Effects of intermittent exposure of marine pollutants on sugar kelp and periphyton

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Opponent: PhD. Sten-Åke Wängberg

ABSTRACT

Pollution in the natural aquatic environment is erratic and manifests as temporary increments of chemical concentrations, pulses. Traditional ecotoxicological testing does not consider intermittent exposures and does not consider the post-exposure period even though latent effects may occur. Sugar kelp (*Saccharina latissima*, L.) was exposed to pulses of seven antifouling compounds with different mechanisms of action and marine periphyton were exposed to pulses of a photosystem-II inhibitor and a sterol synthesis inhibitor. Effects of pulsed exposure that did not appear until after the end of exposure were observed in both systems. The pollution history of periphyton communities was found to alter the effect of the exposure of both irgarol and clotrimazole but with dissimilar consequences. Even brief pulses of chemical pollution needs to be considered as latent effects may appear. Sequences of pulsed exposures are hypothesised to differ in toxicity depending on whether the ecological mechanisms of action of the toxicants are similar or dissimilar.

Keywords: Pulsed exposure, *Saccharina latissima*, periphyton, sequential exposure

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LIST OF PAPERS

This licentiate thesis is based on two papers which are referred to in the text by their Roman numerals. The introductory part of the thesis summarises, merges and further extends the presented topics.

I. Effects of seven antifouling compounds on photosynthesis and inorganic carbon utilisation in sugar kelp, *Saccharina latissima (Linnaeus)*
- Submitted to Archives of Environmental Toxicology and Chemistry

II. Pulsed exposure of irgarol or clotrimazole affects community structure differently depending on periphyton sampling site
- Manuscript

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SUMMARY

Pollution in the aquatic environment is erratic. Concentrations of chemicals are temporarily but greatly increased due to reoccurring or random events such as run-off caused by rain-fall, spray-drift, flooding, discharge of wastewater and shifts of tidal currents. These events may cause pollution that greatly exceeds the background environmental level of those chemicals. Assessing the environmental impact of such events is important but intermittent exposures are unfortunately not addressed in standard ecotoxicological testing and neither are latent effects that appear after the end of exposure.

Intermittent exposure was addressed and sugar kelp (*Saccharina latissima*, Linnaeus) was exposed to pulses of seven antifouling toxicants (chlorothalonil, DCOIT, dichlofluanid, diuron, irgarol, tolylfluanid, zinc pyrithione) and acute effects on photosynthesis were studied. All chemicals except zinc pyrithione were toxic to sugar kelp photosynthesis. Chlorothalonil and tolylfluanid caused photosynthesis inhibition that continued to increase even after exposure had ended. The carbon-concentrating mechanism of sugar kelp was also studied and found to be targeted by chlorothalonil, DCOIT, dichlofluanid and tolylfluanid.

Effects of pulsed exposures were further studied on sessile microalgal periphyton communities sampled on glass discs. The periphyton samples were exposed to a pulse of 1, 12, 24 or 96 hours of either irgarol (a PSII-inhibitor) or clotrimazole (a sterol-synthesis inhibitor) and the pigment composition of the community was evaluated using multivariate methods. The pigment fingerprint reflects taxonomic composition of the algal community as well as the physiology of the algae and these two aspects cannot be readily distinguished. Periphyton from a relatively unpolluted site suffered from inhibited growth and altered adaptations to the light regime in the bioassay during exposure to both toxicants. Effects of clotrimazole remained after exposure had ended. Periphyton from a site polluted by irgarol did not suffer from inhibited growth during irgarol exposure, but the light-adaptations were still disturbed. Periphyton from the irgarol polluted site were more affected by the clotrimazole exposure and effects lasted for a longer period after the end of exposure. Physiological effects on light-adaptations were in some cases evident despite that the periphyton were able to grow at the same rate as the controls. This is an example of an effect that would be hidden if only superior, integrating, endpoints are studied. In line with the community-conditioning hypothesis and the Pollution-Induced Community Tolerance (PICT) concept the periphyton from the irgarol-polluted site reacted differently compared to the periphyton from the less polluted site. It is hypothesised that in a sequence of pulsed exposures the initial exposure shapes the community in a way that the impact of the second exposure depends on the relation of ecological mechanism of action of the toxicants. When they are similar, the community will have become more tolerant to the second exposure, through elimination of sensitive species and individuals. When the toxicants are dissimilar, however, the first exposure causes a decrease of genetic diversity in the community and thus a generally increased sensitivity to subsequent exposure and the second exposure would thus cause a larger toxic effect.

Effects of exposure to irgarol and clotrimazole in periphyton were compared to the range of pigment changes in periphyton samples in field. Periphyton were colonised on glass discs at two sites with similar physical characteristics (shallow muddy bays) but with different irgarol pollution. Periphyton from each site were translocated to the other, respectively, and the pigment composition was monitored over time. The rate and range of succession occurring in field periphyton were compared to the succession patterns of periphyton in microcosms. The rate of change occurring in field periphyton was rather quick as pigment composition after translocation changed to become similar to the reference sample of the new site after 2 days. The range of pigment change in field and bioassays did not overlap which is reasonable since the microcosm setup uses 24-h light regime and growth media with enhanced nutrient level.

INTRODUCTION

In a historical perspective, the science of ecotoxicology is young. In the first half of the 1900s non-biological methods were considered superior to bioassays for estimating hazard to the environment. The single species fish bioassay was introduced in the 1940s, (Hart et al. 1945, as cited in Cairns & Pratt 1989) and from that point, the use of biological evidence in pollution assessment became more common. The science of aquatic ecotoxicology is based on the theories and methods of mammalian toxicology, which is an element of medicine (Slooff 1983, as cited in Cairns & Pratt 1989). An unfortunate consequence of the heritage of mammalian toxicology is that standard bioassays in ecotoxicology sometimes are too simplified for properly assessing effects on high levels of ecological organisation. As medicine, and thus mammalian toxicology, is primarily focused on human health, the practice of single species bioassays was generally accepted as appropriate for ecotoxicology (Cairns & Pratt 1989). This somewhat direct transfer of methods between two different disciplines is not without problems. Even extrapolating toxicological effects between mammals, e.g. from rats and monkeys to humans, is problematic since their physiology differs in certain aspects. Extrapolating toxicological effects from one or a few species to any other species is certainly not an easy task. If the extrapolation should also cover higher levels of ecological organisation, i.e. communities or ecosystems, it becomes problematic since the degree of variation increases drastically. The problems of extrapolating between taxa are mainly due to biological differences. When extrapolating from low to high levels of ecological organisation (i.e. from species to communities), the difficulties are further increased due to the larger amount of information contained in higher levels of ecological organisation (Liess 2002). Single species tests are certainly important and necessary for the understanding of toxicity in organisms and populations. As single species tests tend to be more easily repeatable, they are important tools for hypothesis testing and for clarifying toxicity mechanisms and effect dynamics. By carefully testing toxicological hypotheses, single species tests may be used to increase our detailed understanding of toxicology. However, to be able to properly predict ecological effects of toxicants we need to make toxicity tests on higher levels of ecological organisation, at least on the level of biological communities (Pratt et al. 1997).

INTERMITTENT EXPOSURE

Standard ecotoxicological tests are usually designed for evaluating the effect of a chemical that is present at a constant concentration over a fixed duration of time. This time-frame is often pragmatically determined (e.g. a 96 h test conveniently fits within one working week). Unfortunately, such a design does not reflect environmental pollution well, since pollution is erratic and concentrations fluctuate with time. Neither are latent effects, that appear after the end of exposure, covered in such tests (Zhao & Newman 2004). Input to aquatic environments often occur in pulses when peak concentrations of a pollutant temporarily, but greatly, exceeds the background level (Handy 1994, Liess et al. 1999). The environmental effect of fluctuating concentrations is hard to predict. As pulses with elevated concentrations are brief, to calculate permit-limits for effluents from averages of concentrations over time may not be protective enough (Diamond et al. 2006). In the environment, the concentration fluctuations are driven by variations in input to, distribution within and removal from the various aquatic compartments. Input may come from runoff from agriculture during rain events (Solomon et al. 1996, Liess et al. 1999), sewage treatment plants (e.g. Reemtsma et al. 2006, Martinovic et al. 2008), flooding of polluted areas, spray-drift, shifts of tidal and wind-driven currents, accidental pollution and erratic industrial discharge (McCahon & Pascoe 1990, Handy 1994). Within the aquatic environment chemicals are distributed between water, organic and particulate matter. Removal from the water phase is caused by absorption into biota, physico-chemical breakdown and binding to particles, which eventually sink to the bottom (Newman & Unger 2003).

Several authors in aquatic ecotoxicology have addressed intermittent exposures. Most studies are focussed on single species assays and population effects. Latent effects, that appear after the end of exposure, have been recognised by several authors as a problematic topic within the field of intermittent exposure ecotoxicology (e.g. (Abel 1980, Zhao & Newman 2004). A number of models have been designed that attempt to predict effects of individuals and populations as a consequence of pulsed exposures (Ashauer et al. 2006, Connell & Yu 2008). As these models build upon principles of uptake and redistribution within the organism and relate internal concentrations of chemicals to effect, they fail to recognize latent effects, that may be related to delayed biological events, and indirect effects in ecosystems. There are a few investigations aiming at higher levels of ecological organisation, and among them some general patterns of effects have been recognised. The effects of intermittent exposure to communities have been addressed by Goldsborough & Robinson (1986) who studied microalgal communities exposed to short herbicide pulses. They found that ecosystem functions recover after disturbance due to functional redundancy but community structure was profoundly affected for several weeks. Pusey et al. (1994) exposed artificial streams to the insecticide chlorpyrifos and noted both direct effects of reduced populations of sensitive species and indirect effects of increased populations of species who are early colonisers. The importance of indirect effects on community structure after pulsed exposures was stressed by Lopez-Mancisidor and co-workers (2008) who saw indirect effects several weeks after application of insecticides to mesocosms placed in streams.

Although the importance of studying intermittent exposure has been recognised, few authors attempt to build new hypotheses or describe general patterns. A more generally applicable approach was presented by Cedergreen and her co-workers who discussed whether the effect of pulsed exposures depends on the partitioning coefficient (between octanol and water, k_{OW}), or mode of action of the chemical (Cedergreen et al. 2005). In their study, limited to six compounds, such a distinction could not be clearly made and they concluded that both k_{OW} and mode of action had impact on the effect. However, they emphasised the importance of effects that are not readily reversible, as such effects may cause larger environmental impact compared to effects that are reversed once the compound is removed from the medium. Clearly, vital questions when studying pulsed exposures are whether the exposure causes effects that remain, or appear, after the exposure has ended, and how indirect effects affect the ecosystem after the pollution event.

COMMUNITY ECOTOXICOLOGY

The ecological consequences of environmental contamination may be estimated either with perspective from higher (e.g. ecosystems responses) or lower (e.g. enzymatic, cellular or physiological responses) ecological organisation. Traditionally effects studied on a lower level of organisation are used to predict effects on higher levels, while effects studied on higher levels of ecological organisation are used in epidemiology to retrospectively deduce what caused a certain effect. As mentioned earlier, extrapolating effects to higher level of organisation with precision is difficult, if at all doable, and ecosystem-epidemiology can only be used in retrospect, after certain damage is already done. There are several difficulties of predicting the effects on higher levels of organisation. Toxicants interact with cells, cellular compartments, membranes and enzymes in organs and individuals. This causes certain alterations in the physiology of the individual. As the physiology of the individual depends on the phenotype, and the distribution of phenotypes within a population may be determined by environmental factors (e.g. pollution), two populations of the same species may respond differently to the same exposure. Thus, even extrapolating among different populations of the same species is problematic. Projecting further onto the level of communities (and beyond) is even more difficult as all communities are different and contain varying amounts of information.

A further discussion of community ecotoxicology requires an outlook into community ecology and the different definitions of ecological communities and their inherent properties. The Clementsian paradigm (Clements 1916, as cited in Landis et al. 1996) portrays ecological succession as progressive, moving towards an ideal state. Communities were believed to be stable and to return, with time, to an equilibrium state once it had been disturbed. These ideas are the basis for concepts such as community stability, recovery and resilience. If this was truly the case, it would be possible to define reference scenarios and it would be possible to make accurate evaluations of toxicological impact based on the current status of an ecological system. However, natural communities are continuously changing through succession within the community, as a response to variations in the environment and seasonal changes and as a consequence of disturbance. As communities are in constant change, the evaluation of change due to pollution in ecotoxicological

testing must always be made as a comparison to a control community. The control community should not be a reference of the status of the community before pollutant exposure but rather a reference to the development that takes place in the community during the test period, without being exposed to the studied toxicant.

The complexity of community change connected to community history is addressed by the community conditioning hypothesis which states that ecological communities retain information about all events in their history (Landis et al. 1996, Matthews et al. 1996). Communities carry information that is not strictly biological. Information of a community's history may be stored in a wide array of information, from genetic diversity to physiology, to species composition, consumer webs and assembly order. Thus, to think of communities as a form of "super-organism" is wrong. Ecological systems (populations, communities etc.) do not contain a protected and inherited "genetic blueprint" and they incorporate the impacts of stressor events. Communities are complex in the sense that they are assembled in a certain order which cannot be reversed, they have a direction in time (Landis et al. 1996 and references within). Several authors recognize these properties in communities: Guasch et al. (1997) studied periphyton communities exposed to the photosynthesis-inhibitor atrazine and found that the toxicity of atrazine changed during succession of the community. In another study, Lopez-Mancisidor et al. (2008) evaluated ecological interactions in a community and detected indirect effects of exposure weeks after exposure had ended. Other authors within ecology have also addressed the importance of disturbance history (e.g. Death 1996).

How to describe and measure the health of an ecosystem is not trivial. The health of an individual may be defined as the lack of disease, or if the individual functions well, in every aspect of its life cycle. Due to functional redundancy, measuring functional endpoints is not adequate to assess ecosystem health as effects may be hidden. Sensitive species may have been removed, but gross functions of the community may still be unchanged. Structural endpoints, such as the taxonomic composition of a community, may be used to describe a community, but a reference situation for such a description would not be possible to define. Furthermore, as multispecies toxicity tests and field experiments are non-organismal (i.e. community structure is evaluated, not the health of certain individuals) and build upon unique systems they have the property of not being repeatable (Landis et al. 1996) in a strict sense. However, there are methods to test endpoints at high level of ecological organisation. Although each community is unique and contain information that is not strictly biological, common patterns may still be recognized and even tested. Pollution induced community tolerance (PICT) is a concept that explains how communities are shaped during pollution events and how this can be evaluated in retrospect (Blanck et al. 1988, Blanck 2002). Individuals (i.e. phenotypes) and species that are sensitive to a pollutant are removed by this selection pressure exerted by the polluting compound. The community goes through a toxicant induced succession (TIS) (Blanck 2002, Blanck et al. 2005). As all communities are different, the result of the TIS interpreted as changes of community composition may be hard to overview and predict. Especially since all community changes also bring indirect effects, i.e. in form of changed competition patterns. However, a common pattern of all communities is that the community

through its new selection of species and individuals becomes more tolerant to the pollutant (the PICT concept). PICT is a quantifiable and testable concept (e.g. Blanck & Wangberg 1988, Blanck & Dahl 1996, Dahl & Blanck 1996a, Blanck et al. 2008). As a consequence of the TIS the community may become tolerant to other chemicals than the pollutant in question, co-tolerance. Co-tolerance relates to the community's tolerance mechanisms which evolves during TIS. The community may become tolerant to a second chemical if the evolved tolerance mechanisms may be applied also on the second chemical.

A related, but different topic, is the concept of ecological mechanism of action. The distribution of species and individuals (phenotypes) targeted by a certain pollutant is referred to as its ecological mode of action. Compounds that share a similar ecological mode of action affect the same selection of species. As a contrast, compounds with a dissimilar ecological mode of action do not affect the same mechanisms and species. The concept of ecological mode of action is central in ecologically oriented ecotoxicology when discussing effects on community succession and structure.

END POINTS - PHOTOSYNTHESIS AND COMMUNITY STRUCTURE

This thesis deals with two aspects of aquatic ecotoxicology on different levels of biological organisation: physiological effects on sugar kelp (a marine brown macroalga, *Saccharina latissima)* and effects on community structure in periphyton. *Saccharina latissima* was selected as a model organism, as a representative of algae with carbon-concentrating mechanisms (CCM). CCMs are special adaptations for utilising the bicarbonate pool of dissolved carbon dioxide in marine water. There are several types of mechanisms for carbon uptake and concentration in marine algae. The CCM of *S. latissima* is located partially to the outside of the cells and is thus vulnerable to waterborne toxicants. Since carbon acquisition through the CCM is a limiting process for primary production, algae that rely on such CCMs may be sensitive to certain toxicants that target the CCM.

The periphyton community was chosen as a suitable entity for experimental work with communities. Barranguet and co-workers (2003) describe the periphyton biofilm to truly be a community since it functions as a more complex system than the sum of the species present, since the organisms interact and develop interdependently. The term *periphyton* appears in the literature with slightly varying definitions. This thesis refers to the original definition according to Sladeckova (1962), the "assemblage of biota that grow attached to objects installed in water artificially by man". Diatoms and cyanobacteria usually dominate mature marine periphyton communities. Periphyton also include other eukaryotic algae, heterotrophic bacteria, and several other types of phototrophic and organotrophic unicellular eukaryotes (i.e. protists). The organisms are embedded in a polysaccharide matrix of extracellular polymeric substances (EPS). The EPS acts as a cohesive agent for the biofilm and facilitates interaction within the biofilm (Flemming & Wingender 2001a, b). The EPS has also been shown to function as protection from grazing (Barranguet et al. 2005).

AIM OF THE THESIS

This thesis and the included papers investigate ecotoxicological effects of intermittent exposure. The overarching aim is to discuss ecotoxicology with a perspective of increased realism in the

exposure scenarios. The effects of pulsed exposures of different antifouling toxicants on the photosynthesis of *S. latissima* were studied and that work is presented in Paper I. Does the extent of the exposure period affect the toxicity and does the inhibition go away once the exposure is gone? Furthermore, whether the antifouling toxicants targeted the carbon-concentrating mechanisms was also evaluated.

Pulsed exposure was further studied on periphyton communities, both on periphyton from a relatively unpolluted site and periphyton from a site polluted to the antifoulant irgarol. Does the pollution history of the periphyton, as a consequence of the site where they were sampled, influence the effect of the pulses? These results are presented in Paper II. The effects of pulsed exposures to periphyton were compared with periphyton community changes in field periphyton samples. These experiments were conducted by translocation of periphyton from a relatively unpolluted site to a polluted site, and vice versa. The aim was to present a reference scenario of the magnitude of community change and also to evaluate how rapid periphyton adapt to new conditions. These results are presented below.

EXPERIMENTAL SETUP

All experiments were conducted at the Sven Lovén Centre for Marine Science, Kristineberg, at the west coast of Sweden (Fig. 1). Field experiments were performed in the waters around the mouth of the Gullmar fjord (Fig. 2). HPLC analyses of pigment composition of periphyton were conducted at Gothenburg University, Department of Marine Ecology in Gothenburg.

TESTED COMPOUNDS

The chemical compounds selected for this thesis comes from several different groups of chemicals. Several of them are inhibitors of photosynthesis. All of them are toxic to algae. They are briefly presented in table 1 and further details are found in the papers where they were used.

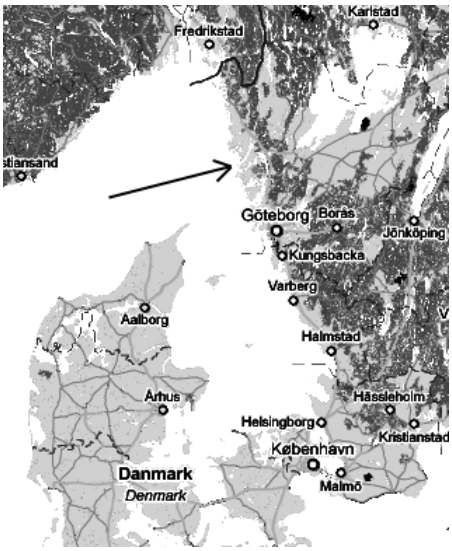
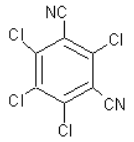
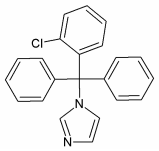
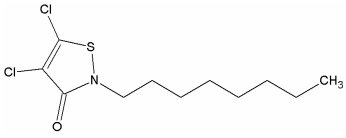
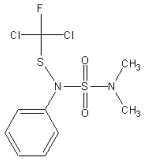
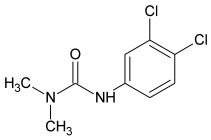
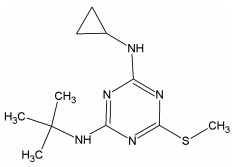
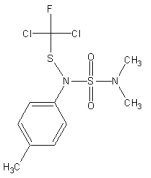
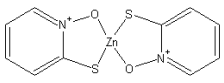


Fig. 1. Map of southern Sweden with the location of the Gullmar fjord indicated with an arrow.

Table 1. Chemical compounds in the thesis, their Chemical Abstracts Service registry number, chemical class, molecular structure and in which paper they are used.

Name	CAS RN	Class	Structure	Reference
Chlorothalonil	1897-45-6	Chloronitrile		I
Clotrimazole	23593-75-1	Azole		II
DCOIT	64359-81-5	Kathon		I
Dichlofluanid	1085-98-9	Phenylsulfamide		I
Diuron	330-54-1	Phenylurea		I, II
Irgarol	28159-98-0	*s*-triazine		I, II
Tolylfluanid	731-27-1	Phenylsulfamide		I
Zinc Pyrithione	12463-41-7	n/a		I

SUGAR KELP PHOTOSYNTHESIS

Sugar kelp, *Saccharina latissima* (Linnaeus) (Lane et al. 2006) (Phaeophyceae), previously known as *Laminaria saccharina* (Linnaeus) (Lamouroux 1813) is a brown macroalga that is common on the west coast of Sweden. Fronds, complete with stipes, were collected by hand and kept in aquaria with flowing natural water. The experimental units that were used were discs of frond tissue that were punched with a cork borer. The toxicity of each of the seven compounds was evaluated by measuring the amount of ^{14}C-labelled carbon dioxide that was incorporated within a certain time interval during exposure to a span of concentrations of each compound. Concentration-response curves were constructed and the median effective concentration (EC50) was calculated through log-linear interpolation. To investigate toxicity kinetics and dynamics with a mechanistically different endpoint, oxygen evolution was measured during exposure to the EC50 of each compound. If the range of tested concentrations provoked less than 50% inhibition, the highest tested concentration was used. Oxygen evolution was measured using platinum electrodes in a setup which allowed five replicates at a time. Light and dark periods were alternated to prevent oxygen related stress during the light phases. During the light phases the gross oxygen evolution was quantified. As I wanted to study whether the tested compounds affected the carbon concentrating mechanism (Paper I, Fig. 1), the ratio of available carbon dioxide in the test medium was increased, by lowering the pH through an injection of MES buffer into the medium. Thus, the CCM was bypassed and the alga had access to enough carbon dioxide to maintain photosynthesis (Axelsson et al. 2000, Mercado et al. 2006). If the rate of photosynthesis increased (as compared to the control) merely as a consequence of increased carbon dioxide availability, it was concluded that the CCM was inhibited.

PERIPHYTON COLONISATION, SAMPLING AND TRANSLOCATION

Natural periphyton communities were allowed to settle on circular glass plates (15 mm diameter) mounted in polyethylene holders and frames which held a total of 170 glass discs. The frames were hung from a buoy and submerged in open water at a depth of 1 – 1.5 m. The method is described in detail in (Blanck & Wangberg 1988, Dahl & Blanck 1996b). Periphyton were sampled in Kalvhagefjorden (Kvh) (Paper II), Kilen (Kil) and in Smalsund (Sms) (paper II). The sites are indicated on the map in Fig. 2. For the SWIFT bioassay (detailed description can be found in Porsbring et al. 2007) the periphyton biofilm had a suitable thickness after 8-9 days. During transport from a field site, for starting a bioassay or during translocation, the periphyton-sample holders were kept in natural water in an insulated box with a cover to protect the algae from excess sunlight.

The sites Kilen and Kalvhagefjorden are thoroughly characterised in (Blanck et al. 2008) (site 1 and 5, respectively). The site Kilen, as briefly discussed earlier, is situated in a marina in a shallow and muddy bay. The other site, Kalvhagefjorden, was chosen as a less contaminated contrast site to Kilen, since it is situated in the scarcely populated archipelago and is also a shallow and muddy bay. Analyses of irgarol contamination show that Kilen has been contaminated by irgarol for several years, while Kalvhagefjorden is less contaminated. This has led to induced tolerance in Kilen periphyton, while the sensitivity of Kalvhagefjorden periphyton to irgarol exposure is

unchanged, at least since 1994 (Blanck et al. 2008). The site Smalsund is situated not far from the Kilen site, but outside the shallow bay and is probably less contaminated by irgarol than Kilen. Due to the relative vicinity of a marina and boating traffic, Smalsund is still regarded as irgarol polluted. It is situated in close vicinity of site 3, as described in (Blanck et al. 2008), which has been moderately irgarol polluted for several years.

SWIFT

The periphyton bioassays in this thesis were made using a 96 h semi-static microcosm design referred to as SWIFT. For details of the SWIFT bioassay see (Porsbring et al. 2007). Here the method is described briefly: samples were sorted to select glass discs that were evenly covered by periphyton. Discs with a periphyton coverage that looked too thin or too thick, compared to the average coverage, were discarded. The glass discs were gently wiped on all sides except the top using a paper tissue, and then placed in rectangular 500 mL glass vessels. After distributing the discs in one vessel, 300 mL of test medium was gently poured in to the vessel and the vessel was placed on a rocking table in a light and temperature-controlled chamber. The temperature was adjusted to the temperature at the site at the time of sample collection. Light was continuously (24 h light period) provided by fluorescent tubes. Test media were changed daily. To be able to follow changes in pigment composition during the bioassay pigment samples were taken daily.

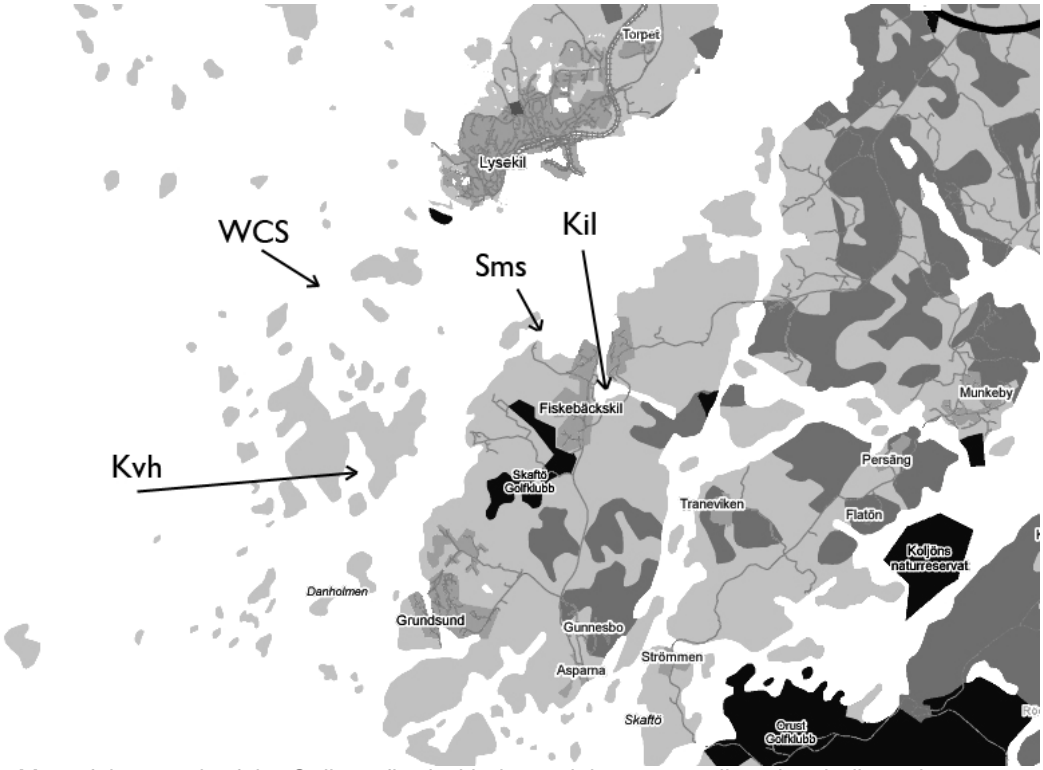


Fig. 2. Map of the mouth of the Gullmar fjord with the periphyton sampling sites indicated. (Kvh = Kalvhagefjorden, Sms = Smalsund, Kil = Kilen, WCS = water collection site)

Test media were prepared from natural seawater collected in an area in the archipelago northwest of the Sven Lovén research station, indicated in Fig. 2 as WCS (water collection site). The water was filtered through a glass fibre filter (Whatman GF/F) to remove particles and phytoplankton and stored cool at +8°C. Ambient concentrations of phosphate and nitrate were increased by 0.7 µmol/L of PO_4^{3-} and 8 µmol/L of NO_3^-. Stock solutions of the tested chemicals were prepared in methanol, and later added to the test media as a 1:12000 dilution which was allowed to dissolve for one day before use in the bioassay.

PIGMENT ANALYSIS – HPLC

To sample the pigment composition of the periphyton, periphyton samples were randomly selected and placed in cold methanol (HPLC grade) in darkness and stored in -20°C until analysis could be performed. The samples were analysed using HPLC equipped with a diode-array detector using the absorbance at 436 nm wavelength for quantification and comparing the spectrum diagram to a database with pigment standards for identification of the peaks.

MULTIVARIATE STATISTICS

The pigment composition datasets consist of a list of several parameters for each sample. Two different methods were used for condensing the multivariate datasets into graspable information: Bray-Curtis dissimilarity index (BCDI) and multidimensional scaling. BCDI was originally constructed for evaluating differences between forest communities in Wisconsin (Bray & Curtis 1957). Multivariate datasets are collapsed into a univariate number which allows for comparison of several samples and construction of concentration-response curves or time-response curves. Essentially, all parameters of the two communities are compared pair-wise and the dissimilarity between them is quantified as a value between 0 (totally similar) to 1 (totally dissimilar). For describing relations of exposure (time and/or concentration) and effect, individual treatment samples were compared to an average control community (ACC). The ACC is a construct of the arithmetic means of pigment peak areas or the median species abundances in the individual control samples. BCDI was calculated according to equation 1.

$$BCDI = \frac{\sum_{i=1}^{n} \left| X_{ACC,i} - X_{sample,i} \right|}{\sum_{i=1}^{n} \left(X_{ACC,i} + X_{sample,i} \right)}$$

(Equation 1. Bray-Curtis index of dissimilarity)

$X_{ACC,\ i}$ is the value of the *i*th variable in the ACC and $X_{sample,\ i}$ is the value of the corresponding variable in an individual sample. An implication of the use of BCDI is that the level of no-effect is always above zero, because a score of zero would imply that the control replicates are exactly identical. The ACC is a construct based on pooled information from the individual controls. Natural variations in structure cause any sample, control or treatment, to always be slightly dissimilar to the ACC.

To be able to compare several communities to each other the multivariate ordination multidimensional scaling (MDS) was used. MDS is an iterative technique to condense and map

multivariate data in few dimensions, usually two. It originates from the field of psychometrics (e.g. (Torgerson 1952, Shepard 1957, Kruskal 1964)) but is also utilised in ecology and ecotoxicology (e.g. (Field et al. 1982, Clarke 1999)). MDS can be thought of as backwards constructing a map using the distance table in the appendix of a roadmap. Biological data can be used to calculate a measure of dissimilarity, pair-wise, between all communities. This table of dissimilarity is analogous to the distance table in a road-map. When plotted, the MDS then shows the samples (or analogously, cities) plotted at relative distances that as good as possible fit the data in the distance table. Pigment composition data were compared pair-wise and a table of dissimilarities was calculated. The degree of which the available data could not be perfectly fitted to the selected number of dimensions (usually two) is termed the "stress" of the plot. The distances in the MDS plot is based on rank similarities between samples (i.e. "sample *A* is more similar to sample *B* than it is to sample *C*"). When interpreting an MDS plot, two items located close together in the ordination are presumed to be closer related than two items that are located far from each other. MDS has been used to link community structure to environmental and experimental gradients of anthropogenic perturbation (e.g. Dahl & Blanck 1996b, Clarke 1999, Porsbring et al. 2007).

MOST SIGNIFICANT FINDINGS

EFFECTS OF PULSED EXPOSURE TO SUGAR KELP PHOTOSYNTHESIS

Pieces of fronds of sugar kelp (*Saccharina latissima)* were exposed to seven different antifouling compounds from different classes of chemicals. The acute effect of continuous exposure for 3 h 15 min on photosynthetic carbon-incorporation was evaluated as concentration-response curves (Paper I, Fig. 2). In a separate set of experiments, algal discs were pulse-exposed to each of the seven compounds and the degree of inhibition over time and post-exposure effects were studied as oxygen evolution (Paper I, Fig. 3). Effects on the function of the CCM were also studied using oxygen evolution as endpoint. The seven compounds proved to be differently toxic to different aspects of photosynthesis of *S. latissima,* which is outlined in table 4 of Paper I. Four of the compounds, chlorothalonil, DCOIT, dichlofluanid and tolylfluanid, inhibited the CCM. The CCM is a vital component of algal photosynthesis, especially in densely populated areas with high influx of light where competition for dissolved CO_2 is high. Two of the tested compounds, chlorothalonil and tolylfluanid, proved to be of special interest as the inhibition of oxygen evolution continued to increase even after end of exposure. Remaining inhibition after end of exposure could be a sign of irreversible damage. Those results are similar to the investigation by Cedergreen et al. (2005) who also showed that certain toxicants may inhibit photosynthesis after end of exposure. Since certain toxicants may cause inhibition that lasts even after exposure has ended, it is problematic to rigidly stick to ecotoxicology tests with a fixed time period. Post-exposure effects need to be considered in chemical risk assessment.

COMMUNITY EFFECTS OF PULSED EXPOSURE

Periphyton communities were exposed to pulses of irgarol and clotrimazole. General patterns of the controls of all experiments showed adaptations to the specific conditions of the bioassay. This is a consequence of the elevated concentration of nutrients and the 24 h light-regime. Even though the start samples from the different experiments were not very similar due to natural variations, the SWIFT bioassay conditions forced the succession of the periphyton samples into a similar direction and they all end up in the same area, if pigment profiles are interpreted using MDS. The changes reflect both physiological adaptations (e.g. xanthophyll content shifts as response to high light) and taxonomic succession. To be able to compare the magnitude of response by periphyton communities to the different exposures the multivariate index Bray-Curtis index of dissimilarity (BCDI) (Bray & Curtis 1957) was used. BCDI is a means for condensing multivariate datasets into univariate numbers which allowed for comparing communities and, thus, grading the effect level caused by different exposures. Some key pigments were studied in detail, chlorophyll *a*, which reflects algal biomass, and the xanthophylls. The xanthophyll cycle is a means for photoacclimation in plants and algae (Jin et al. 2003, Latowski et al. 2004, Van Leeuwe et al. 2008). In diatoms the pigments that are mainly responsible for this mechanism are diadinoxanthin and diatoxanthin (Jin et al. 2003, Van Leeuwe et al. 2008). During high-light conditions diadinoxanthin is transformed into diatoxanthin through de-epoxidation (Hager 1980). During the extent of the SWIFT bioassay, control communities showed adaptation to the specific conditions within the microcosms by shifting to a high ratio of diatoxanthin over diadinoxanthin and an increase of the total pool of xanthophylls (Fig. 4). This is a sign of adaptation to high-light conditions (Demmig-Adams & Adams 1992).

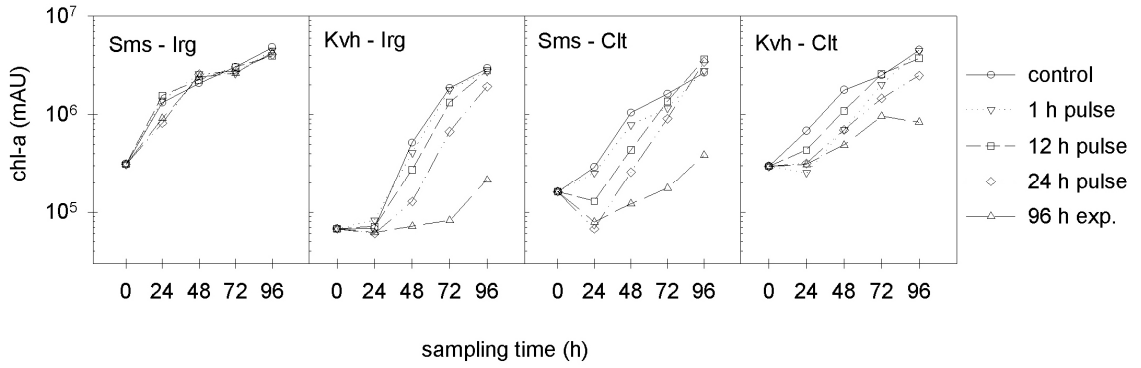


Fig 3. Relative amount of chlorophyll *a* (chl-a) in periphyton samples in SWIFT. Values are arithmetic means (n=3) given as relative absorbance observed at 436 nm and expressed as Absorbance Units (AU). Sampling sites of periphyton: Sms – Smalsund, Kvh – Kalvhagefjorden are shown in the map in Fig. 2. Exposure concentrations: Irg – 5 nM irgarol, Clt – 1 µM clotrimazole.

Periphyton that were sampled in a relatively unpolluted site, Kalvhagefjorden, were exposed to one pulse of irgarol or clotrimazole for a period of 1, 12, 24 or 96 h. Exposure to irgarol delayed or inhibited the process of optimisation to bioassay conditions. After exposure had ended the previously exposed periphyton became more similar to the control communities with time. Chlorophyll *a* values of irgarol-exposed samples from Kalvhagefjorden show that growth was inhibited during exposure and that the periphyton continued to grow once irgarol exposure ended (Fig 3.). During exposure to irgarol the change of xanthophylls, as observed in the controls, was delayed and periphyton exposed to irgarol had a lower ratio of diatoxanthin over diadinoxanthin (Fig. 4). This is a consequence of irgarol being a PSII-inhibitor as such toxicants inhibit the light-powered electron-transport during photosynthesis and thus also inhibits the build up of a proton gradient over the thylakoid membrane (Moreland 1980, Hall et al. 1999), which is the force that induces the transformation of diadinoxanthin to diatoxanthin (Hager 1980). The periphyton from Kalvhagefjorden that were continuously exposed to clotrimazole became slightly dissimilar to their controls (Paper II, Fig. 1), their growth was inhibited (Fig. 3) and the xanthophyll response to high light was different from the controls (Fig. 4). The periphyton exposed to pulses of clotrimazole suffered from inhibited growth for some time after end of exposure (Fig. 3) and they showed affected xanthophyll adaptation, at least at the 72h sampling (Fig. 4).

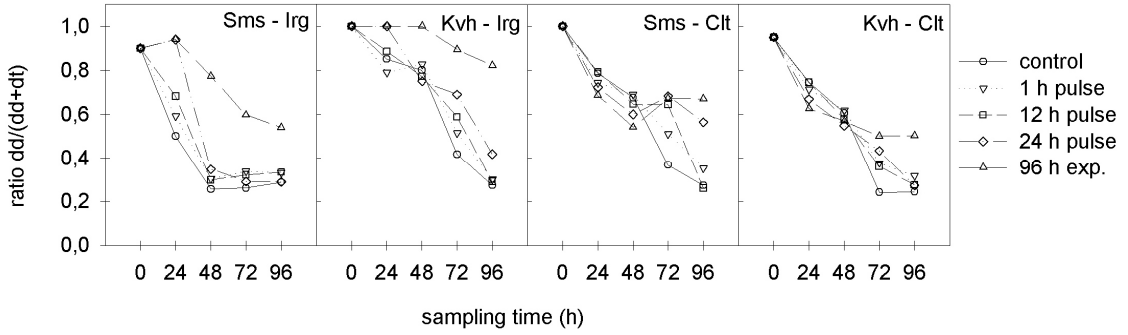


Fig. 4. Ratio of xanthophylls (diadinoxanthin and diatoxanthin) in periphyton, dd = diadinoxanthin, dt = diatoxanthin. dd and dt values are arithmetic means (n = 3) of their absorbance values at 436 nm.

Compared to periphyton from Kalvhagefjorden, periphyton from Smalsund, which is a presumably irgarol-polluted site, reacted differently when exposed to similar pulses of irgarol or clotrimazole. Studying the chlorophyll *a* values, it is revealed that growth of the Smalsund periphyton was not affected by irgarol exposure (Fig. 3). The distribution of xanthophylls in the Smalsund periphyton was, however, affected by irgarol exposure (Fig. 4). This may be an indication that the Smalsund periphyton were adapted to irgarol exposure and that their homeostasis-maintaining functions worked and growth could continue. That the xanthophyll distribution was affected is, however, a sign of some disturbance that may be costly and affect the performance of certain species and individuals in a longer perspective. The affected xanthophyll distribution during a period of sustained growth is an example of an effect that would not have been noticed if only growth, which is regarded as a superior endpoint, would have been studied. Especially problematic in this respect are endpoints that are superior in the sense that they are functionally redundant.

This means that if some pathways of a certain function (e.g. metabolism) are blocked, others may still be working and the integrating endpoint (e.g. growth) may seem unaffected. In a longer perspective this may however be problematic for the affected organisms as energy is spent on maintaining homeostasis rather than growth, reproduction or competition in an ecological perspective. Another type of effect that could have passed unnoticed was presented by Hurd et al. (1996) who showed that population dynamics were affected post-exposure in non-target species. It was not until the moulting process began, two weeks after insecticide application, that any effects were noted. Hidden effects are also recognized in discussions of the community-conditioning hypothesis. According to Matthews et al. (1996) an implication of community conditioning is that information about a community may be retained in community properties that remain hidden, or, more accurately, unmeasured. It is thus important to measure several parameters of communities and not only rely on integrating endpoints.

Interestingly, the clotrimazole exposed periphyton from Smalsund were more affected than the periphyton from Kalvhagefjorden (Paper II, Fig. 1). Chlorophyll *a* values were affected to a larger degree and it took longer time before the periphyton that had been exposed to pulses of clotrimazole started to grow once the exposure had ended (Fig. 3). Even the xanthophylls were more different from their controls, both during continuous and after pulsed exposures, compared to the Kalvhagefjorden periphyton (Fig. 4).

The community conditioning hypothesis states that communities exposed to pulses of contaminants are presumed to react according to shaping events (i.e. pollution) in their history (Landis et al. 1996, Matthews et al. 1996). The difference in response to irgarol exposure between the different periphyton communities can be explained as PICT response. The effects of clotrimazole exposure, however, show the opposite pattern with regards to community sensitivity. A possible explanation is that as the Smalsund community has become more tolerant to irgarol, through TIS, the genetic diversity of the community has been reduced as sensitive species and individuals (phenotypes) have been removed. The specific tolerance mechanisms may also include an increase in metabolic energy spent on detoxification or recycling of damaged proteins. Thus, the induced tolerance comes with a cost. Since clotrimazole has a dissimilar ecological mode of action than irgarol, the subsequent exposure to clotrimazole causes a larger effect than a subsequent exposure to a similar compound, like another PSII-inhibitor, would.

This discussion may be expressed as a hypothesis that a sequence in time of two pulse exposures to different chemicals, one at a time, may cause different degrees of damage to a community depending on the ecological mechanism of action of the chemicals (Fig. 5). More specifically, if the chemicals have a similar ecological mechanism of action, as sensitive species and individuals are targeted by the first toxicant, the community will become less sensitive to such toxicants and the second exposure will not add much to the overall damage. But, if the toxicants have dissimilar mechanisms of action, the first exposure would cause an overall decrease of genetic diversity of the community and thus increase the general sensitivity to toxic exposure and the toxic effect of the second toxicant will be enhanced (Fig. 5). To summarise, a sequence of dissimilarly acting toxicants will cause greater toxic effect than a sequence of similarly acting chemicals. This

reasoning is also in line with the works of Vinebrooke et al. (2004) who have discussed ecosystem effects of multiple stressors. A sequential exposure to toxicants with dissimilar ecological mechanisms of action, such as the clotrimazole exposure to the irgarol-polluted Smalsund periphyton, would with their nomenclature be a case of negatively correlated species co-tolerance to the two toxicants. Thus, Vinebrooke and co-workers describe the same concepts, but with a different perspective. In this thesis, I discuss ecological mode of action as a property of a chemical in relation to a certain community. Vinebrooke and co-workers regard the same issue from the species within in the community and whether their tolerances to the disturbance in question are correlated or not.

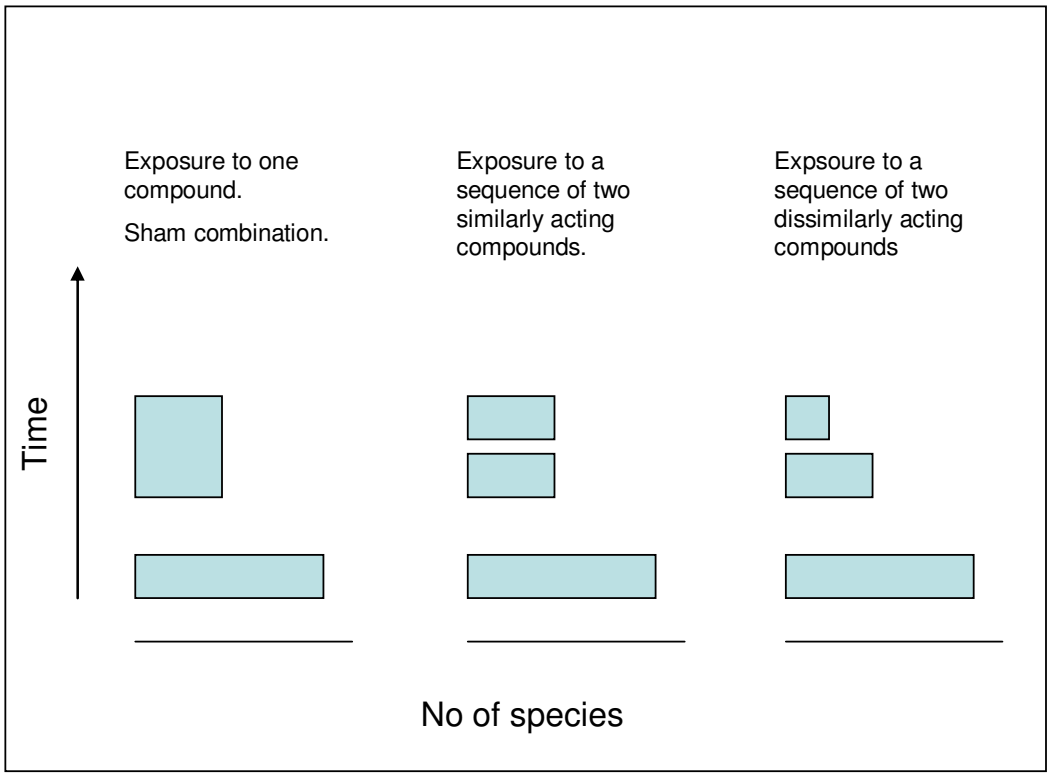


Fig. 5. Conceptual explanation of how sequential exposure of similarly and dissimilarly acting chemicals affects communities. The second exposure in a sequence of similarly acting compounds does not further shape the community. If the compounds act in a dissimilar way, the second exposure does not target the same species and individuals as the first exposure, hence the larger effect.

TRANSLOCATED PERIPHYTON IN FIELD

To compare the range of biological variation that can be evaluated within the SWIFT bioassay to variation in natural communities, periphyton samples were studied in the field. Periphyton communities were sampled at two sites, Kilen and Kalvhagefjorden, which are similar in several aspects but differ in respect to pollution history as Kilen is contaminated by irgarol and Kalvhagefjorden is less contaminated, as described earlier. Periphyton from the two sites were translocated to the other site, respectively, and the succession following the translocation was evaluated at both sites. The successions over time of pigment composition of the translocated

periphyton and reference samples (periphyton that remained at the site of initial colonisation) are shown as an MDS in Fig. 6. The reference samples are separated along the entire period, indicating differences induced by site specific properties (i.e. pollution, available colonising species, nutrient regime, light differences due to particles in the water). Periphyton from Kilen that were translocated to Kalvhagefjorden quickly became similar to the Kalvhagefjorden reference community. After one day the transition of pigment composition had started and after two days, the translocated periphyton were very similar to the Kalvhagefjorden reference sample (Fig. 6). The periphyton from Kalvhagefjorden that were translocated to Kilen, however, moved in another direction the first day after translocation. The second day after translocation, the sample had become more similar to the Kilen reference. Further on, the translocated sample continuously succeeded parallel to the Kilen reference (Fig. 6).

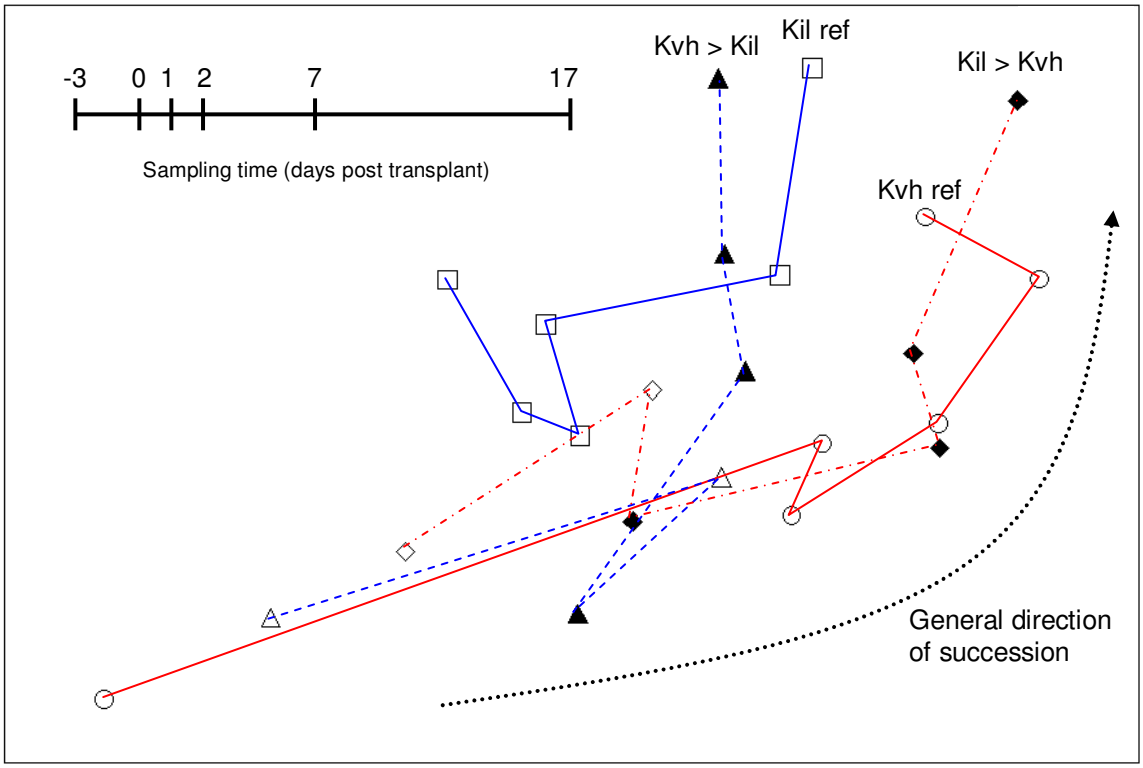


Fig. 6. Multidimensional scaling of pigment samples (arithmetic means, n=2) from field periphyton. Periphyton from two sites (Kvh = Kalvhagefjorden, Kil = Kilen) were translocated to the other site, respectively, at day 0. Samples were taken pre and post translocation according to the indicated timeline. Samples labelled ref are reference samples that remained in the original location.

Since the site Kilen is contaminated by irgarol the periphyton that had colonised those discs were tolerant to irgarol, in consistence with the PICT concept. Hypothetically, translocation to a site with less irgarol contamination does not necessarily require re-structuring of the community but probably physiological adaptations to new nutrient levels and perhaps a different quality of light. Colonisers from the new site will certainly attach and slowly shape the structure of the periphyton, but the initial transition is unproblematic. This is reflected in the quick adjustment of pigment

composition to become similar to the Kalvhagefjorden reference. As shown in the MDS, already after 2 days the samples were similar and continued to progress in related trajectories (Fig. 6). Pigment fingerprints of periphyton reflect both physiological and taxonomic composition of the algal part of the community. Physiologically changed pigment as response to different light regimes and changing irgarol exposure are relatively quick and followed by changes related to taxonomic composition.

For the periphyton that originated in Kalvhagefjorden the sequence of pigment fingerprints after translocation is more complex (Fig. 6). The colonising community in Kalvhagefjorden was presumably not adapted to irgarol contamination and translocation to Kilen probably called for restructuring of the community as sensitive species were removed and new tolerant colonisers attached. As observed in the MDS ordination, the shift was dynamic and took several days. After 2 days in Kilen the samples had started to become more similar to the Kilen samples than the Kalvhagefjorden reference and after 7 days the translocated sample was similar to the Kilen reference and they continued to develop in parallel (Fig. 6).

MICROCOSM PERIPHYTON AND FIELD-SAMPLED PERIPHYTON

A multidimensional scaling of the reference samples from the translocation experiment and samples from two SWIFT experiments are plotted in (Fig. 7). The SWIFT samples that are plotted are controls and samples that were continuously exposed to either 5 nM irgarol or 1 µM clotrimazole (see Paper II for details of the microcosm bioassay). Plotting these different sets of data in the same ordination allows for a comparison of the range of pigment composition changes in periphyton from field sites and periphyton sampled during microcosm bioassays. The field-samples were taken from Kilen and Kalvhagefjorden at the same dates in August of 2007 while the SWIFT samples originated from Kalvhagefjorden and were sampled at different times during August of 2006. The span of natural variation of periphyton is reflected in the dissimilarity between the start samples (samples in lower/left end of trajectories in Fig. 7). Unlike the natural reference samples that move upwards in the plot, the SWIFT bioassay conditions caused the control samples to shift to the right in the plot (Fig. 7). It is expected that they would be different since the nutrient status and light-regime in SWIFT is clearly different from the natural environment. It is noteworthy that the transition of the SWIFT control samples slowed down between days 3-4. The control samples from one of the experiments (diamonds) from days 3 and 4 are in fact on top of each other.

Samples exposed to clotrimazole (black diamonds) progress along the same route as the controls for the first day but then they stop and remain within short distance (Fig. 7). As clotrimazole is a growth inhibitor (Porsbring et al. 2008) it is possible that the periphyton are able to perform physiological pigment changes during the first day but are incapable of any community succession involving dominance of new species.

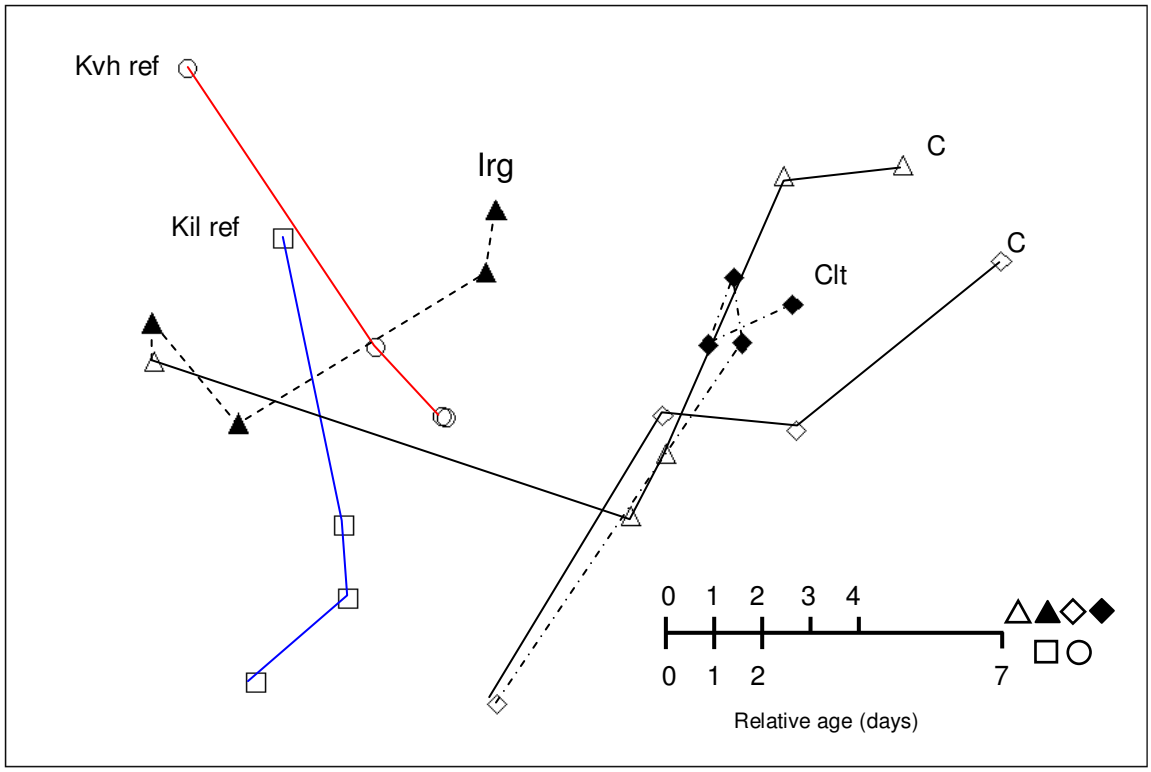


Fig. 7. Multidimensional scaling of field periphyton from Kalvhagefjorden (Kvh ref) and Kilen (Kil ref) and SWIFT samples that originate from Kalvhagefjorden. Labels of SWIFT samples show controls (C) and samples that were continuously exposed to 5 nM irgarol (Irg) or 1 µM clotrimazole (Clt). Trajectories connect samples over time and the last sampled date carries the label. Relative ages are indicated in the timeline and refer to the first sample, which was equally old in all samples (8 days).

The effect of irgarol exposure is noticeable already after one day of exposure in the ordination. As irgarol directly interferes with the physiological adaptations to high-light conditions in SWIFT the response is quick. The irgarol-exposed samples, unlike the clotrimazole-exposed, continued to develop slowly over time (Fig. 7). During the period of 72-96 h even the growth rate increased (Fig. 3). This may be a case of community restructuring, slowly allowing the few irgarol tolerant species (phenotypes) that existed in Kalvhagefjorden to become abundant and grow. Irgarol tolerance in periphyton has taken several years to develop, and Blanck and co-workers (2008) suggested that the potential to form irgarol-tolerant communities may now exist in low abundances even in communities that are not continuously exposed to irgarol. During extended exposure, such as in SWIFT, it is possible that the few tolerant species become more abundant during the TIS. This reasoning is also in line with the community conditioning hypothesis (Landis et al. 1996, Matthews et al. 1996), which states that communities are shaped by events in their past and that such properties may remain for indefinite time periods and possibly alter the future of the community.

CONCLUDING REMARKS AND OUTLOOK

Pulsed exposures to either single species or communities may cause effects that last longer than the exposure, and may even cause effects that appear after exposure has ended. The extent of remaining effect seems to be a property of both the compound and the community. The pollution history of communities may cause different community responses to the same exposure. When studying community responses, effects may go unseen simply by not measuring all possible parameters. Integrating endpoints, such as growth, may be too redundant to catch certain effects.

Communities may respond to previous exposure events with increased tolerance or, in fact, increased sensitivity to subsequent exposures. It is hypothesised here that during exposure to a sequence of dissimilarly acting compounds, communities suffer increased sensitivity to the second exposure as a consequence of decreased diversity. Correspondingly, during a sequence of similarly acting compounds it is hypothesised that the first exposure alone, more or less, shapes the community and that the second exposure thus does not add much to the overall effect.

Algae with carbon-concentrating mechanisms, such as *Saccharina latissima*, may be specifically targeted by enzyme-inhibiting compounds. Presently, the increasing atmospheric carbon dioxide and decreasing oceanic pH causes the advantage of carbon-concentrating mechanisms to decrease. In addition to the pressure from worldwide abundant pollutant chemicals, this might be an underlying cause of shifts of species abundance due to changed competitive patterns.

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REFERENCES

Abel PD (1980) Toxicity of hexachlorocyclohexane (Lindane) to Gammarus pulex. Freshwater Biology 10:251-259

Ashauer R, Boxall A, Brown C (2006) Predicting effects on aquatic organisms from fluctuating or pulsed exposure to pesticides. Environmental Toxicology and Chemistry 25:1899-1912

Axelsson L, Mercado JM, Figueroa FL (2000) Utilization of HCO3- at high pH by the brown macroalga Laminaria saccharina. European Journal of Phycology 35:53-59

Barranguet C, van den Ende FP, Rutgers M, Breure AM, Greijdanus M, Sinke JJ, Admiraal W (2003) Copper-induced modifications of the trophic relations in riverine algal-bacterial biofilms. Environmental Toxicology and Chemistry 22:1340-1349

Barranguet C, Veuger B, van Beusekom SAM, Marvan P, Sinke JJ, Admiraal W (2005) Divergent composition of algal-bacterial biofilms developing nder various external factors. European Journal of Phycology 40:1-8

Blanck H (2002) A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Human and Ecological Risk Assessment 8:1003-1034

Blanck H, Arrhenius A, Porsbring T, Kuylenstierna M, Backhaus T, Scholze M (2005) Predicted and observed mixture effects of three antifoulants on periphyton community structure in marine microcosms. In: Arrhenius A, On the ecotoxicology of chemical mixtures. Doctoral thesis. University of Gothenburg, Gothenburg

Blanck H, Dahl B (1996) Pollution-induced community tolerance (PICT) in marine periphyton in a gradient of tri-*n*-butyltin (TBT) contamination. Aquatic Toxicology 35:59-77

Blanck H, Eriksson KM, Grönvall F, Dahl B, Guijarro KM, Birgersson G, Kylin H (2008) A retrospective analysis of contamination and periphyton PICT patterns for the antifoulant irgarol 1051, around a small marina on the Swedish west coast. Marine Pollution Bulletin (In press)

Blanck H, Wangberg SA (1988) Validity of an ecotoxicological test system - short-term and long-term effects of arsenate on marine periphyton communities in laboratory systems. Canadian Journal of Fisheries and Aquatic Sciences 45:1807-1815

Blanck H, Wängberg S-Å, Molander S (1988) Pollution-induced community tolerance - A new ecotoxicological tool. In: Cairns Jr J, Pratt JR (eds) Functional testing of aquatic biota for estimating hazards of chemicals. American Society for Testing and Materials, Philadelphia, p 219-230

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349

Cairns J, Pratt JR (1989) The scientific basis of bioassays. Hydrobiologia 188:5-20

Cedergreen N, Andersen L, Frihauge Olesen C, Spliid HH, Streibig JC (2005) Does the effect of herbicide pulse exposure on aquatic plants depend on Kow or mode of action? Aquatic Toxicology 71:261-271

Clarke KR (1999) Nonmetric multivariate analysis in community-level ecotoxicology. Environmental Toxicology and Chemistry 18:118-127

Clements FE (1916) Plant succession: An analysis of the development of vegetation. In: Publication 42. Carnegie Institute, Washington, DC, USA

Connell D, Yu J (2008) Use of exposure time and life expectancy in models for toxicity to aquatic organisms. Marine Pollution Bulletin 57:245-249

Dahl B, Blanck H (1996a) Pollution-induced community tolerance (PICT) in periphyton communities under tri-*n*-butyltin (TBT) stress in marine microcosms. Aquatic Toxicology 34:305-325

Dahl B, Blanck H (1996b) Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms. Marine Pollution Bulletin 32:342-350

Death RG (1996) The effect of patch disturbance on stream invertebrate community structure: the influence of disturbance history. Oecologia 108:567-576

Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43:599-626

Diamond JM, Klaine SJ, Butcher JB (2006) Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. Environmental Science & Technology 40:5132-5138

Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. Marine Ecology-Progress Series 8:37-52

Flemming HC, Wingender J (2001a) Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. Water Science and Technology 43:1-8
Flemming HC, Wingender J (2001b) Relevance of microbial extracellular polymeric substances (EPSs) - Part II: Technical aspects. Water Science and Technology 43:9-16
Goldsborough LG, Robinson GGC (1986) Changes in periphytic algal community structure as a consequence of short herbicide exposures. Hydrobiologia 139:177-192
Guasch H, Munoz I, Roses N, Sabater S (1997) Changes in atrazine toxicity throughout succession of stream periphyton communities. Journal of Applied Phycology 9:137-146
Hager A (1980) The reversible, light-induced conversions of xanthophylls in the chloroplast. In: Czygen FC (ed) Pigments in plants. Fischer, Stuttgart, p 57-79
Hall LW, Giddings JM, Solomon KR, Balcomb R (1999) An ecological risk assessment for the use of Irgarol 1051 as an algaecide for antifoulant paints. Critical Reviews in Toxicology 29:367-437
Handy RD (1994) Intermittent exposure to aquatic pollutants - Assessment, toxicity and sublethal responses in fish and invertebrates. Comparative Biochemistry and Physiology C - Pharmacology Toxicology & Endocrinology 107:171-184
Hart WB, Doudoroff P, Greenbank J (1945) The evaluation of the toxicity of industrial wastes, chemicals and other substances to freshwater fishes, Waste Control Laboratory, Atlantic Refining Co., Philadelphia
Hurd MK, Perry SA, Perry WB (1996) Nontarget effects of a test application of diflubenzuron to the forest canopy on stream macroinvertebrates. Environmental Toxicology and Chemistry 15:1344-1351
Jin E, Polle JEW, Lee HK, Hyun SM, Chang M (2003) Xanthophylls in microalgae: From biosynthesis to biotechnological mass production and application. Journal of Microbiology and Biotechnology 13:165-174
Kruskal J (1964) Nonmetric multidimensional scaling: A numerical method. Psychometrika 29:115-129
Lamouroux JVF (1813) Essai sur les genres de la famille des thalassiophytes non articulées. Annales du Muséum d'Histoire Naturelle, Paris 20:21-47, 115-139, 267-293, Plates 117-113
Landis WG, Matthews RA, Matthews GB (1996) The layered and historical nature of ecological systems and the risk assessment of pesticides. Environmental Toxicology and Chemistry 15:432-440
Lane CE, Mayes C, Druehl LD, W. SG (2006) A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. Journal of Phycology 42:493-512
Latowski D, Grzyb J, Strzalka K (2004) The xanthophyll cycle - molecular mechanism and physiological significance. Acta Physiologiae Plantarum 26:197-212
Liess M (2002) Population response to toxicants is altered by intraspecific interaction. Environmental Toxicology and Chemistry 21:138-142
Liess M, Schulz R, Liess MHD, Rother B, Kreuzig R (1999) Determination of insecticide contamination in agricultural headwater streams. Water Research 33:239-247
Lopez-Mancisidor P, Carbonell G, Fernandez C, Tarazona JV (2008) Ecological impact of repeated applications of chlorpyrifos on zooplankton community in mesocosms under Mediterranean conditions. Ecotoxicology 17:811-825
Martinovic D, Denny JS, Schmieder PK, Ankley GT, Sorensen PW (2008) Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance. Environmental Science & Technology 42:3421-3427
Matthews RA, Landis WG, Matthews GB (1996) The community conditioning hypothesis and its application to environmental toxicology. Environmental Toxicology and Chemistry 15:597-603
McCahon CP, Pascoe D (1990) Episodic Pollution: Causes, Toxicological Effects and Ecological Significance. Functional Ecology 4:375-383
Mercado JM, Andria JR, Perez-Llorens JL, Vergara JJ, Axelsson L (2006) Evidence for a plasmalemma-based CO2 concentrating mechanism in Laminaria saccharina. Photosynthesis Research 88:259-268
Moreland D (1980) Mechanisms of action of herbicides. Annual Review of Plant Physiology 31:597-638
Newman MC, Unger MA (2003) Environmental contaminants. In: Newman MC, Unger MA (eds) Fundamentals of ecotoxicology. CRC Press, Boca Raton, p 21-50

Porsbring T, Arrhenius A, Backhaus T, Kuylenstierna M, Scholze M, Blanck H (2007) The SWIFT periphyton test for high-capacity assessments of toxicant effects on microalgal community development. Journal of Experimental Marine Biology and Ecology 349:299-312

Porsbring T, Blanck H, Tjellström H, Backhaus T (2008) Toxicity of the pharmaceutical clotrimazole to marine microalgal communities. Aquatic Toxicology:In press, published online

Pratt JR, Melendez AE, Barreiro R, Bowers NJ (1997) Predicting the ecological effects of herbicides. Ecological Applications 7:1117-1124

Pusey BJ, Arthington AH, McLean J (1994) The effects of a pulsed application of chlorpyrifos on macroinvertebrate communities in an outdoor artificial stream system. Ecotoxicology and Environmental Safety 27:221-250

Reemtsma T, Weiss S, Mueller J, Petrovic M, Gonzalez S, Barcelo D, Ventura F, Knepper TP (2006) Polar pollutants entry into the water cycle by municipal wastewater: A European perspective. Environmental Science & Technology 40:5451-5458

Shepard R (1957) Stimulus and response generalization: A stochastic model relating generalization to distance in psychological space. Psychometrika 22:325-345

Sladeckova A (1962) Limnological investigation methods for the periphyton ("aufwuchs") community. Botanical Reviews 28:286-350

Slooff W (1983) Biological effects of chemical pollutants in the aquatic environment and their indicative value. Dissertation, University of Utrecht

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. Environmental Toxicology and Chemistry 15:31-76

Torgerson W (1952) Multidimensional scaling: I. Theory and method. Psychometrika 17:401-419

Van Leeuwe MA, Brotas V, Consalvey M, Forster RM, Gillespie D, Jesus B, Roggeveld J, Gieskes WWC (2008) Photoacclimation in microphytobenthos and the role of xanthophyll pigments. European Journal of Phycology 43:123-132

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104:451-457

Zhao Y, Newman MC (2004) Shortcomings of the laboratory-derived median lethal concentration for predicting mortality in field populations: exposure duration and latent mortality. Environmental Toxicology and Chemistry 23:2147-2153