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Experimental warming and toxicant exposure can result in antagonistic effects in a shallow-water sediment system

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ABSTRACT: It has been suggested that future warming will exacerbate the effect of local stressors such as toxicants. In this study the individual and combined effects of warming (+4°C above ambient) and a toxicant (the antifouling substance copper pyrithione) on natural intact shallow-water sediment were studied in an outdoor flow-through facility. Functional (oxygen and inorganic nutrient fluxes in light and dark, bacterial production) and structural (biomass and composition of microphytobenthos and meiofauna) variables were measured. Warming was found to modify the toxicant response antagonistically, i.e. warming removed the negative effect of the toxicant exposure. This antagonism was found for functions depending on light (gross primary production, 24 h net oxygen fluxes, oxygen and silica fluxes). Most functional variables were, however, affected by warming alone, while structural variables were affected by the toxicant alone. At the end of the experiment, the system had 2 types of microalgal communities, a typical benthic algal mat and a floating periphytic mat. Both the benthic and floating microalgal mats were significantly affected by the toxicant alone, but in opposite directions. The biomass of the benthic algal mat was significantly higher under toxicant exposure, whereas the biomass of the floating periphytic mat was lower. Our results suggest that the effects of toxicants in aquatic environments may be reduced (rather than amplified) by warming. We also show that autotrophic communities can respond differently within the same ecosystem and that habitat may determine the mode of response to warming–toxicant exposures in aquatic environments.

KEY WORDS: Multiple stressors · Global warming · Toxins · Sediment · Microphytobenthos

INTRODUCTION

Coastal shallow-water ecosystems are continuously exposed to a range of anthropogenic stressors, including increased nutrient loading, physical disturbance and toxicants (Halpern et al. 2008, Defeo et al. 2009). Superimposed on these local stressors are stressors related to climate change, such as increased temperature, increased CO_2, freshwater discharge and storm frequency (IPCC 2007). We know very little about the mode of interaction between local and global stressors, particularly when it comes to shallow-water sediment systems (Crain et al. 2008, Halpern et al. 2008). Interacting stressors may strengthen (synergism) or weaken (antagonism) effects of individual stressors, resulting in often unexpected non-additive effects (Folt et al. 1999). Synergistic interactions between multiple stressors are often anticipated (Crain et al. 2008). For example, it has been suggested that future warming will exacerbate the effect of local stressors such as toxicants (Noyes et al. 2009, Lovett 2010) and, particularly in the marine environment, multiple stressors tend to interact synergistically (Crain et al. 2008).

Temperature–toxicant interactions are complicated, involving both physical and physiological mechanisms that influence bioavailability, toxicant kinetics and sensitivity of the organisms (Heugens et al. 2001). Until recently, most experiments applied a classical toxicological approach, with individual spe-

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cies being tested under controlled laboratory conditions (Crain et al. 2008, Holmstrup et al. 2010). Such experiments are crucial for establishing limits of tolerance for individual species and are therefore useful tools in risk assessment. The response to toxicants in natural communities, however, may be different (Heugens et al. 2001, Fleeger et al. 2003), and consequently the numbers of toxicological, as well as multiple-stressor, studies under ecologically relevant conditions have increased during the last decade (e.g. Christensen et al. 2006, Dueri et al. 2009, Fitch & Crowe 2011). Here we present results from a study on the combined effects of warming and a toxicant, using intact illuminated sediments under naturally varying light and temperature.

Shallow coastal areas where light reaches the sea floor not only provide resources and shelter for secondary producers, such as fish, but also function as coastal filters in the transport of nutrients between land and sea (McGlathery et al. 2007). Important supporting ecosystem services include primary production, production of oxygen, and cycling and retention of nutrients (Millennium Ecosystem Assessment 2005). Because of the shallow-water column, these key ecosystem processes are benthic, mainly driven by microscopic organisms in the sediment (microphytobenthos, bacteria and archeons), and modified by the activity of macro- and meiofauna (Dyson et al. 2007, Kristensen et al. 2012, Nascimento et al. 2012). By measuring primary and bacterial production, and sediment–water fluxes of oxygen and nutrients in natural undisturbed sediment, we can determine the integrated net response of the sediment community to multiple stressors. Moreover, provided that the experiment runs for long enough, we can also include indirect, food-web-related effects (Fleeger et al. 2003, Chapman 2004, Alsterberg et al. 2007).

Different trophic levels have been found to vary in sensitivity and mode of response to multiple stressors (Christensen et al. 2006, Crain et al. 2008). Previous experiments exposing intact sediment to single and combined stressors suggested that heterotrophic compartments, including benthic fluxes in the dark, were more sensitive than the autotrophic compartment (Underwood & Paterson 1993, Sundbäck et al. 2007, Alsterberg et al. 2011). Benthic diatoms in particular appear to be resistant to multiple stressors, contributing to the resilience of the benthic community as a whole (Hicks et al. 2011, Alsterberg et al. 2012). Previous experiments studying the recovery of a sediment system after toxicant exposure (copper pyrithione) showed that microphytobenthos quickly recovered, contributing to the resilience of the entire sediment system (Alsterberg et al. 2007, Larson et al. 2007).

The aim of this study was to investigate whether a temperature rise of 4°C above ambient (predicted by IPCC according to the A1FI scenario for the year 2100; IPCC 2007) interacts with a model toxicant, changing the structure and function of an illuminated, non-tidal shallow-water sediment system. Copper pyrithione (CuPT) was chosen as the model toxicant for 2 reasons. Firstly, CuPT is an antifouling substance used in modern boat paints (Maraldo & Dahllöf 2004) and its mode of action is disruption of cell membranes and pH gradients, which further disrupt ATP synthesis and solute transport through membranes (Chandler & Segel 1978, Dinning et al. 1998). Secondly, we have previous experience of the effects of CuPT in shallow-water sediments (Alsterberg et al. 2007, Larson et al. 2007, Sundbäck et al. 2007). We hypothesized that: (1) warming would significantly change the community response to CuPT; (2) the interactive effects would be non-additive, mainly synergistic; and (3) the effects would be stronger in the heterotrophic compartments. Our approach was to expose natural, intact sediment in a flow-through system to a low-level concentration of CuPT and warming (+4°C over ambient) under natural variation of light and temperature.

MATERIALS AND METHODS

Approach and experimental overview

The experiment was run for 1 mo (29 d) in spring (April–May). Intact sediment cores were exposed to flow-through seawater using 4 treatments (n = 5 replicates): ambient temperature with no toxicant (Amb), elevated temperature by +4°C (Warm), ambient temperature with toxicant addition (Tox), and elevated temperature with toxicant (WarmTox). Both functional (oxygen and inorganic nutrient fluxes in light and dark and bacterial production) and structural variables (biomass and composition of benthic microalgae and meiofauna) were measured. Structural variables were measured in the top 0.5 cm sediment and samples were always taken after the flux measurements.

Sediment collection and experimental set-up

Sediment was collected from a boat with an Olausson box-corer (30 × 30 cm) at a depth of 2 m in

Munkeby Bay (58° 14′ N, 11° 32′ E) on the west coast of Sweden (SE Skagerrak). Cylinders of black acrylonitrile butadiene styrene plastics (height = 25 cm, i.d. = 25 cm) were used to sample one core from each box-corer and subsequently used for sediment-water incubations in the experimental flow-through setup. Visible epibenthic macrofauna, such as crabs and mussels, were removed from the cylinders. The depth of the overlying water in the experimental cylinders was ~10 cm, resulting in a water volume of ~5 l. A total of 25 cylinders were used, 5 for initial sampling and 5 replicates for each of the 4 treatments (Amb, Warm, Tox and WarmTox).

The experiment was run in a greenhouse using a flow-through seawater supply directly from an adjacent shallow bay in the Gullmar Fjord. Seawater was continuously pumped into 2 elevated, isolated and dark plastic containers of ~1000 l each (turnover time 0.7 h) placed just outside the greenhouse. After passing a 1 mm mesh cartridge filter the containers supplied a flow of ~20 l h^{-1} (turnover time 4 h) to each experimental cylinder. Incoming seawater was monitored for temperature, salinity, pH, oxygen and flow rate in the 2 elevated tanks and in each experimental cylinder. Manipulations of seawater temperature were performed as described in Alsterberg et al. (2011), using an immersion heater (6 kW, Energi Ekonom i Höör) mounted in one of the 2 elevated tanks and connected to a computerized control unit. Sensors in 2 experimental cylinders (ambient and heated) in the greenhouse measured temperature continuously. Warm seawater was maintained at 4 ± 0.15°C above the temperature of the ambient seawater. The inner walls of the cylinders were cleaned every 2nd day to prevent the growth of a biofilm. The toxicant, CuPT, with 100% dimethyl sulfoxide (DMSO) as solvent (final concentration of 0.0019%), was added to the relevant treatments on Days 1, 8 and 16 of the experiment. To ensure that the toxicant came into rapid contact with the sediment, we used a slurry of dried sediment from the original collection site mixed with the DMSO–toxicant mixture (Sundbäck et al. 2007). During each toxicant addition, water flow was stopped approximately for 3 h. Toxin was added at night to avoid photolysis of CuPT (Petersen et al. 2004). The final concentration was 1 nmol CuPT per gram dry weight sediment, equaling the lowest observed effect concentration found in laboratory microcosms (Petersen et al. 2004). To control for any bias created by toxicant addition, a sediment slurry with only 100% DMSO (i.e. without CuPT) was added to treatments with no toxin.

Sampling and analyses

On the final day of the experiment (Day 29), sediment–water fluxes of oxygen and inorganic nutrients were measured during the day and night. All other samples were taken on the following day. During the experiment, a floating microalgal mat had formed in all but one cylinder (see 'Results: Physical variables and visual observations'). The areal cover of this mat varied and was documented by taking photographs of all cylinders on Days 13 and 29. At the end of the experiment, these floating mats were carefully sampled (before taking the other samples), and their wet and dry weights were determined. Small subsamples of the mat were frozen for subsequent microscopy. After this, the overlying water was carefully removed and samples from the top 5 mm of sediment were taken for assessment of structural variables and bacterial production. Finally all sediment was sieved (1 mm) to check for macroscopic infauna.

Structural variables

Water content (by weight) of the sediment was calculated from the weight loss after drying ca. 5 ml of wet sediment for 24 h at 60°C. Organic matter content was measured as loss on ignition at 550°C.

Chlorophyll *a* (chl *a*) content of the sediment was measured as a proxy for the biomass of microphytobenthos (MPB). Two sediment cores (5 mm deep) were taken from each replicate cylinder using a 5 ml cut-off plastic syringe (area 0.785 cm^2) and immediately frozen (–20°C). Sediment chl *a* was extracted with acetone (final concentration 90%) overnight and measured spectrophotometrically (UV-2401PC, Shimadzu) using the methods and equations of Lorenzen (1967), which correct for degradation products of chl *a* (phaeopigments). These results were further converted to carbon content by applying a C/chl *a* ratio of 30 (de Jonge 1980, Sundbäck & Miles 2000). For sediment microalgal counts and identification, 2 samples from the top 5 mm sediment were taken from each cylinder with a cut-off 2 ml syringe (area 0.594 cm^2) and stored frozen (–20°C). After thawing, each sample was diluted with filtered seawater and shaken by hand for 1 min. Large particles were allowed to sink, and the supernatant (= loose fraction) was decanted into a disposable test tube. The remaining sample was then further diluted and ultrasonicated for 10 min (Branson 2510) while cooling with ice to detach cells from mineral particles. Sand particles were allowed to sink and the supernatant was mixed

with the loose fraction. Live (fluorescing) cells were counted in a Bürker counting chamber using epifluorescense microscopy (500× magnification; Olympus BH-2). Cells were identified to the nearest taxonomic level possible, measured and allocated to size groups.

For meiofaunal biomass, 2 sediment cores (5 mm deep) were taken from each replicate cylinder, using a 5 ml cut-off plastic syringe (area 0.785 cm^2). The samples were pooled and preserved in formalin with Rose Bengal. Before counting, samples were fractionated into size groups by sieving through 500, 200, 100 and 40 µm mesh sieves. Meiofaunal abundance for each sieving step was converted to ash-free dry weight (Widbom 1984) and further converted to carbon using the conversion factor of 0.43 (Båmstedt 1986). No meiofauna samples were taken from the floating algal mat. After finished sampling, all sediment in each cylinder was sieved (1 mm mesh) to collect macrofauna.

Functional variables

Sediment–water fluxes of oxygen and nutrients

During flux incubations, water flow was stopped and the cylinders were sealed airtight with a transparent plastic lid equipped with a Teflon-coated stirring bar (~60 rpm). Fluxes were determined from the concentration change in the overlying water at the start and end of the incubation. Daytime incubations were made between ~11:00 and ~13:00 h and nighttime incubations between ~22:00 and ~04:00 h. Oxygen concentrations were measured using the Winkler technique and nutrient samples (ammonium, nitrate and nitrite, dissolved inorganic phosphorus [DIP], and silica) were measured with an automatic analyzer (Smart Chem, Westco Scientific Instruments) according to standard colorimetric procedures (Strickland & Parsons 1972).

Benthic oxygen fluxes during light and dark were used as measures of net primary production (NPP) and community respiration (CR), respectively. Gross primary production (GPP) was calculated by subtracting CR (a negative number) in the dark from NPP. Since respiration in the light is generally higher than in the dark (Kühl et al. 1996), our GPP values are probably somewhat underestimated (Carvalho & Eyre 2012) but this should not influence our interpretation of the results. GPP was further converted to fixed carbon using a photosynthetic quotient of 1.2 (Kirk 1994, Glud et al. 2002). The 24 h oxygen flux was calculated by adding NPP for the light period and CR during the dark period. Daily values of GPP were calculated by multiplying flux rates per incubation time by a light factor calculated as a ratio between total daily irradiance and irradiance during the incubation period (light data from the Swedish Meteorological and Hydrological Institute).

Bacterial production

Bacterial production (BP) was estimated only for the surface sediment by incubating 0.1 ml sediment with ^{3}H-leucin according to Ask et al. (2009). Using 20 µl radioactive leucin L-[4, 5-^{3}H] (Amersham, TRK 510) and 80 µl non-radioactive leucin (Sigma-Aldrich, L-Leucin, non-animal source), the final leucin concentration was 70 µM with a specific activity of 22.86 µCi mol^{-1}; these values were chosen based on a saturation curve performed prior to the experiment. Two subsamples from each cylinder were pooled and incubated in darkness at *in situ* temperature for 40 min. Controls were treated with 65 µl 100% trichloroacetic acid (TCA, final concentration 5%) immediately before the incubation. Incubations were terminated by adding 65 µl 100% TCA. Samples were then repeatedly centrifuged and washed using TCA and ethanol. Scintillation liquid (Hionic fluor™, Perkin Elmer) was added and samples were analysed in a TRI-CARB 2900TR scintillation counter (Quanta Smart v.1.1 software). Leucin incorporation rates were converted to bacterial protein production (Simon & Azam 1989) and further to BP rates using a conversion factor of 1.44 kg C mol^{-1} (Buesing & Gessner 2006).

Statistical analysis

Data were analyzed using a 2-factor ANOVA with the fixed factors temperature and toxin. Initial samples for bacterial production and sediment chl *a* were not included in the statistical analyses but are reported in the 'Results'. The type I error rate was set to 0.05 and the term 'significant' is used throughout to refer to $p \leq 0.05$. All data were checked for homogeneity of variances and normality using Cochran's test and box and residual plots. Raw data were normally distributed and variances were not significantly heterogeneous. Six outliers were identified and removed from the data set, which did not change the interpretation of the results. Statistical analyses were performed in the R environment (R Development Core Team 2010).

Two-way permutational multivariate ANOVA (PERMANOVA) (Anderson 2001, McArdle & Anderson 2001), with temperature and toxin as fixed factors, was used to analyze the sum of effects on functional variables. We refer to these results as 'integrated community function'. Three data sets were run. Two data sets (light and dark) included 6 functional variables: hourly fluxes of oxygen, and inorganic nutrients (NH_4^+, NO_3^- + NO_2^-, DIP, $Si(OH)_4$) and bacterial production. The third data set analyzed temperature and toxin effects on the composition of benthic microalgae (relative abundance based on cell counts). The raw data were not transformed but were standardized through normalization. Principal coordinate analysis (PCOA) was carried out and the results were plotted. The analyses were run using the programs PERMANOVA v.1.6 and PCO3 (Anderson 2001).

RESULTS

Overview of results

Among the 23 variables assessed (20 individual, 3 integrated), a significant warming–toxicant interaction was observed for 4 individual variables and 1 integrated variable: daily GPP, 24 h net oxygen flux, oxygen and silica fluxes in light, and integrated community function in light (Table 1). The mode of interaction was interpreted as antagonistic because the effects of warming completely removed the negative

Table 1. Effects of warming and CuPT on measured variables tested by 2-way ANOVA and permutational multivariate ANOVA (PERMANOVA). Functional variables are divided into daily rates and light and dark fluxes. Functions in light include light fluxes and bacterial production. Functions in the dark include dark fluxes and bacterial production. Only significant effects ($p < 0.05$) of warming, toxin or their interaction are shown. Positive (+) or negative (–) effects of treatments or mode of interaction (antagonistic) are also shown

Variable	Significant factor	df	MS	*F*	p	Effect direction/ mode of interaction
2-way ANOVA						
State variables						
Chlorophyll *a*	Toxin	1	1557.6	5.5	0.036	+
Phaeopigments						
Floating algal mat biomass	Toxin	1	50.3	5.8	0.029	–
Meiofaunal biomass						
Polychaete abundance	Toxin	1	653.6	4.8	0.037	–
Nematode abundance						
Harpacticoid copepods						
Macrofauna abundance						
Functional variables						
Daily rates						
24-h net oxygen flux	Warming × Toxin	1	5004.5	5.9	0.027	Antagonistic
Gross primary production	Warming × Toxin	1	22042	15.2	0.002	Antagonistic
Bacterial production	Warming	1	5744.3	5.4	0.036	–
Fluxes in light						
O_2	Warming × Toxin	1	85.1	3.9	0.002	Antagonistic
NH_4^+	Warming	1	1441.9	5.8	0.033	+
NO_3^-+ NO_2^-	Warming	1	19367	16.5	0.001	+
DIP	Toxin					–
$Si(OH)_4$	Warming × Toxin	1	7201.9	11.7	0.005	Antagonistic
Fluxes in dark						
O_2	Warming	1	48.7	5.5	0.037	+
NH_4^+						
NO_3^-+ NO_2^-	Warming	1	181.5	11.4	0.005	+
DIP						
$Si(OH)_4$	Toxin					–
PERMANOVA						
Benthic microalgal composition						
Functions in light	Warming × Toxin	1	18.9	5.7	0.001	
Functions in dark	Warming	1	17.5	3.7	0.012	
	Toxin	1	18.2	3.8	0.011	

effects of the toxicant. No synergism was observed. Most of the significantly ($p < 0.05$) affected variables were influenced either by warming or by the toxicant, with no significant interaction between the 2 factors. Warming alone significantly affected the functional variables BP, CR, ammonium flux (light) and nitrate flux (light and dark) (Table 1). A significant toxicant effect was found for the structural variables chl *a*, biomass of the floating algal mat and abundance of polychaete larvae (Table 1). While the integrated community function showed an interactive effect in light, in the dark it was affected independently by warming and toxicant. Eight variables were not affected at all by the treatments (Table 1).

Physical variables and visual observations

The sediment was silty, with initial water content of 77% (w/w) and an organic matter mean content (loss on ignition) of 13.7%. At the start of the experiment, the ambient water temperature at noon was 9.6°C, and varied between 9 and 16°C over the course of the experiment (Fig. 1). In the heated treatment, temperature varied between 11 and 19.5°C. There were 2 temporal minima in the heated treatment (Fig. 1) caused by a failure of the water supply. These failures lasted only a few hours and should not have affected the overall result from the 29 d experiment. A first sign of sediment surface flaking due to oxygen bubbles entrapped in the microphytobenthos mat was observed after ca. 1 wk. However, floating algal mats were observed only after Day 16 of the experiment. The mats were 1 to 10 mm thick and seldom covered the entire sediment surface area. Thus, at the end of the experiment, 2 types of microalgal mats existed, a cohesive benthic mat, and a floating mat, whose species compositions differed (see below).

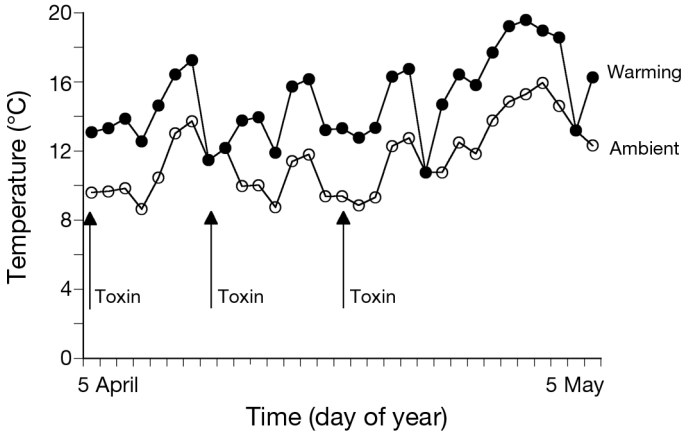

Fig. 1. Temperature of ambient (○) and warmed seawater (+4°C above ambient; ●) measured at 12:00 h each day during the experimental period (29 d). Arrows indicate addition of toxicant (1 nmol CuPT per gram dry wt sediment)

Algae

Both the benthic (MPB) and floating microalgal mats were significantly affected by the toxicant alone (Table 1), but in opposite ways. MPB biomass (based on chl *a*) was significantly higher under toxicant exposure, whereas the biomass of the floating mat was lower (Table 1, Fig. 2). In the ambient treatment, the chl *a* content was 77 mg chl *a* m^{-2} and in the initial samples the chl *a* content was 101 mg chl *a* m^{-2} ($p = 0.42$). Since the biomass of the floating mat (6 to 19 g C m^{-2}) dominated total algal biomass, the overall effect of CuPT on algal biomass was negative (Fig. 2).

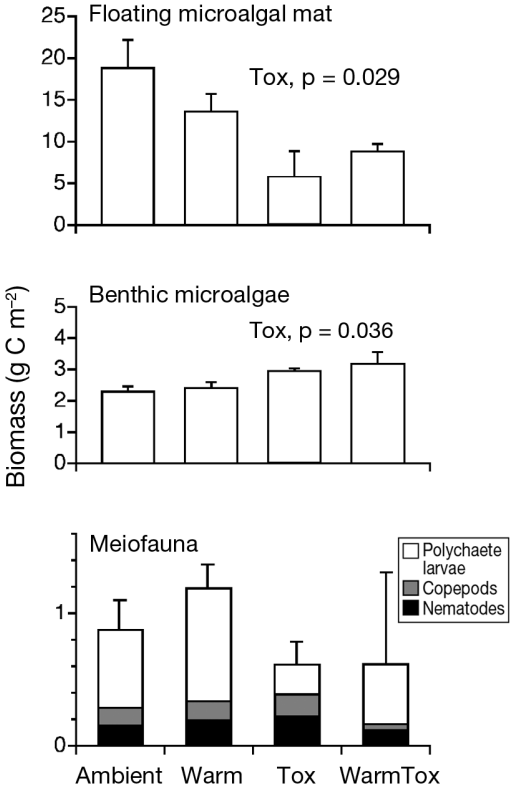

Fig. 2. Biomass of floating microalgal mats, benthic microalgae and meiofauna. Data are means + SE (n = 5) for floating and benthic microalgae as well as for each meiofaunal major taxa in ambient temperature with no toxicant (Amb), raised temperature (+4°C) (Warm), ambient temperature with toxicant addition (Tox), and raised temperature with toxicant (WarmTox). Tox denotes a significant toxin effect ($p < 0.05$, 2-way ANOVA)

Algal species that dominated in the floating mat were colony-forming diatoms, especially chains of *Fragilaria* sp. and tube-forming *Berkeley* sp., but also *Licmophora* sp. and *Melosira* sp. Thus, in composition the mat resembled a 'periphytic'—though floating—community. Visually, *Fragilaria* sp. appeared to dominate at ambient temperature, while the diversity seemed higher in the 2 heated treatments, where filaments of macroalgae and cyanobacteria were also present. Benthic mats were dominated by typical solitary, mainly motile, benthic diatoms belonging to the genera *Navicula* (sensu lato), *Nitzschia*, *Cylindrotheca* and *Amphora*. PERMANOVA, based on 57 taxonomical units, did not identify any compositional effects of the experimental treatments on the benthic mat (Table 1).

Fauna

Neither the total biomass of meiofauna nor the biomass of individual major taxa responded significantly to the treatments (Table 1, Fig. 2). Total meiofaunal abundance was also unaffected by the treatments, and there was no difference between the relative abundance of size groups of nematodes, copepods and polychaete larvae (Table 1). The only significant effect on meiofauna was that the CuPT addition resulted in a lower abundance of polychaete larvae (Table 1). Macrofauna consisted mainly of the polychaete *Hediste* sp. and the amphipod *Corophium volutator*. The total density of macrofauna was in the range of 100 to 200 ind. m^{-2}. No significant pattern for any of the treatments was observed (Table 1).

Primary and bacterial production

One of the few significant Warm × Tox interactions was found for primary production. CuPT decreased both GPP and NPP at ambient temperature by a factor of 8, but in heated aquaria this negative effect was totally absent (Table 1, Figs. 3, 4). The toxicant effect on GPP was further strengthened by the fact that community respiration was lower (though not significantly) in the Tox treatment (Fig. 4). Bacterial production, which remained similar to the initial samples (180 and 170 mg C m^{-2} d^{-1}, respectively, p = 0.59), was almost one order of magnitude lower than GPP except in the Tox treatment. However, in contrast to GPP, warming decreased BP (Table 1, Fig. 3), with no significant treatment interaction.

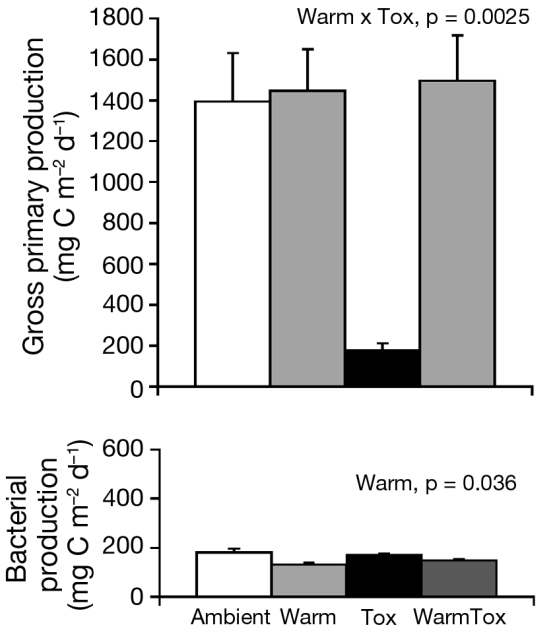

Fig. 3. Daily gross primary production (estimated from sediment–water oxygen fluxes) and sediment bacterial production. For treatments see Fig. 2 legend. Data are means + SE (n = 5). Warm denotes a significant warming effect and Warm × Tox a significant interaction between warming and toxicant ($p < 0.05$, 2-way ANOVA)

Oxygen and nutrient fluxes

In light, CuPT turned the system slightly heterotrophic (Fig. 4), but warming removed the Tox effect (Fig. 4). At first sight, the pattern of oxygen consumption in the dark appeared similar to that in the light, but CuPT did not reduce CR significantly, and only the increasing effect of warming remained in the dark (Table 1, Fig. 4). After 24 h, Tox replicates were significantly net heterotrophic, but when warming was combined with the toxicant (WarmTox) the replicates were significantly net autotrophic (Table 1).

Nutrients were consumed both in the light and in the dark (Fig. 4). Daytime nitrogen uptake was much higher than during the night, reflecting an active algal community (Fig. 4). P and Si fluxes did not differ between day and night (Fig. 4). At first sight, the pattern for NH_4^+ flux in light resembled that of O_2 production, but NH_4^+ was significantly affected only by warming (Table 1, Fig. 4). For NO_3^-, warming stimulated sediment uptake in the light and this effect was also found in the dark. DIP fluxes were low, and Tox was the negative factor that was closest to being significant (p = 0.1 and 0.09; Table 1). In contrast, uptake of silica was significantly stimulated by the Warm × Tox interaction (Table 1).

When combining 6 functional variables (all fluxes and BP), PERMANOVA separated all 4 treatments from each other in the light. In the PCOA plot, the separation was most clearly seen for the Amb and Tox treatments, which were separated both from each other and from the Warm and WarmTox treatments (Table 1, Fig. 5). Warm and WarmTox treatments in the light overlapped in the PCOA, supporting results showing an antagonistic interaction. In the dark, there was no significant Warm × Tox interaction (Table 1), and generally, there was more overlap between treatments in the dark, where Amb was separated from WarmTox and Warm from Tox (Fig. 5).

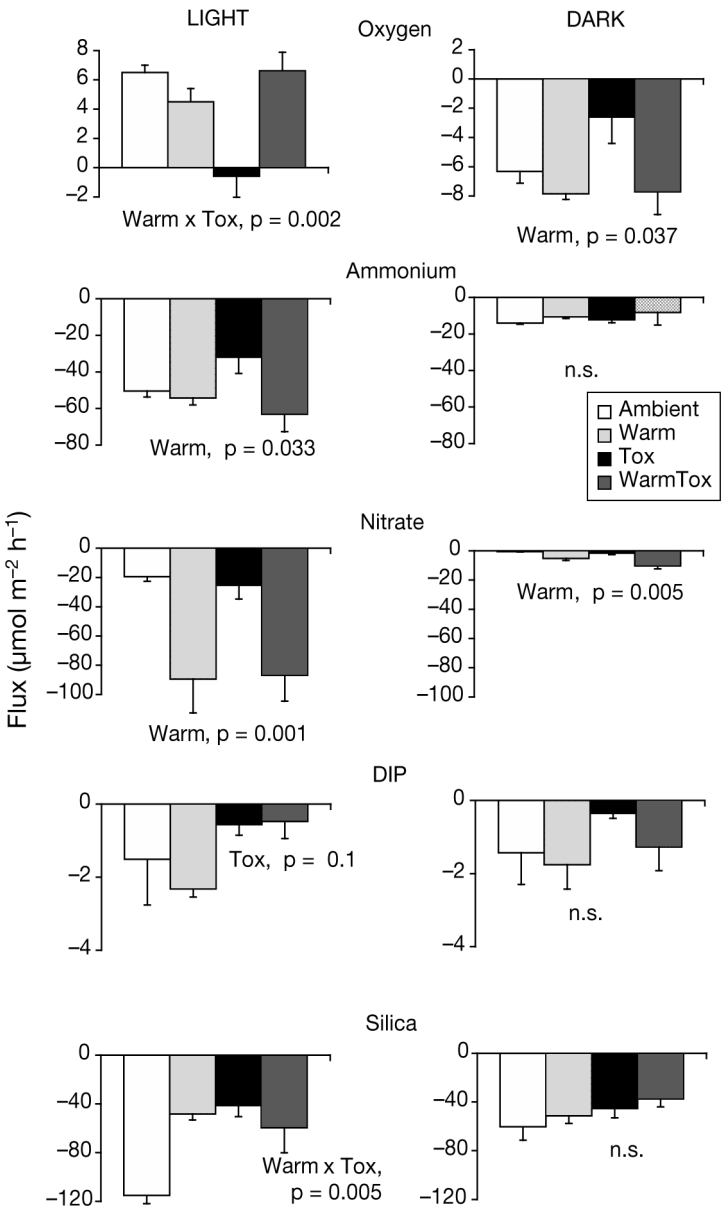

Fig. 4. Hourly sediment–water fluxes for oxygen, ammonium, nitrate + nitrite (Nitrate), dissolved inorganic phosphorus (DIP) and silica in light and dark. For treatments see Fig. 2 legend. Data are means + SE (n = 5). Warm × Tox and Warm denote significant interaction and single effects at $p < 0.05$; n.s. indicates a nonsignificant treatment effect (2-way ANOVA)

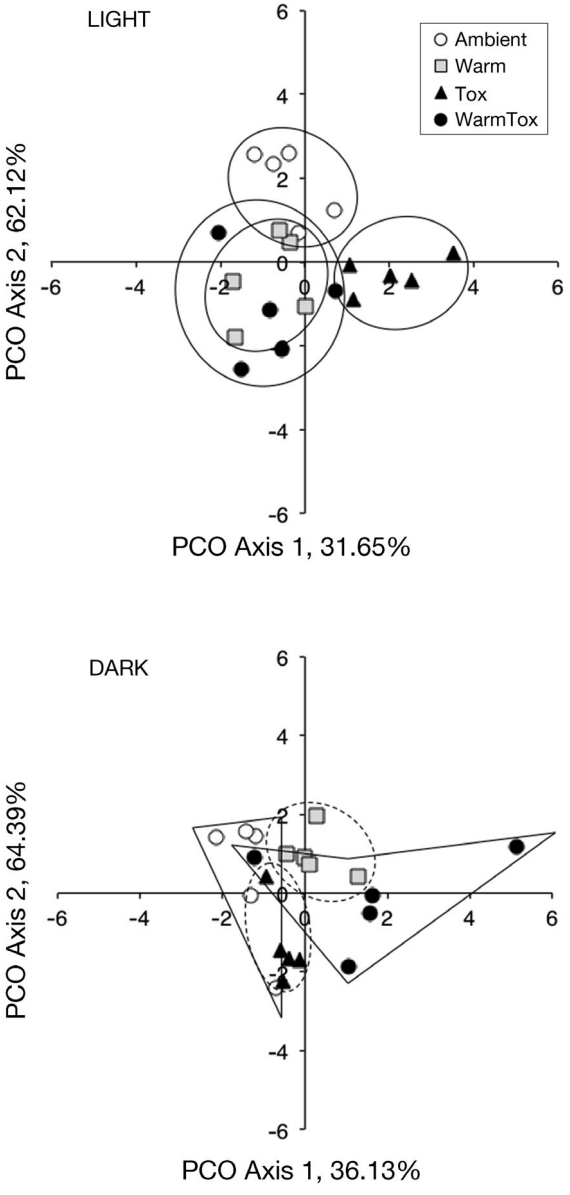

Fig. 5. Integrated system functioning analyzed by permutational multivariate ANOVA (PERMANOVA) including 6 variables (hourly fluxes of oxygen, and inorganic nutrients [NH_4^+, $NO_3^- + NO_2^-$, DIP, $Si(OH)_4$] and bacterial production) depicted by a principal coordinate analysis (PCOA) plot. Clusters are circled when treatments are significantly separated from each other. In the dark PCO, clusters with dashed lines differ significantly from each other but not from the clusters surrounded by solid lines and vice versa. Values for the 2 axes give the percent of total variation explained by the 2 coordinates. See Fig. 2 legend for treatments

DISCUSSION

Warming influenced the toxicant response, but not always

We hypothesized that warming would change the response of the community to the toxicant exposure. Significant Warm × Tox interactions were only found for 5 variables out of 23 considered, all 5 of which were for variables that depended on light (Table 1). The majority of variables were affected either by warming or by toxicant, and some variables were not significantly affected at all. Thus, our first hypothesis about interactive effects was only partly supported. The observed variation in response is not unexpected: indigenous organisms of a natural system differ in their sensitivity and resilience, depending on trophic level (e.g. Christensen et al. 2006, Crain et al. 2008). Moreover, in natural multitrophic communities, direct (acute) toxicant effects can later be replaced by indirect, food-web-mediated effects, often resulting in changed direction of effects (e.g. Fleeger et al. 2003, Larson et al. 2007, Alsterberg et al. 2007). As our measurements were made at the end of a 4-wk experiment — and 2 wk after the last toxicant addition — we cannot evaluate direct initial effects. Also, when interpreting our results, it must be kept in mind that at the time of measurements (end of experiment), the studied community was no longer a pure benthic community since a floating algal mat had developed. This algal mat dominated in terms of biomass, and can also be expected to have influenced the functional variables (sediment–water fluxes). Since this flaking phenomenon is also common in non-tidal shallow bays (Alsterberg et al. 2011), its presence is not trivial (Admiraal 1984, Sutherland et al. 1998, Gerbersdorf et al. 2004).

Interactive effects were antagonistic

No synergistic warming–toxicant effects were observed, leading to the rejection of our second hypothesis. All interactive effects were antagonistic in the sense that the negative effects of toxicant were absent in the heated treatment (Figs. 3 & 4). Crain et al. (2008) found that antagonism was the most common type of temperature–toxicant interaction. There are several possible explanations for this antagonism. The mode of interaction (additive, synergistic or antagonistic) may depend on (1) how temperature affects the toxicant itself and its bioavailability, (2) changes in chemical interactions (Noyes et al. 2009), (3) the physiology and trophic level of the organisms (Christensen et al. 2006, Crain et al. 2008, O'Connor 2009) and (4) the experimental approach. Mesocosm experiments tend to result mainly in antagonistic effects while laboratory experiments tend to show synergism (Crain et al. 2008). A plausible explanation is that the more complex the experimental community is, the more indirect effects there will be, hiding or mitigating direct stressor effects (Fleeger et al. 2003, Crain et al. 2008). This especially applies to our experiment, which, in addition to being a mesocosm experiment, also used intact sediment cores with all their natural heterogeneity and complexity.

We can only speculate about the mechanisms behind the antagonism observed in our experiment. One explanation could be an increased turnover due to warming, leading to a dilution effect and a lower bioavailability of the toxicant to individual cells (Heugens et al. 2001). Increased turnover was in our experiment evidenced partly by increased oxygen consumption with warming, i.e. increased nitrogen mineralization (cf. Fitch & Crowe 2011). The availability of nitrogen through increased nitrogen mineralization may have resulted in higher algal and bacterial nitrogen uptake, which probably prevented an increased efflux of nitrogen. The implication of the increased turnover could be a faster recovery after acute CuPT effects at higher temperature. A previous laboratory experiment showed that although low-level CuPT exposure initially affected photosystem II of benthic microalgae, recovery occurred within days (Alsterberg et al. 2007). This recovery may even be faster with warming.

Although statistically significant antagonistic interactions were seen in only 4 individual variables, an antagonistic tendency appeared also for other variables (community respiration, ammonium flux in light; Fig. 4). However, because of high variation among replicates, only warming emerged as a significant factor (Table 1). Still, this tendency gives further support to the findings that warming counteracted the negative toxicant effects.

Both autotrophic and heterotrophic components were affected — though differently

In previous experiments on intact sediments, heterotrophic compartments were more frequently and strongly influenced by stressors (including warming) than autotrophic compartments (Larson et al. 2007, Sundbäck et al. 2010, Alsterberg et al. 2011).

Since temperature dependence of photosynthesis is weaker than that of respiration (e.g. Yvon-Durocher et al. 2010), it would have been natural to find more temperature effects for 'heterotrophic' variables. This was partly the case. Warming significantly increased oxygen consumption and decreased bacterial production (Fig. 1, Table 1). In addition, nitrogen flux (controlled both by autotrophs and heterotrophs) was influenced only by warming. Given that temperature, together with organic matter content, is one of the main controlling factors of BP (Sander & Kalff 1993), it was surprising that we did not find any positive effects of warming on BP. We have presently no good explanation for this negative effect of warming, but it may have been caused by a temperature-induced indirect effect mediated by food-web interactions (Kordas et al. 2011). Thus, the potential stimulatory effect of warming on BP could have been counteracted by a strengthened grazing pressure by meiofauna (Delaney 2003, Nydahl et al. 2013).

Neither the total biomass of meiofauna nor the biomass of the major taxa was affected by warming (Table 1). The biomass of meiofauna, especially of polychaete larvae, has been shown to respond positively to increased temperature (Coull 1999, Alsterberg et al. 2011). However, polychaete larvae are temporary meiofauna and the effects of increased temperature on this group could have been missed because many larvae that were present initially in the sediment had grown to be larger than meiofauna by the end of the experiment. The negative effects of CuPT on the abundance of polychaete larvae were also surprising since most of the previously observed toxicant effects were on harpacticoid copepods (Alsterberg et al. 2007, Larson et al. 2007), which were not affected in the present experiment (Table 1). Previous studies mainly observed sublethal effects of CuPT on meiofaunal species, such as reduced grazing pressure on microphytobenthos (Alsterberg et al. 2007). Therefore, the negative effects on polychaete larvae could have been an indirect, sublethal effect of the toxicant that with time reduced the total abundance of polychaete larvae.

Light-dependent functions (GPP, 24 h net oxygen flux, oxygen production, silica uptake and integrated light function) and algal biomass were affected either by the temperature–toxicant interaction (Table 1) or the toxicant alone. Note, however, that the positive effect on benthic chl *a* was probably food-web mediated through negative sublethal effects by CuPT on meiofaunal grazing pressure, resulting in stimulation of microphytobenthic growth (Alsterberg et al. 2007, Sundbäck et al. 2010). The results suggest that autotrophs were mainly affected by the toxicant and heterotrophs by warming. Also, toxicant alone affected mainly state variables, while process rates were affected by temperature alone or by the combination of temperature and toxicant. This is perhaps logical, since the 2 factors affect different processes in auto- and heterotrophic organisms: CuPT affects membrane permeability (Al-Adham et al. 1998, Dinning et al. 1998) while temperature affects metabolic processes (Yvon-Durocher et al. 2010).

The strong negative response of primary production to CuPT at ambient temperature is in stark contrast to our previous results using naturally occurring low-level concentrations of toxicants (Larson et al. 2007, Alsterberg et al. 2007, Sundbäck et al. 2010). Also, the biomass of the floating mat was lower with toxicant addition, but the difference was not as dramatic as for primary production (Fig. 2). The probable reason for the effect of toxicant on primary production observed here was that the floating periphyhtic community — which dominated algal biomass (Fig. 2) — was more sensitive to the toxicant than MPB. Alternatively, if availability of the toxicant differed, the sediment might have been a more 'protected' habitat than the water column. Comparisons of the sensitivity of 3 different types of microalgal communities to herbicides and a new antifouling substance showed that epipsammic (attached to sand grains) communities were less sensitive than periphyton, with phytoplankton being most sensitive (Bonilla et al. 1998, Ohlauson et al. 2012). The floating mat can be regarded as detached periphyton living in a planktonic environment. Interestingly, raised temperature reduced the effects of toxicant on primary production. Although higher temperature may increase the degradation of some toxicants, total degradation of CuPT by a temperature rise of only 4°C is unlikely because of the thermal stability of the pyrithione complex (which has a decomposition temperature above 240°C; Wang et al. 2010). A more likely explanation is that warming accelerated pollution-induced community tolerance of the microphytobenthos by selecting for CuPT-tolerant species (Admiraal et al. 1999, Blanck 2002). We did not carefully analyze the species composition of the floating mat, but there appeared to be some observable differences in species composition.

Since we did not measure primary production of MPB and the floating algal mats separately (since the measurements were based on oxygen evolution), we do not know whether MPB production was affected by the toxicant. Judging from the increased biomass of MPB (interpreted as an indirect effect) and on re-

sults from previous experiments using CuPT (Sundbäck et al. 2007, Alsterberg et al. 2007), we conclude that CuPT did not negatively affect MPB, and that MPB composition was not affected by the toxicant or temperature. A temperature rise of only 4°C in a habitat that normally experiences much higher short-term fluctuations cannot be expected to induce any measurable effects within the time frame of the experiment. Testing 25 diatom species isolated from tidal flats, Scholz & Liebezeit (2012) showed that growth rates were similar within the range 10–30°C. Again, it was shown that the MPB community of shallow-water sediments is robust, being relatively insensitive to environmental variations compared with microalgal communities in the water column.

CONCLUSIONS

Community and ecosystem responses to multiple stressors have been referred to as unexpected ecological surprises (Christensen et al. 2006). The results from our experiment are not an exception to this statement. The antagonistic, rather than synergistic, interaction and the fact that the 2 algal communities showed different responses to warming and toxicant exposure are interesting. The effects of toxicants on aquatic organisms under a warmer climate will not necessarily be amplified, but rather the opposite. These results also suggest that photoautotrophic communities can respond differently within the same ecosystem, and that the habitat of seemingly similar communities can determine the mode of response during warming–toxicant exposure.

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