University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Valery Forbes Publications | Papers in the Biological Sciences

1-1-2003

Long-term effect of Sea-Nine on natural coastal phytoplankton communities assessed by pollution induced community tolerance

Dorthe K. Larsen
Roskilde University

Inge Wagner
Roskilde University

Kim Gustavson
DHI-Institut for Water and Environment, Hørsholm, Denmark ,

Valery E. Forbes
University of Nebraska-Lincoln, vforbes3@unl.edu

Torben Lund
Roskilde University

Follow this and additional works at: http://digitalcommons.unl.edu/biosciforbes

Larsen, Dorthe K.; Wagner, Inge; Gustavson, Kim; Forbes, Valery E.; and Lund, Torben, "Long-term effect of Sea-Nine on natural coastal phytoplankton communities assessed by pollution induced community tolerance" (2003). *Valery Forbes Publications.* Paper 52.
http://digitalcommons.unl.edu/biosciforbes/52

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Valery Forbes Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Published in *Aquatic Toxicology* 62:1 (January 10, 2003), pp. 35–44; doi: 10.1016/S0166-445X(02)00065-6
Copyright © 2003 Elsevier Science B.V. Used by permission.
Submitted November 19, 2001; revised April 15, 2002; accepted April 17, 2002; published online June 4, 2002.

Long-term effect of Sea-Nine on natural coastal phytoplankton communities assessed by pollution induced community tolerance

Dorthe K. Larsen,[1,2] Inge Wagner,[1,2] Kim Gustavson,[1]
Valery E. Forbes,[2] and Torben Lund [2]

1. DHI-Institut for Water and Environment, The International Agency for 14C determination, Agern Allé 11, 2970 Hørsholm, Denmark

2. Department of Life Sciences and Chemistry, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark

Corresponding author — V. E. Forbes

Abstract

Sea-Nine™211 has been introduced as a new biocide in antifouling paints with an immediate degradation when it is released from ship hulls. The active component of Sea-Nine™211 is 4,5-dichloro-2-n-octyl-isothiazoline-3-one (DCOI). In the present study, the toxicity of DCOI and the occurrence of Pollution Induced Community Tolerance (PICT) were tested in microcosms containing eutrophic coastal water with its natural composition of phytoplankton. The experiment was performed in closed systems with a single addition of the nominal concentrations 0, 3.2, 10, 32 and 100 nM DCOI, for a period of 16 days. Pollution induced community tolerance (PICT) was observed in the phytoplankton communities exposed to the nominal concentrations 32 and 100 nM DCOI. Chemical analysis of DCOI in the coastal water utilized in the toxicity and PICT experiment was performed by GC-MS using a solid- phase extraction method. Half-life was calculated to be 2.5 days for the nominal concentrations 32 and 100 nM DCOI. The results of the present study show that nominal concentrations of 32 and 100 nM DCOI significantly increased the community tolerance already after 2 days of exposure and that the tolerance was maintained for a period of 16 days even when DCOI was degraded during this period. The causes for the persistent tolerance are discussed in relation to the degradation of DCOI and structural changes in the phytoplankton communities.

Keywords: Antifouling paints, 4,5-Dichloro-2-n-octyl-isothiazoline-3-one, DCOI, Biodiversity, Microcosms, PICT-method

1. Introduction

In 1996 the new antifouling agent Sea-Nine™ 211 Biocide was introduced. The active ingredient of Sea-Nine™ 211 Biocide, 4,5-dichloro-2-n-octyl-4-isothiazoline-3-one (DCOI), has shown potential effects against a wide spectrum of bacteria, fungi and algae (Miller and Lovegrove, 1980; Vasishtha et

al., 1995; de Nys et al., 1996; Willemsen et al., 1998). A rapid degradation of DCOI in natural seawater has been observed with a half-life of generally less than 24 h (Shade et al., 1993). The environmental risk of Sea-Nine is considered by Shade et al. (1993) to be relatively low, due to the very rapid degradation when released from ship hulls.

However, present knowledge about effects and degradation of DCOI is insufficient since effects of DCOI are based on single species toxicity tests and evaluated from nominal concentrations of DCOI (Shade et al., 1993; Björk and Karlsson, 1992). As far as the degradation of DCOI, this has been estimated by the producer, but to a certain extent performed under non-realistic conditions and never directly related to the effects of DCOI (Shade et al., 1993; Jacobson et al., 1993). Thus, the sparse amount of effects data measured under realistic conditions and in response to actual concentrations of DCOI, highlights the need for further information about the fate and effects of DCOI in the environment.

The aims of the present work were to investigate long-term effects of DCOI on natural marine phytoplankton communities and to investigate whether DCOI was degraded during the course of the experiment. The combination of measuring the toxicity of DCOI and tolerance of phytoplankton toward DCOI at the community level and simultaneously performing chemical analysis of DCOI concentrations in the microcosms provided detailed knowledge about the concentration level of DCOI that could be expected to affect phytoplankton communities in the environment. Furthermore, to evaluate whether DCOI exposure resulted in structural changes of the phytoplankton communities in the microcosms, the taxonomic composition, diversity and richness of the phytoplankton were determined.

Analysis of PICT combined with analytical chemical measurements of DCOI concentrations by solid phase extraction using gas chromatography combined with mass spectroscopy (GC-MS) were performed. The PICT methodology, developed by Blanck et al. (1988), provides information on the potential impact of a toxicant at the community level. PICT measurements have been demonstrated to be both sensitive and ecotoxicologically relevant (Petersen and Gustavson, 1997; Gustavson and Wängberg, 1995; Dahl and Blanck, 1996). The degree of pollution induced community tolerance is dependent on exposure concentration and can be detected with a series of short-term tests, which show the direct effect of the toxicant on the community. Validation of the PICT methodology used to identify tolerance of phytoplankton communities has been performed in other studies (Gustavson and Wängberg, 1995; Petersen and Gustavson, 1997; Gustavson et al., 1999).

2. Methods and materials

2.1. Location and sampling

Coastal water was collected in Tempelkrogen (55°40′ N, 11°47′ E) at the inner part of Ise Fjord in Denmark on November 30th, 1998. In situ physical and chemical properties of the coastal water were determined. The temperature was 1 °C, pH 8.2 and salinity 11‰. A thin layer of ice was observed in the water surface. The greater part of Tempelkrogen is shallow with a depth of less than 2 m, and it has only one connection to Ise Fjord through a narrow mouth at its northern end.

Eighty-six litres of coastal surface water were collected and filtered through a 150 μm net in the laboratory. The filtered water was acclimatized to laboratory conditions for 5 days before the experiment, allowing the algae to increase in biomass. Chlorophyll a concentration was determined according to Riemann and Ernst (1982) by UV spectroscopy and was 5.5 μg chla/l on day 0. The filtered coastal water with its natural content of phytoplankton was randomly distributed by adding 1 l into each of 15 non-toxic polyethylene bottles (microcosms) until a total volume of 5 l was obtained. All bottles were washed in acid and rinsed three times in filtered coastal water before being used in the experiment.

2.2. *Design of the experiment*

The experiment was conducted over 16 days, 5–20 December 1998. The effects of DCOI in the microcosms were recorded as photosynthetic activity relative to control. The concentration of DCOI in the microcosms was assessed by chemical analysis of the coastal water by GC-MS. Number of species and composition of the phytoplankton communities in the microcosms were determined using an inverted microscope (400–600×).

Stock solutions of DCOI in acetone (formula $C_{11}H_{17}Cl_2NOS$, molecular weight 282.2 g/mole) were added in a single addition on day 0, giving a final acetone percent of 0.01 in the microcosms. Control microcosms were given the same amount of acetone. Microcosms pre-exposed to nominal concentrations 0, 3.2, 10, 32 and 100 nM DCOI in three replicates were utilized in the experiment. The duration of the pre-exposure was between 1 and 15 days, depending on which day tolerance was measured. The microcosms were held at 16 °C in 12 h light cycle:12 h dark cycle with a light intensity of 250 (μmol/m^2/s). They were placed on a shaking table at 50 rpm and loosely capped through the entire experiment. The microcosms were rearranged on the sample days to obtain equal light exposure.

2.3. *Pollution induced community tolerance*

The tolerance towards DCOI was detected during a short-term concentration-response test of photosynthesis inhibition in the microcosms with phytoplankton communities. Tolerance was determined on days 2, 5, 8, 12 and 15 in the laboratory. By comparing the response from the microcosms containing pre-exposed phytoplankton communities with the response from the control microcosms, tolerance was considered to have occurred if photosynthetic activity in the pre-exposed community was significantly higher, than photosynthetic activity in the control community. The short-term test was performed for each microcosm with addition of a geometric nominal concentration series of six DCOI concentrations with three replicates plus two blind samples from the control microcosms.

Volumes of 9.9 ml samples from each microcosm were transferred into glass scintillation vials (20 ml) and 100 μl of stock solution with the geometric concentration series (0, 10, 32, 100, 320 and 1000 nM) were added. This gave a final acetone percent of 0.01 in the vials. Likewise, 100 μl 1% acetone solution was added to the blind samples, giving a final acetone percent of 0.01. All samples were preincubated in a thermostatic water bath at 11–12.5 °C. The vials were gently shaken, and the samples received light from fluorescent tubes (250 μmol/m^2/s) for 1 h. Subsequently, 100 μl (14.3 μCi/ml) ^{14}C-labeled bicarbonate (^{14}C International Agency, Denmark) was added, and the samples were incubated for 2 h. In the meantime, 500 μl glutardioaldehyde (25%) was added to the blind samples to stop all biological activity, and 100 μl ^{14}C-labeled bicarbonate (14.3 μCi/ml) was added. Immediately after incubation, 200 μl 1M HCl was added to all samples including the blind samples to stop the incubation and to remove unincorporated ^{14}C-bicarbonate. The blind samples were used to correct for abiotic ^{14}C-carbon fixation. After 18–24 h, 11 ml of scintillation cocktail (Instagel II® plus, Packard Bioscience Company) was added, and the vials were thoroughly agitated. The incorporated radioactivity was measured by liquid scintillation counting (WALLAC LS 1409). The photosynthetic activity in the samples was expressed as disintegrations per min (DPM), and was calculated from an external calibration standard and corrected for quench and background.

2.4. *Long-term effect*

The long-term effect of DCOI on the function of the phytoplankton communities, expressed as photosynthetic activity, was followed in the pre-exposed microcosms and the control microcosms during the course of the PICT experiment. It was established from the test concentration 0 nM in the short-term concentration–response test in the PICT experiment performed on days 2, 5, 8, 12 and 15.

2.5. Chemical analysis

Chemical analysis was carried out at the same time as the tolerance experiment to determine the actual concentration of DCOI in the microcosms. A volume of 200 ml coastal water from each microcosm was transferred to a 500 ml glass flask and stored at −20 °C until further treatment.

Chemical analysis was performed by solid extraction using Oasis™ HLB extraction cartridge 6 cc 200 mg (Waters). Cartridges were conditioned with 10 ml HPLC grade methanol and 10 ml millipore water without flow. A 100 ml sample of coastal water was loaded and a constant flow (20 ml/min) was maintained until the cartridges were dry. Twenty ml of HPLC grade dichloromethane was added to the cartridges. The eluent was collected and evaporated until dryness and thereafter dissolved in 1.5 ml HPLC grade dichloromethane. Desmetryne (2-isopropyl-amino-4-methylamino-6-methylthio-1,3,5-triazine) 99.4% purity (Bie & Berntzen) was used as internal (surrogate) standard. Naphthalene 98% purity (Aldrich) was used as external standard and was added directly into the GC-vial.

The samples were analyzed by a Hewlett Packard Gas Chromatograph 5890 series II coupled to a Hewlett Packard mass selective detector 5971A using an HP-5 column, selective ion monitor (SIM) splitless injection and an injection volume of 1 μl. The temperature program was 70/2-10/300/5. Recovery was between 34 and 113% and the detection limit was 1 nM (S/N=10).

The concentration of DCOI was determined by searching for the ions of DCOI (ions 169 and 182), desmetryne (ions 213 and 198) and naphthalene (ion 128). If the ion ratio of DCOI and the ion ratio of desmetryne differed by ±20% from a standard sample, the sample was not used to calculate the concentration of DCOI. The standard samples consisted of a known concentration of DCOI, desmetryn and naphthalene and were analyzed before and after the measurements of the actual DCOI concentrations in the samples.

2.6. Taxonomic composition in the phytoplankton

Qualitative and quantitative taxonomic analyses were conducted by transferring 100 ml of coastal water from one control microcosm and from one microcosm pre-exposed to 3.2, 10, 32 and 100 nM DCOI into 100 ml medicine flasks to which 1 ml Lugol solution without acid was added. Samples were stored at 4 °C until preparation.

Direct counting of the preserved phytoplankton communities was carried out by Utermöhl's technique (Utermöhl, 1958) using a counting chamber and an inverted microscope (Olympus and Leica 400×). Sedimentation tubes contained between 3 and 30 ml coastal water depending on the density of the phytoplankton cells. The procedure was carried out as described in Chorus and Bartam (1999). A deviation from the procedure was the sedimentation time; all samples were allowed to sediment for at least 24 h before counting. One thousand cells were counted for each sample, giving a maximum error of 7–10% (Chorus and Bartam, 1999). The phytoplankton cells were identified to the level of order except for cyanophyceae, prymnesiophyceae and prasinophyceae, which were identified to the level of class. The last two classes were stated together due to difficulty in separating them. Cyanophyceae were counted in colonies containing more or less than 20 cells. Taxonomic diversity in the microcosms was determined by the Shannon–Wiener diversity index.

2.7. Statistical analysis

Statistical significance of the differences in DCOI tolerance expressed as differences in the photosynthetic activity between the control and the pre-exposed communities was determined by a two-factor analysis of variance (ANOVA), followed by a Tukey HSD test. The two factors were the pre-exposure concentration and the short-term test concentration. Statistical analysis was performed in Statistica version 5.0 for each of the days on which tolerance was measured. The analysis was performed on log-transformed data (Fowler and Cohen, 1997) and a significance level of 0.05 was used for all analyses, unless otherwise stated.

3. Results

3.1. Pollution induced community tolerance

In the experiment PICT was found in the phytoplankton communities pre-exposed to the two highest DCOI concentrations (32 and 100 nM) as the photosynthetic activity in these microcosms showed significant differences from the control communities on all of the days tolerance was measured. This was seen from the short-term test, where test concentrations of 100, 320 and 1000 nM DCOI did not inhibit photosynthetic activity in these microcosms as was the case for the control microcosm and the microcosms pre-exposed to 3.2 and 10 nM DCOI. The results are presented in Figure 1 for each of the tolerance measurements on days 2, 5, 8, 12 and 15.

The tolerance was already significant on day 2 (F=9.3 df=4,60 P<0.05) and became more distinct by day 5 (F=263.6 df=4,60 P<0.05). On day 8 the tolerance was most pronounced, as the tolerance already could be detected at test concentration 32 nM DCOI (see Figure 1, day 8) (F=99.5 df=4,60 P<0.05). After day 8 the tolerance slowly decreased, though it was still significant on day 15 (F=17 df=4,60 P<0.05). Tolerance was not observed in the microcosms pre-exposed to the nominal concentrations 3.2 and 10 nM DCOI (P>0.05).

3.2. Photosynthetic activity in the microcosms

The photosynthetic activity in the control microcosms and the DCOI exposed microcosms on days 2, 5, 8, 12 and 15 is shown in Figure 2.

Photosynthetic activity in the microcosms with phytoplankton communities exposed to 10, 32 and 100 nM DCOI was initially affected relative to the control microcosm, though a recovery was seen as the effects vanished over time. The photosynthetic activity in the microcosms exposed to 3.2 nM DCOI followed the photosynthetic activity in the control microcosm. Initially in the experiment there was a noticeable increase in the photosynthetic activity in the control microcosms and the microcosms exposed to the two lowest DCOI concentrations, indicating population growth in these microcosms. However, the photosynthetic activity decreased slowly again after 8 days in these microcosms, probably due to nutrient limitation. The opposite picture was found in the microcosms exposed to 32 and 100 nM of DCOI. Inhibition of photosynthetic activity was observed at the beginning of the experiment, but then the activity recovered after 8 days, and after 15 days the photosynthetic activity was at the same level as in the control community. The reason for this recovery might be a lower DCOI concentration but could also be due to a delayed nutrient exploitation. Furthermore, the photosynthetic activity increased by a factor of three in all microcosms during the course of the experiment (from 2500 to 8000 DPM). Thus, the results indicated that DCOI had no effects on the function of phytoplankton communities after 16 days at 10.5–11 °C.

3.3. Concentrations of DCOI

Actual concentrations of DCOI in the microcosms were analyzed throughout the course of the PICT experiment (Figure 3).

The analysis of the actual DCOI concentrations showed degradation of DCOI with half-lives determined to be 2.5 days (60 h) for the nominal concentration of 32 nM DCOI and 2.6 days for the nominal concentration of 100 nM DCOI. Half-lives were calculated according to first order kinetics (r=0.94, n=9, P=0.01). On day 15 the actual DCOI concentration was 1 nM in the microcosms exposed to the nominal DCOI concentration 100 nM, whereas the actual DCOI concentration was under the detection limit in the microcosms exposed to the nominal DCOI concentration 32 nM.

3.4. Taxonomic composition of the phytoplankton communities

The taxonomic composition of the phytoplankton communities in the microcosms from the PICT experiment is shown in Figure 4. Effects of DCOI on taxonomic composition are assessed qualitatively as

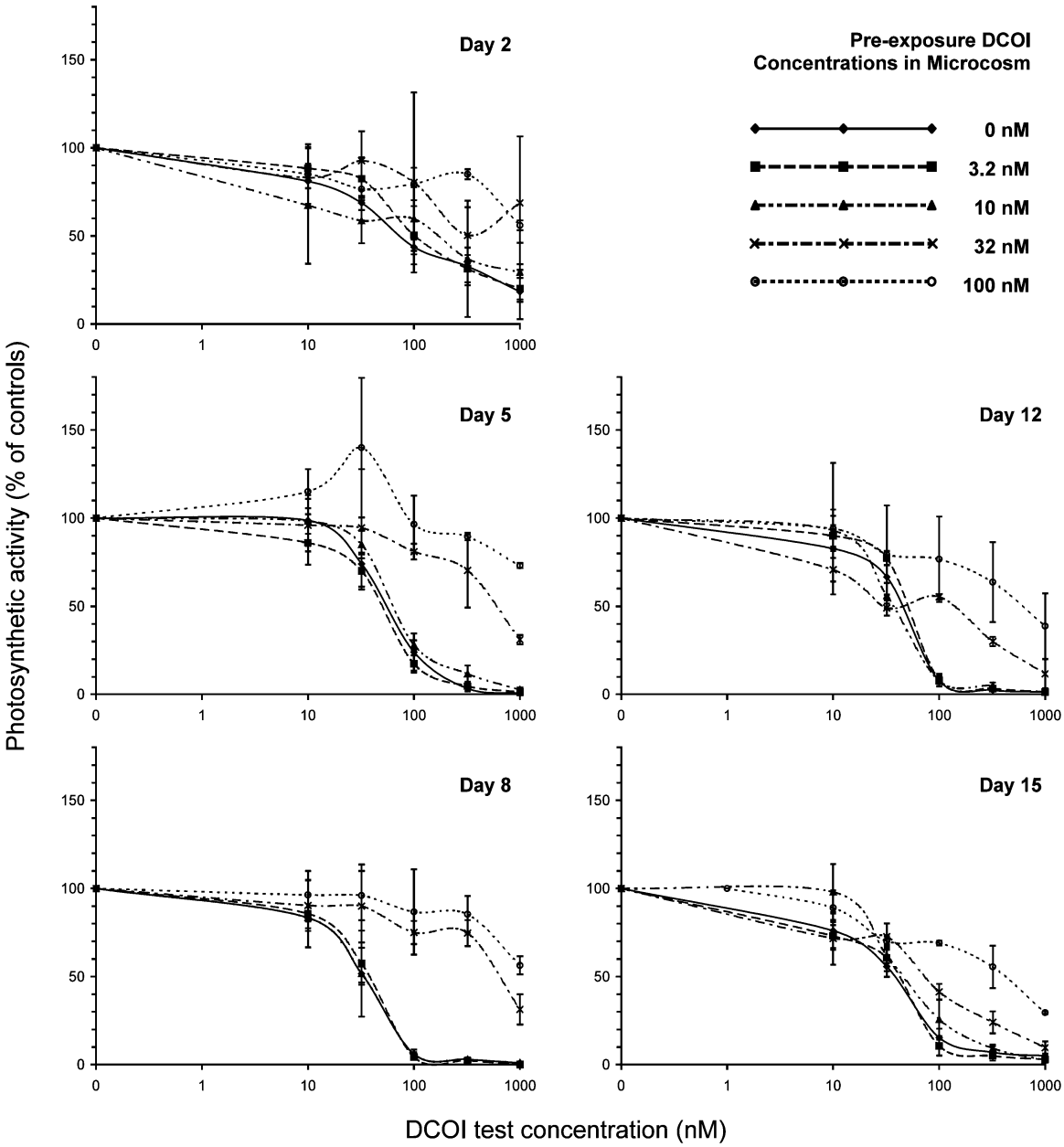

Figure 1. Pollution induced community tolerance. Tolerance of the pre-exposed phytoplankton communities on different days. The tolerance was established in a short-term photosynthetic concentration-response test. The test concentrations were 0, 10, 32, 100, 320 and 1000 nM (DCOI) Error bars represent standard deviations (n=3). Note the X-axis is logarithmic.

our design did not permit statistical comparisons of this parameter among microcosms to be made.

By day 15, the dominant phytoplankton order in the control microcosm was Eupodiscales. The phytoplankton communities exposed to the two lowest nominal concentrations 3.2 and 10 nM DCOI had a similar taxonomic composition as the control community on day 15, even though 3.2 nM de-

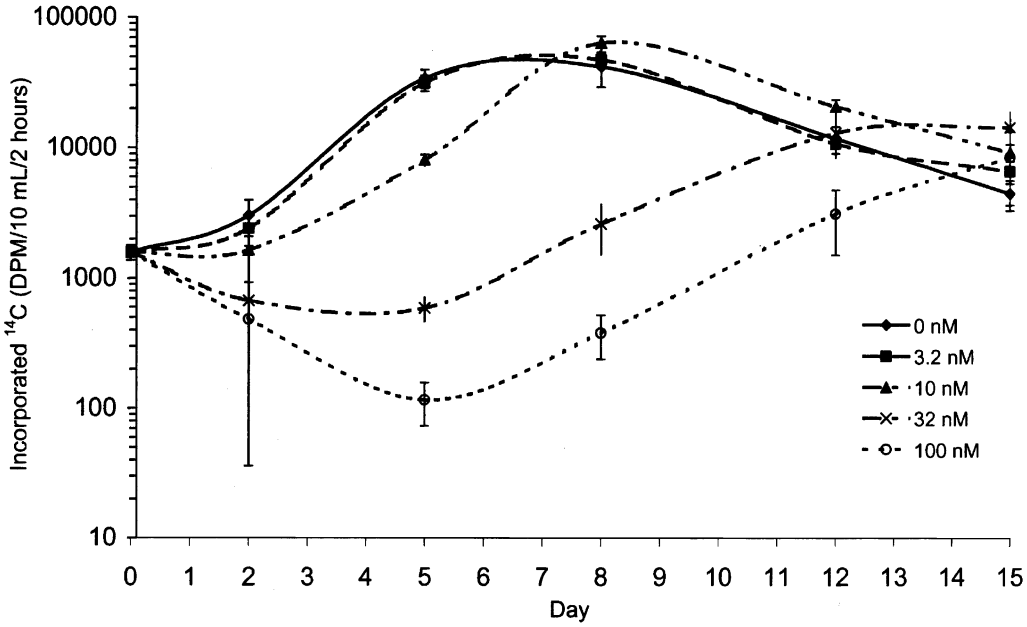

Figure 2. Photosynthetic activity over time. Photosynthetic activity in the control and the pre-exposed microcosms on different days established from the short-term test concentration 0 nM of DCOI. The photosynthetic activity was assessed in a 2 h test by incorporation of ^{14}C-bicarbonate. Vertical error bars represent standard deviations (n=3). Note the Y-axis is logarithmic.

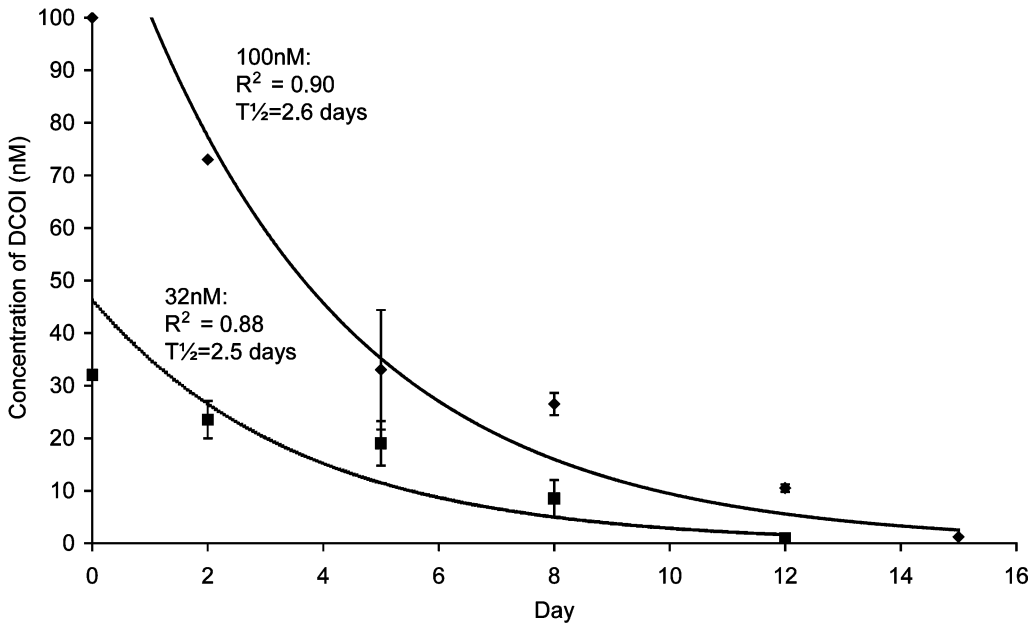

Figure 3. Actual DCOI concentrations over time. Actual concentrations of DCOI in the PICT experiment on different days. Samples from the microcosms pre-exposed to 32 and 100 nM DCOI were analyzed by GC-MS. Vertical error bars represent standard deviations (n=2). Half-lives are calculated from first-order kinetics, illustrated by the solid exponential regression curves.

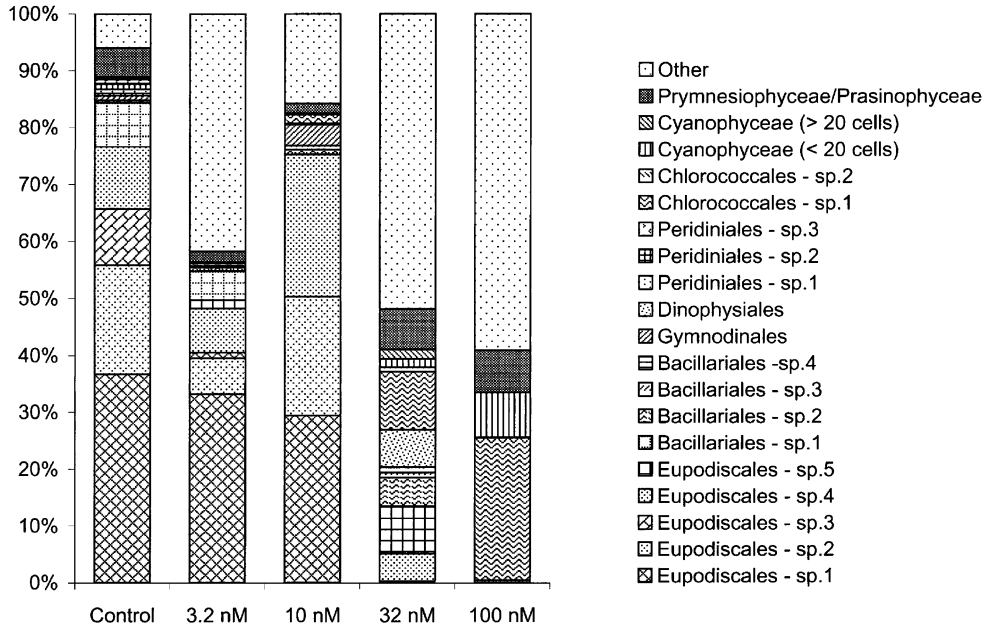

Figure 4. Taxonomic composition. Percentage of different taxonomic groups on day 15 of the control and the phytoplankton communities (microcosms) pre-exposed to the nominal concentrations 3.2, 10, 32 and 100 nM DCOI. Direct counting was carried out using inverted microscopy. One Thousand cells were counted for each microcosms.

viates slightly because of a high abundance of the taxonomic group 'others'. The phytoplankton communities changed considerably when exposed to the nominal concentrations 32 and 100 nM DCOI in terms of differences in taxonomic composition and percentage distribution of the major orders. These phytoplankton communities were dominated by the taxonomic group 'others', which represents small, unidentified algae. Thus, a shift toward small algae was observed in response to DCOI exposure. The succession in the phytoplankton, in which some taxonomic orders were excluded and others appeared, was associated with DCOI exposure. In the microcosm exposed to 32 nM the disturbance was obvious in relation to the control on day 15 by the appearance of several new taxonomic orders. However, the exclusion of taxonomic orders was already observed in the microcosm exposed to 3.2 nM DCOI where Peridiniales species 1 and Gymnodinales no longer occurred. In the microcosm exposed to the highest DCOI concentration (100 nM) a decrease in the number of phytoplankton orders was perceptible.

3.5. Diversity of phytoplankton orders in the microcosms

The diversity of the phytoplankton was determined by the Shannon–Wiener diversity index to the level of order. The diversity provides information about the abundance of individuals in each order (evenness) and the number of different orders (richness) in the phytoplankton. The results are presented in Figure 5.

The diversity in the phytoplankton exposed to the nominal DCOI concentrations 3.2, 10 and 32 nM was almost unaffected relative to the control, whereas the diversity in the phytoplankton exposed to the nominal DCOI concentration 100 nM decreased markedly and was associated with a sharp decrease in number of orders (see Figure 5).

4. Discussion

An initial inhibition in photosynthetic activity in the microcosms with phytoplankton communities exposed to 32 and 100 nM DCOI was detected.

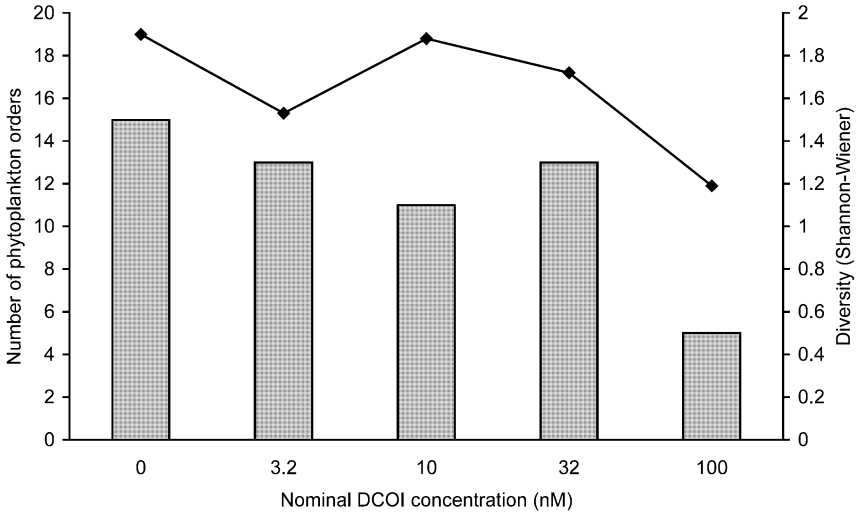

Figure 5. Diversity and number of phytoplankton orders day 15. Diversity and number of phytoplankton orders on day 15 in the microcosms exposed to 0, 3.2, 10, 32 and 100 nM DCOI. To determine diversity the Shannon–Wiener Diversity Index was used. Columns represent number of taxonomic groups and the solid line depicts diversity.

However, a recovery was observed and by the end of the experiment the level of photosynthetic activity in all of the exposed microcosms was equal to that of the control, and furthermore, higher than the initial photosynthetic activity on day 0. Thus, if the long-term effects of DCOI were based on the functional measurement of photosynthesis it would result in a conclusion of no effects of DCOI on phytoplankton communities. However, by using the PICT methodology it was found that a single addition of 32 nM DCOI was capable of inducing an increased community tolerance for a period of at least 15 days.

In the present study, the measurements of the actual DCOI concentrations in the microcosms in which PICT was detected indicated that DCOI was degraded during the course of the experiment, and the actual DCOI concentrations in these microcosms were 1 nM after 12 and 15 days, respectively. However, despite the degradation of DCOI in these microcosms, PICT was still significant 15 days after a single addition of DCOI. The NOEC (No Observed Effect Concentration) was determined to be 10 nM from the PICT experiment. Thus, the actual DCOI concentration of 1 nM, was a factor of ten lower than the NOEC, and no effect of DCOI was, therefore, expected. The reason for the persistence in tolerance is probably due to the changes in community structure (changes in taxonomic composition of the phytoplankton communities) observed in the microcosms exposed to 32 and 100 nM DCOI.

Alternatively continued tolerance could be caused by metabolites of DCOI remaining in the microcosms. In that case, the PICT methodology had been able to detect co-tolerance. According to Blanck et al. (1988) co-tolerance can occur for compounds closely related to the tested toxicant, either chemically in structure and properties, or in their mode of action. Molander et al. (1992), however, detected co-tolerance between tributyltin (TBT) and diuron, which differ in chemical structure, chemical properties and mode of action. From the information of the metabolites of DCOI (Björk and Karlsson, 1992; Jacobson et al., 1993) it can not conclusively be established, whether co-tolerance from the metabolites of DCOI can take place.

Diversity decreased markedly at 100 nM due to a reduction in the number of orders of phytoplankton in the community. The high taxonomic richness observed in the phytoplankton community exposed to 32 nM DCOI can be explained as a moderately disturbed situation giving opportunities for more species. There seems to be a relationship between species richness and community tolerance

e.g. the lowest concentration giving a detectable tolerance at 32 nM, also had the highest taxonomic richness. A further increase in DCOI exposure concentration (100 nM) showed PICT, but was accompanied by a decrease in taxonomic richness. The above phenomena were also detected by Molander and Blanck (1992) in periphyton communities exposed to diuron.

5. Conclusion

The changes in taxonomic composition in the DCOI exposed microcosms and the PICT results are very consistent. From these results it is proposed that DCOI is able to exert a long-term effect on natural marine phytoplankton communities. Such effects could lead to more homogeneous phytoplankton communities which could have important knock-on effects in aquatic ecosystems. Therefore, continued use of DCOI in the future should be considered carefully, and investigations for other antifouling methods should continue.

Acknowledgments — We thank DHI-Institut for Water and Environment, The International Agency for ^{14}C determination for providing working facilities and technical assisstance. This work was supported mainly by funds through ACE (Assessment of antifouling agents in coastal environments) in an EU-project (MAS3-CT98-0178 ACE).

References

Björk and Karlsson, 1992 • M. Björk and B. Karlsson, *Ecotoxicological evaluation of 4,5-dichloro-2-n-octyl-4-isothiazoline-3-one*, National chemical inspectorate, Sweden (1992).

Blanck et al., 1988 • H. Blanck, S. Wängberg, S. Molander, 1988. Pollution-Induced Community Tolerance–A new ecotoxicological tool. In: J. Cairns & J. R. Pratt, eds., *Functional testing of aquatic biota for estimating hazard of chemicals*. ASTM STP 988, Philadelphia, pp. 219-230.

Chorus and Bartam, 1999 • I. Chorus and J. Bartam *Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management*, E & FN Spon, London (1999), pp. 352–361.

Dahl and Blanck, 1996 • B. Dahl and H. Blanck, Pollution-Induced community tolerance (PICT) in periphyton communities established under tri-n-butyltin (TBT) stress in marine microcosms. *Aquatic Toxicology*, **34** (1996), pp. 305–325.

de Nys et al., 1996 • R. de Nys, T. Leya, R. Maximilien, A. Afsar, P.S.R. Nair and P.D. Steinberg, The need for standardised broad scale bioassay testing: A case study using the red alga *Laurencia rigida*. *Biofouling*, **10:**1–3 (1996), pp. 213–224.

Fowler and Cohen, 1997 • J. Fowler and L. Cohen *Practical statistics for field biology*, Wiley & Sons, England (1997), pp. 83–85.

Gustavson and Wängberg, 1995 • K. Gustavson and S. Wängberg, Tolerance induction and succession in microalgae communities exposed to copper and atrazine. *Aquatic Toxicology*, **32** (1995), pp. 283–302.

Gustavson et al., 1999 • K. Gustavson, S. Petersen, B. Pedersen, F. Stuer-Lauridsen, S. Pedersen and S. Wängberg, Pollution-Induced community tolerance (PICT) in coastal phytoplankton communities exposure to copper. *Hydrobiologia*, **416** (1999), pp. 125–138.

Jacobson et al., 1993 • A. Jacobson, L. S. Mazza, L. J. Lawrence, B. Lawrence, S. Jackson, A. Kesterson, 1993. Fate of an Antifoulant in an Aquatic Environment. Rohm and Haas Company. ACD Symposium Series No. 522, Chapter 12. Pesticides in Urban Environments: Fate and Significance. American Chemical Society.

Miller and Lovegrove, 1980 • G. A. Miller and T. Lovegrove, 3(2H)Isothiazolone, a new class of antifouling toxicant. *Journal of Coating Technology*, **52:**661 (1980), pp. 69–72.

Molander and Blanck, 1992 • S. Molander and H. Blanck, Detection of pollution-induced community tolerance (PICT) in marine periphyton communities established under diuron exposure. *Aquatic Toxicology*, **22** (1992), pp. 129–144.

Molander et al., 1992 • S. Molander, B. Dahl, H. Blanck, J. Jonsson and M. Sjöström, Combined effects of tri-n-butyl tin (TBT) and diuron on marine periphyton communities detected as pollution-induced community tolerance. *Archives of Environmental Contamination and Toxicology*, **22** (1992), pp. 419–427.

Petersen and Gustavson, 1997 • S. Petersen and K. Gustavson, Toxic effects of tri-butyl-tin (TBT) on autotrophic pico-, nano-, and microplankton assessed by size fractional pollution-induced community tolerance (SF-PICT) concept. *Aquatic Toxicology*, **00** (1997), pp. 1–12.

Riemann and Ernst, 1982 • B. Riemann and D. Ernst, Extraction of chlorophyll a and b from phytoplankton using standard extraction techniques. *Freshwater Biology*, **12** (1982), pp. 217–223.

Shade et al., 1993 • W. D. Shade, S.S. Hurt, A.H. Jacobson and K. H. Reinert, Ecological risk assessment of a novel marine antifoulant. *Environmental Toxicology and Risk Assessment*, **2** (1993), pp. 381–408.

Utermöhl, 1958 • H. Utermöhl, Zur vervollkommnung der quantitativen phytoplanktonmethodik. *Mitteilungen International Vereiningung Limnology*, **9** (1958), pp. 1–38.

Vasishtha et al., 1995 • N. Vasishtha, D. Sundberg and D. Rittshof, Evaluation of release rates and control of biofouling using monolithic coatings containing an isothiazolone. *Biofouling*, **9:**1 (1995), pp. 1–16.

Willemsen et al., 1998 • P. R. Willemsen, K. Overbeke and A. Suurmond, Repetitive testing of TBTO, Sea-Nine 211 and farnesol using *Balanus amphitrite* (Darwin) cypris larvae: variability in larval sensitivity. *Biofouling*, **12** 1-3 (1998), pp. 133–147.