my results have successfully been used that way in a risk assessment of the antifouling compound ZPT.

Some of the main arguments against community studies in effect studies and risk assessment have been their tendency to grow in complexity and workload, economic expense and the lack of quick and easy screening methods to assess effects at community levels. I have developed, tested and applied a novel approach to estimate effects on the function of natural zooplankton communities (manuscript I), which I believe is a valuable contribution as a tool in community studies. In general, this work also shows that it is possible to perform effect studies in complex communities on a realistic financial basis and time period and still be able to subtract useful information from the natural variance.

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The thesis analyses effects of pollutants on natural plankton communities on the basis of three independent mesocosm experiments and a series of laboratory experiments performed in Denmark and Greenland. The work focus on integrating functional and structural measures of community responses to reveal indirect effects and co-effects with the abiotic environment on three trophic levels, namely bacteria, phytoplankton and zooplankton. The role of mesocosms and community studies in risk assessment and their usefulness in integrating ecological knowledge into ecotoxicology is discussed with examples of work done on natural communities of phytoplankton and zooplankton. Abiotic conditions such as UV light and nutrient concentrations are shown to influence pollutant effects.

Responses of marine plankton to pollutant stress

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