**Selective and context-dependent effects of chemical stress across trophic levels at the basis of marine food webs**

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## Abstract

Human activities increasingly impact the functioning of marine food webs, but anthropogenic stressors are seldom included into ecological study designs. Diet quality, as distinct from just diet quantity, has moreover rarely been highlighted in food web studies in a stress context.

We measured the effects of metal and pesticide stress (copper and atrazine) on the contribution of a benthic intertidal diatom community to two processes that are key to the functioning of intertidal systems: biomass (diet quantity) and lipid (diet quality) production. We then examined if stressors affected diatom functioning by selectively targeting the species contributing most to functioning (selective stress effects) or by changing the species’ functional contribution (context-dependent effects). Finally, we tested if stress-induced changes in diet quality altered the energy flow to the diatoms’ main grazers (harpacticoid copepods).

Diatom diet quantity was reduced by metal stress but not by low pesticide levels due to the presence of an atrazine-tolerant, mixotrophic species. Selective effects of the pesticide reduced diatom diet quality by 60% and 75% at low and high pesticide levels respectively, by shifting diatom community structure from dominance by lipid-rich species towards dominance by an atrazine-tolerant, but lipid-poor species. Context-dependent effects did not affect individual diatom lipid content at low levels of both stressors, but caused diatoms to lose 40% of their lipids at high copper stress.

Stress-induced changes in diet quality predicted the energy flow from the diatoms to their copepod consumers, which lost half of their lipids when feeding on diatoms grown under low and high pesticide and high metal stress. Selective pesticide effects were a more important threat for trophic energy transfer than context-dependent effects of both stressors, with shifts in diatom community structure affecting the energy flow to their copepod grazers at stress levels where no changes in diatom lipid content were detected.

**Key words:** chemical stress, marine, diatoms, copepods, energy flow, fatty acids, atrazine, copper

## Introduction

The impact of human activities on biological communities and their contribution to ecosystem functioning has become a central topic in ecological research (Halpern et al. 2008, Cardinale et al. 2012, Gamfeldt et al. 2015). Although conservation research is framed within the context of anthropogenic change, exposure to anthropogenic stressors is however rarely included in the design of studies focussing on biodiversity effects on ecosystem functioning (McMahon et al. 2012, De Laender et al. 2016).

Stress can affect ecosystem functioning by causing biodiversity loss in terms of species richness, as well as through changes in community structure, without necessarily causing species to go extinct (Hillebrand et al. 2008, Wittebolle et al. 2009, Mensens et al. 2015, De Laender et al. 2016). Selective stress effects on community structure (hereafter ‘selective stress effects‘, Wittebolle et al. 2009) can influence ecosystem functioning if stressed communities are dominated by tolerant species with a low functional contribution (Larsen et al. 2005, Mensens et al. 2015). If the functionally most important species are also the most stress-tolerant, loss of functioning under stress will be limited (Radchuk et al. 2016). In addition, functioning in stressed communities can be altered by ‘context-dependent effects’, i.e. changes in the species’ functional contribution (Fox 2006, Fox and Harpole 2008, Tylianakis et al. 2008b, Hiddink et al. 2009). Context-dependent effects can arise from direct effects of the environmental drivers on the species’ functional contribution (Fox and Harpole 2008, e.g. physiological stress, Schimel et al. 2007), as well as from environmental drivers altering species interactions (Fox 2006, Fox and Harpole 2008).

The majority of experiments designed to address ecosystem functioning under anthropogenic change have focused on single trophic levels, usually primary producers (Raffaelli 2006, Cardinale et al. 2011). Stressors that alter functioning at the producer level can however have concomitant impacts on their consumers (Rohr and Crumrine 2005, McMahon et al. 2012). The trophic impacts of anthropogenic stressors are most often examined with regard to diet quantity, which has been linked to the abundance and biomass of consumers (e.g. Wendt-Rasch et al. 2004, Rohr and Crumrine 2005). In the frame of the subject of quantity of available food, some recent studies proposed methods to evaluate the abundance of trophic resources also according to the impacts of anthropogenic stressors (Zupo et al. 2017). Far less attention has been devoted to diet quality, which considers a diet’s biochemical composition, for example lipid content (Jodice et al. 2006, Guo et al. 2016), and which strongly affects growth, reproduction and energy profile of the consumers (Österblom et al. 2008, Taipale et al. 2013). The biochemical composition of primary producers is increasingly affected by human disturbance (Guo et al. 2016, Sanpera-Calbet et al. 2016), which has made the integration of food biochemistry into traditional studies of diet quantity a key challenge for estimating food web functioning under stress (Arts and Wainmann 1999, Guo et al. 2016).

Benthic diatoms are the main primary producers in many soft-sediment intertidal habitats, and diatom diet quality in terms of essential fatty acid (EFA) content plays a crucial role in trophic energy transfer (Arts et al. 2001, Taipale et al. 2013). EFAs cannot be synthesized by animals but are key determinants of the growth and energy content of aquatic consumers (Brett and Müller-Navarra 1997, von Elert 2002, Arendt et al. 2005, Litzow et al. 2006). Moreover, the oxidation of polyunsaturated C16- and C20-FAs (polyunsaturated Fas or PUFAs) to short-chain polyunsaturated aldehydes (PUAs) (Miralto et al. 1999) and other non-volatile oxylipins (NVOs), such as hydroxy-fatty acids, epoxy-hydroxy-fatty acids, and oxo-acids (D’Ippolito et al. 2005) can cause deleterious effects of diatom diets for consumers.

Next, we test if stressor-induced changes in EFA production in the diatom community were caused by selective or context-dependent stress effects. Context-dependent effects are measured by comparing EFA concentrations in experimental diatom communities with those in synthetic communities. The latter are computed for all control and stress treatments from the individual species’ EFA production values in unstressed monocultures, and as such uniquely mimic the selective stress effects while excluding all context-dependent effects.

Harpacticoid copepods are among the main consumers of benthic diatoms and incorporate large amounts of EFAs from their algal diet, making them key players in the energy transfer from primary producers to higher trophic levels (Alheit and Scheibel 1982, Buffan-Dubau and Carman 2000, Andersen et al. 2005). Therefore, any potential change in grazer EFA content, due to context-dependent stress effects on diatom diet quality or due to selective effects leading to dominance of diatoms with high or low lipid content, could impact the energy flow in intertidal systems. Therefore, Next to its dominance in the study area, this harpacticoid copepod is an efficient grazer on epipelic diatoms forming a biofilm on the intertidal sediment in the study site and is relatively easy to manipulate in lab experiments (e.g. De Troch et al. 2012b). Atrazine is a herbicide which binds to the plastoquinone binding protein of photosystem II, causing the disruption of photosynthetic electron flow (Legrand et al. 2003, Knauert 2008) and thus the growth and photosynthesis aquatic primary producers, such as microalgae (Pennington et al. 2001, Larras et al. 2016). Atrazine is commonly not acutely toxic to aquatic consumers, but has adverse chronic effects on consumers due to food limitation, hormonal disruption and reduced reproduction, although these reproductive and hormonal effects are not consistently observed (e.g. Hayes et al. 2011). Despite its Europe-wide ban in 2001, atrazine is still a common pollutant European estuaries (Noppe et al. 2007) and remains one of the most-used pesticide worldwide (Benbrook 2016). In contrast to organic pesticides, heavy metals occur naturally in the environment, and several of them are essential for organism physiology (Hänsch and Mendel 2009). This is the case for copper which is involved in several metabolic pathways in microalgae, as an essential micronutrient and component of proteins and enzymes (Hänsch and Mendel 2009). However, copper concentrations above the required levels are toxic to marine organisms at all trophic levels (e.g. Real et al. 2003, Manimaran et al. 2012). As copper enters coastal environments through river run-off, it affects both primary producers and consumers through the formation of reactive oxygen species (ROS) which can lead to cell death by damaging cell membranes and nucleic acids (e.g. Rhee et al. 2013). Copper also affects marine primary consumers by inhibiting membrane transport proteins (Bianchini et al. 2004) and by limiting the quantity of their algal diet (Pinho et al. 2007).

## Methods

**Experimental organisms & culture conditions.** The harpacticoid copepod *Microarthridion littorale* (family Tachidiidae) was collected from intertidal mud at the polyhaline Paulina site in the Westerschelde estuary (SW Netherlands, 51° 21’N, 3°43’E), where it represented the dominant grazer (~ 90% of all harpacticoid individuals). *M. littorale* specimens were extracted alive from the sediment using a mixed technique of sediment decantation and extraction via white light attraction. Adult specimens were randomly collected with a glass pasteur pipette using a Wild M5 binocular. Copepods were washed 3 times over a 38 µm sieve and placed in glass jars with filtered and autoclaved natural seawater (salinity: 32±1) overnight in order to empty their intestines prior to the start of the experiment.

The diatom community was composed of six species representing the most abundant genera (i.e.*Nitzschia, Amphora, Cylindrotheca, Gyrosigma* and *Navicula*) observed at the sampling site***.*** (Supporting Information Table S1). All diatom species were obtained from the culture collection of the Protistology and Aquatic Ecology Research Group (UGent) (http://bccm.belspo.be). Prior to the experiments, the diatoms were grown in tissue bottles (Greiner BioOne, CELLSTAR® TC, 175 cm2 growth surface) during 10 days in a climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons m-2 s-1, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients (Guillard 1975). In spite of their possibly different optimal requirements, applying the same conditions (e.g. in terms of irradiance) was found to be suitable (see previous experiments of De Troch et al.) as the species also co-occur together in the field.

**Diatom experiments.** The experimental diatom communities were exposed to five treatments to 0, 200 (hereafter ‘low’) and 500 (hereafter ‘high’) µg/l atrazine and copper, respectively. Concentrations were based on trial tests (data not shown) as well as published sensitivity data for marine benthic diatoms (Pistocchi et al. 1997, Levy et al. 2007, Wood et al. 2014). Atrazine treatments were prepared from a stock solution obtained by dissolving 50 mg commercial atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, 99.8% pure, Sigma-Aldrich Chemie Gmbh, Munich, Germany) in 10 ml acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.01% acetone in the treatments. An acetone control treatment of 0.01% acetone was included and compared to an acetone-free control to test for carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a Cu[II]Cl2 solution, analytical grade; VWR International) was spiked directly into the culture medium before exposure of the diatoms. F/2 culture medium was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The obtained atrazine and copper concentrations that were finally applied in the experiment are listed in Table S2. Additionally, the six diatom species were grown in monoculture under control conditions, to quantify each species’ biomass and EFA production in the absence of the stressors (Table S1). All treatments were run in tissue culture flasks (Greiner BioOne, CELLSTAR® TC, 175 cm2 growth surface), with nine replicates per treatment (three replicates in the monoculture treatments). Each microcosm (100 ml) was inoculated with a total cell density of approximately 5000 diatom cells/ml (belonging to the same species in the monocultures, between 800-850 cells/ml per species in the diatom communities) from exponentially growing cultures, and incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons m-2s-1. Culture medium was renewed after eight and 15 days. Diatom biofilms as food for the copepod experiment as well as for the EFA analyses (see below) were harvested after 15 days (late exponential growth phase). The experiments were terminated after 25 days.

Diatom biomass was quantified as biovolume after 0, 2, 5, 10, 15, 20 and 25 days of incubation. Biovolume was calculated from cell densities, linear dimensions (measured digitally using ImageJ, Schneider et al. 2012) and formulas representing the closest approximation of geometric shape for each genus (Hillebrand et al. 1999, Table S1). Cell densities (in cells/ml) were determined by digitally counting the cells (ImageJ cell counting software) in photographs obtained by magnifying and photographing (x100) an area of 0.66 mm2 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11). All analyses below use the biomass on day 15.

Diatom biofilms from three replicates per treatment were harvested as food for the corresponding treatments in the copepod experiment (see below), and purified from copper and atrazine by centrifugation at 50*g* for 10 minutes. The supernatant was replaced with f/2 culture medium, and the suspension was centrifuged again at 50*g* for 10 minutes. A concentrated pellet containing 2.05 mm3 diatom biovolume per replicate was transferred to nine Eppendorf microtubes (0.23 mm3 per microtube), freeze-dried and preserved at -80 °C. The individual microtubes contained the food aliquot for each day of the respective treatments in the copepod experiment (see below).

**Copepod experiment.** We tested the effect of diatom diet quality by offering *M. littorale* diatom diets of equal biomass under unstressed conditions. The copepod experiment consisted of five treatments, each with three replicates of 100 *M. littorale* copepods (a natural mix of adult males and (gravid) females), that were fed for 10 d an equal biomass of diatoms grown under unstressed conditions and low and high atrazine and copper stress, respectively. The experiment was conducted in glass jars containing 100 ml of filtered and autoclaved seawater in a climate room at 15±1 °C with a 12:12 h light:dark cycle and 40 to 50 µmol photons m-2 s-1. To ensure a constant food supply, each treatment was inoculated with a concentrated diatom pellet of 0.23 mm3 biovolume every day, and unconsumed diatoms were removed from the bottom of the jars. Over the whole duration of the experiment, a total diatom biovolume of 2.05 mm3 was applied per experimental unit, corresponding to 3 to 5 x 106 diatom cells per treatment. At the end of each day, there was no food depletion in any of the treatments. Based on our previous experiments (De Troch et al. 2005, 2007), the provided quantity of diatoms can be considered as above the feeding saturation level. Copepod mortality was determined at the end of the experiment, and surviving (85-100%) individuals from each experimental unit were washed in natural seawater to remove food particles, left 12h to empty their gut, and stored at – 80 °C for further fatty acid analysis.

**EFA analyses**. Essential fatty acids (EFAs) as marker of diatom diet quality were quantified as the content of eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). These EFAs were selected as proxies to quantify the energy transfer in view of their relevance for the next trophic level (copepods). Three replicates of 8 ml suspended diatom culture per treatment of the diatom communities and monocultures were collected after 15 days. The samples were centrifuged for 10 minutes at 10 °C at 50*g*. After undergoing the same purification process as the diatom food samples for the grazer experiment, pellets were resuspended, placed in a glass vial and stored at -80 °C for fatty acid analysis.

Copepod and diatom EFA content was measured through hydrolysis of total lipid extracts and methylation to FA methyl esters (FAME), followed by the analysis of the obtained FAME using a gaschromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973) according to the protocol described in De Troch et al. (2012a) for copepods and Mensens et al. (2015) for diatoms. The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 ng/ml). All EFA concentrations were standardized to diatom biomass (see above) or the number of copepod individuals for diatoms and copepods, respectively.

**Data analysis.** An analysis of the diatom community structure among the treatments (control, low and high atrazine and copper) was conducted with a non-metric multidimensional scaling method based on Bray-Curtis similarity. A one-way analysis of similarity (ANOSIM) was used to test for significant biomass differences between the treatments. Subsequently, percentages of similarity (SIMPER) were calculated to determine the main species contributing to any differences in community structure.

Differences in diatom biomass and EFA production among the treatments were tested with a generalized least squares model, with biomass and EFA production as response variables and treatment type as categorical predictor (Equation 1)

Y ∼ βT ∙ T [1]

where Y is the response variable (biomass or EFA production per unit biomass), T is the treatment type (control, low and high atrazine and copper), and the beta coefficient βT is the slope measuring the effect of the treatment type on biomass or EFA production. Biomass and EFA production among treatments were compared with pairwise Tukey’s tests correcting p-values for multiple comparisons by the single-step method.

Next, we tested if potential changes in diatom EFA production were due to selective or context-dependent effects of atrazine and copper. Selective and context-dependent effects were quantified by comparing the EFA production in experimental and synthetic diatom communities. The synthetic communities have the same community structure as observed in the corresponding treatments of the experimental communities, but are computed from the EFA production in unstressed monocultures of each species (Equation 2). The synthetic communities thus reflect the EFA production expected at the same community structure as induced by copper and atrazine, however without any stress exposure or species interactions.

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| $$Y\_{SYN, j}= \frac{\sum\_{i=1}^{N}M\_{i, j=0} ×B\_{i, j} }{B\_{T, j}}$$ | [2] |

YSYN,j is the EFA production per unit biomass in synthetic communities of the same structure as the experimental communities at atrazine or copper concentration *j*. M*i, j=0*is the mean EFA production per unit biomass of species *i* in monoculture under unstressed conditions (*j*=0). B*i, j* is the biomass of species *i* observed in the experimental community at the stress level *j*. B*T,j*is the total biomass observed in the experimental community at stress level *j*.

Selective and context-dependent effects of both stressors were analysed with a generalized least squares model (Equation 3) and pairwise comparisons of the EFA production in experimental and synthetic diatom communities.

Y ∼ βT ∙ T [3]

where Y is the diatom EFA production per unit biomass, T is the treatment type (control, low and high atrazine and copper in the experimental and synthetic diatom communities), and the beta coefficient βT is the slope measuring the effect of the treatment type on EFA production. Pairwise comparisons were performed with a Tukey's test correcting p-values for multiple comparisons by the single-step method.

Context-dependent effects occur when synthetic and experimental communities within the same treatment differ in their EFA production per unit biomass. Since both community types have the same structure, any differences in EFA between the two community types result from direct stress effects or species interactions in the experimental community. Consequently, any differences between experimental and synthetic communities of the same treatment point to context-dependent effects.

Selective stress effects occur when synthetic communities reflecting the control community structure differ in their EFA production from synthetic communities reflecting the community structure under stress. Since EFA values for synthetic communities are computed from those of unstressed monocultures, any differences between synthetic communities are related to differences in community structure rather than direct stress effects or species interactions. Consequently, any differences among synthetic communities are linked to selective rather than context-dependent effects. Fig. S1 provides a scheme visualizing the quantification of context-dependent and selective stress effects.

The response of copepod fatty acid content to stressor-induced alterations in the quality and community structure of their diatom diet was analysed with generalized least squares models, with copepod fatty acid content as response variable and diatom diet quality and community structure as predictors (Equation 4). Diatom diet quality was quantified as EFA production, diatom community structure as the Bray-Curtis percent similarity to the average community structure in the controls (Equation 5). Models were fitted separately for copepods feeding on atrazine- and copper-exposed diatoms respectively, to test if the effects of either stressor on copepod fatty acid content can be predicted from changes in diatom diet quality or community structure.

EC ∼ a + b∙ED + c∙CD [4]

EC is the copepod fatty acid content (EFA content per copepod individual), ED is diatom diet quality (EFA production per unit biomass), CD is diatom community structure (see below), a is the intercept, b and c represent the slopes, i.e. the relation of copepod EFA content to diatom diet quality and diatom community structure. If ED and CD were correlated (correlation factor > 0.5), models were fitted separately for both predictors.

CD = 100 ∙ {1 - Σ |B*i,j* - µB*i*,*j*=0| / Σ(B*i*,*j* + µB*i*,*j*=0)} [5]

B*i,j* is the biomass of species *i* at the atrazine or copper concentration *j* and µB*i,j*=0 is the mean biomass of species *i* in the control (*j*=0).

For all least squares model fits, normality and homogeneity of model residuals were inspected by evaluation of quantile-quantile plots and Shapiro-Wilk’s test, and by Levene’s test and plotting residuals versus explanatory variables respectively. Untransformed data did not violate normality (Shapiro-Wilk’s test, α > 0.1). If indications of deviations from normality were detected (0.1 < α < 0.15), an optimal Box-Cox transformation was applied to maximize normality of model residuals (Box and Cox 1964, Venables and Ripley 2002). If homogeneity was violated, the model was refitted using an exponential variance structure allowing residuals to change with the continuous predictor X (weights = varExp(form ∼ 1 | X) or allowing different variances according to the categorical predictor P (weights = varIdent(form ∼ 1 | P). By means of likelihood ratio testing, the significance of these structures was tested (α = 0.05).

Multivariate, ANOSIM and SIMPER analyses of diatom community structure were performed using Primer 6 software (Clarke and Gorley 2006). All other calculations were done in R 3.0.1. using RStudio (R Development Core Team 2016). The package nlme (Pinheiro et al. 2016) was used for the fitting of generalized least squares models and optional variance structures. Optimal Box-Cox transformations were performed using MASS (Venables and Ripley 2002). Pairwise Tukey’s tests on the fitted models were performed with the package multcomp (Hothorn et al. 2008), using the general linear hypothesis test (glht) function, correcting p-values for multiple comparisons by the single-step method (default procedure in multcomp).

## Results

**Diatom community structure.** The structure of diatom communities under atrazine differed from the structure of communities grown under control conditions and copper exposure (see non-metric multidimensional scaling in Fig. S2, ANOSIM global R = 0.833, p=0.001). The community structure at both copper levels resembled the community structure observed under control conditions (14% and 18% dissimilarity, respectively), with *N. acicularis* and *N. arenaria* contributing the most biomass in both types of communities (Fig. 1, Table S3). In contrast, both atrazine levels induced a change in community structure (70% and 76% dissimilarity from the control) due to an increase in biomass of *C. closterium*, which compared to the control showed a 6- and 12-fold increase in biomass in the high and low atrazine treatments, respectively. This resulted in a dominance by *C. closterium* in the atrazine-exposed communities, as it contributed more than 70% of the total biomass at both atrazine levels (Fig. 1, Table S3). Within the control, copper and atrazine treatments, the community structure of diatom communities showed little variance (within-treatment similarities between 84% and 92%, Table S4).

**Diatom biomass and EFA production.** Diatom biomass and EFA production changed depending on treatment type (all p<0.0001, Fig. 1, Fig. 2). The post-hoc analyses showed that diatom biomass was reduced at both low and high copper as well as at high but not at low atrazine concentrations (Fig. 1, Table 1). Diatom EFA production was reduced at both levels of atrazine, but only at high copper stress (Fig. 2 grey bars, Table 1). High copper stress reduced the EFA production of diatom communities by 40%, low and high atrazine stress by 60% and 75%, respectively (grey bars in Fig. 2).

**Selective and context-dependent stress effects on diatom EFA production.** The EFA production in the experimental and synthetic diatom communities changed depending on treatment type (both p<0.0001). Pairwise comparisons of the EFA production in experimental and synthetic communities did not show differences among the two community types in the control and low copper treatments (Fig. 2, Table 2). At high copper stress, the EFA production in the experimental community was lower than in the corresponding synthetic community (Fig. 2, Table 2). The EFA loss induced by high copper stress in experimental communities was thus not reflected in unstressed synthetic communities of the same structure, whose EFA production did not differ from the control (Fig. 2, Table 2).

In the atrazine treatments, both the experimental and synthetic communities had a lower EFA production than the control (Fig. 2, Table 2). Within each atrazine treatment, the EFA production of experimental and synthetic communities did not differ (Fig. 2, Table 2). The EFA loss induced by atrazine in the experimental communities was thus reflected by the synthetic communities, which mimicked the community structure under atrazine without actual exposure to the herbicide (Fig. 2, Table 2).

**Diet quality effect on copepods.** The EFA content of *M. littorale* was related to the stressor-induced changes in diatom diet quality (both stressors) and diatom community structure (atrazine only, Fig. 3, Table 3). Copepods maintained their control EFA content when feeding on diatoms from the low copper treatment, but lost half of their EFAs when feeding on diatoms grown under high copper stress (Fig. 3). This resulted in a positive correlation of copepod EFA content and diatom EFA content (Table 3). When offered diatoms from the low and high atrazine treatments, copepods also lost half of their EFA content, which was predicted not only by the diatoms’ EFA content, but also by the changes in diatom community structure (Fig. 3, Table 3). Fig. S3 shows the EFA content per diatom biomass and per copepod individual, as well as the relative proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In diatoms, the essential fatty acids consisted mainly of EPA, whereas DHA was the main component in copepods (EPA:DHA ratio 4 to 6 in diatoms, 0.3 to 0.6 in copepods, Fig. S3). The copepod survival rate in the experimental units was between 85-100%. The EFA content of copepods feeding on diatoms from control conditions (65.3 ± 6.1 ng copepod-1) did not differ significantly from copepods at the start of the experiment (i.e. animals collected in the field, 68.8 ± 11.8 ng copepod-1, t-test, p=0.99, Fig. S3).

## Discussion

Biomass production is the most widespread functional endpoint in research on ecosystem functioning under anthropogenic change, and producer biomass (diet quantity) has been the main focus in most trophic experiments (Arts and Wainmann 1999, Balvanera et al. 2006, Cardinale et al. 2011). The loss of diatom biomass induced by copper corresponds to previous findings on the toxicity of copper to marine diatoms (Pistocchi et al. 1997, Masmoudi et al. 2013). Diatom biomass was not affected at low atrazine concentrations, although atrazine has been shown to reduce diatom biomass at concentrations lower than those used in our study (DeLorenzo et al. 1999, 2001, Magnusson et al. 2008). The capacity of our experimental diatom communities to maintain their biomass under low atrazine exposure was related to a change in community structure, with *C. closterium* becoming dominant in all atrazine-stressed communities. Atrazine blocks the electron transport chain of Photosystem II (PSII, Dorigo and Leboulanger 2001). Some diatom species can however reduce their dependency on photosynthesis and thus their sensitivity to PSII inhibitors by mixotrophic growth (i.e. the uptake of organic substrates, Debenest et al. 2009, Larras et al. 2014). *C. closterium* is capable of mixotrophic growth (Vanelslander et al. 2009), which reduces its sensitivity to herbicide stress (Mensens et al., in press). The presence of a mixotrophic, atrazine-tolerant species thus caused changes in community structure which underpinned the absence of biomass loss at low atrazine levels.

Selective atrazine stress determined the diet quality of diatom communities, which lost 60% and 75% of their EFA content at low and high levels of the pesticide. The same extent of energy loss was observed in synthetic communities which reflected the community structure under atrazine without exposure to the pesticide. As diet quality in atrazine-exposed experimental communities and unexposed synthetic communities did not differ, the energy loss under atrazine was caused by selective changes in community structure rather than by context-dependent atrazine effects on diatom diet quality. This selective atrazine stress could be attributed to the dominant species *C. closterium*, which produced three and eight and times less EFAs than the species contributing the most biomass under control conditions, i.e. *N. arenaria* and *N. acicularis*, respectively (see fatty acids per species in Table S1). In the presence of copper, communities were dominated by the same lipid-rich species as in the control, and as a result no selective effects on diet quality were observed. The lower diet quality in experimental compared to synthetic communities at high copper levels was therefore caused by context-dependent rather than selective copper effects. Context-dependent effects also likely caused the further loss of EFAs at high compared to low atrazine stress, since community structure at the two herbicide levels did not differ. This loss of diet quality at high levels of both stressors could be due to physiological stress effects such as an alteration of photosynthesis and thus of the carbon supply for fatty acid synthesis, inhibition of the enzymes involved in lipid biosynthesis or an increase in the degree of fatty acid saturation, which are all reported to reduce the microalgal EFA production under metal and pesticide stress (Böger et al. 2000, Guschina and Harwood 2006, Chia et al. 2013).

In our study system, selective stress proved to be the main driver of microalgal diet quality. Selective atrazine stress caused a more important loss of diatom diet quality than the context-dependent effects of both stressors, at chemical concentrations where no context-dependent effects on individual EFA content were recorded. All copper and atrazine concentrations used in our experiments are respectively far above field concentrations, or have only been recorded in extreme pollution events or at chronically contaminated sites (Graymore et al. 2001, Pennington et al. 2001, Lockert et al. 2006). Apart from scenarios of severe chemical pollution, pesticide and metal stress are thus unlikely to reduce the energy flow in intertidal systems by inhibiting diatom EFA synthesis. Conversely, chemical pollution can cause shifts in algae community structure at stress levels lower than those used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010). The impact of such selective chemical stress on ecosystem functioning will largely depend on the functional importance of the most stress-tolerant species (Larsen et al. 2005, Mensens et al. 2015, Radchuk et al. 2016). Not only pollution, but also long-term changes in environmental variables are causing considerable shifts in microalgae community structure (Pomati et al. 2012, Litchman et al. 2015). Due to the pronounced differences in lipid profiles within and among algal classes (Taipale et al. 2013, Guo et al. 2016), these changes in community structure rather than context-dependent changes in algal diet quality could represent a potentially stronger driver of trophic energy flow under anthropogenic change.

The EFA content of the copepod *M. littorale* closely tracked that of its diet. Selective and context-dependent stress effects on diatom diet quality resulted in a concomitant loss in the EFA content of their main copepod grazer, confirming algal EFAs as being a key component of diet quality which is directly linked to trophic energy transfer. The DHA:EPA ratio of *M. littorale* was higher than in the diatom communities, which corresponds to previous findings on the relative concentrations of both EFAs in copepods and their algal diets (De Troch et al. 2012a, Arndt and Sommer 2014). Diatoms are characterised by a high EPA content (Taipale et al. 2013, Guo et al. 2016), but DHA appears to be the most important fatty acid for copepods (Taipale et al. 2013). Planktonic primary consumers such as cladocerans or calanoid copepods directly depend on the DHA taken up from their diet (Bell et al. 2007, Bell and Tocher 2009, De Troch et al. 2012a), but several harpacticoid copepod species are able to bioconvert EPA to the longer chain DHA, a capacity which has notably been demonstrated in *M. littorale* (De Troch et al. 2012a). While the total EFA content of *M. littorale* reflected that of its different diatom diets, this capacity to convert EPA to DHA likely enabled *M. littorale* to maintain high relative levels of DHA.

Our design of calculating synthetic communities from unstressed monocultures eliminated diversity effects such as species interactions, which can drive the functional contribution of communities along environmental gradients (Tylianakis et al. 2008a, Maestre et al. 2010). It should thus be noted that while our experiments highlight potential functional impacts of selective stress, they do not allow to quantify diversity effects on diatom functioning. Our results also have to be treated carefully due to the limited number of stress levels and replicates. Also, offering *M. littorale* preserved rather than live diatom food might have influenced food uptake. Freeze-drying does not alter the biochemical composition within diatoms cells, but modifies the exterior of diatom cells through the loss of exudates or bacteria associated to the diatom frustule, which can affect the ingestion of diatoms by harpacticoid copepods (Cnudde et al. 2011). Feeding *M. littorale* live diatom cultures under unstressed conditions would however have resulted in a dissimilar diatom community structure than that induced by the stressors: atrazine and copper did not eliminate any of the diatom species, but caused alterations of community evenness, which typically cannot be maintained in the absence of the stressors (De Laender et al. 2016).

Since their EFA content ranks among the highest of all algae classes, diatoms are regarded as high-quality food source and crucial link for the energy flow at the basis of aquatic food webs (Guo et al. 2016). Here, diatom diet quality was more affected by chemical stress than diet quantity. The selective and context-dependent stress effects on diatom diet quality were caused by the large interspecific differences in EFA content and the loss of diatom EFA content under stress. Indeed, the diet quality of benthic diatoms shows more interspecific variation and is more affected by chemical pollutants than their contribution to diet quantity (Mensens et al. 2015). Losses in diet quality occurred at copper concentrations which also impacted the survival of *M. littorale* when the copepods where directly exposed to copper (Table S6). In addition to indirect effects on copepod EFA content through changes in diet quality, high metal stress can thus be expected to reduce copepod abundance through direct toxic effects. Conversely, atrazine changes microalgal community structure at concentrations lower than the 200 µg/L used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010), whereas atrazine has been found to only acutely affect copepods at concentrations higher than 1 mg/L (Hall et al. 1995, Bejarano and Chandler 2003). Atrazine could thus first affect copepods indirectly through selective changes in the structure of their diatom diet rather than through direct effects on the copepods themselves.

The loss of diet quality did not result in increased harpacticoid mortality. Low diet quality rarely causes acute copepod mortality, but reduces copepod EFA content and, on a longer term, growth rate and reproduction (Müller-Navarra 1995, Müller-Navarra et al. 2000, Arendt et al. 2005, Gonçalves et al. 2011). The EFA content of copepods is crucial for their main consumers, especially larval fish whose development can depend on the EFAs taken up from their copepod prey (Sargent et al. 1995, Payne et al. 1998). Whilst in this study losses in diet quality did not eliminate consumers, low diet quality could thus reduce the energy transfer at the plant-animal interface, which is a key limiting factor for the functioning of aquatic ecosystems (Brett and Müller-Navarra 1997, De Troch et al. 2012b). Nonetheless, the importance of algal diet quality, as distinct from just diet quantity, is rarely highlighted in research on food web functioning (Guo et al. 2016). Due to its sensitive response to selective stress, algal diet quality in terms of EFA production and community structure provides a powerful approach to integrate our understanding of coastal ecosystem functioning under anthropogenic change.

Our results support two conclusions. First, chemical stress differentially affects the contribution of marine primary producers to diet quantity and diet quality, with diet quality being more sensitive in this study. Second, selective stress caused a more important loss of diatom diet quality in our study system than context-dependent stress effects, and thus represented the main risk for energy flow to their copepod consumers. The integration of diet quality into traditional studies of diet quantity is recommended to assess energy flow in marine food webs under anthropogenic change.

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**Tables**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Process | Treatment | Treatment | Est | lwr | upr | Z | P |
| Biomass | Control | Atr, low | -6.756 | -9.18 | -4.33 | 2.78 | 0.059 |
| Atr, high | 23.327 | 20.90 | 25.75 | 9.61 | **<0.001** |
| Cu, low | 7.273 | 4.85 | 9.70 | -3.00 | **0.036** |
| Cu, high | 10.533 | 8.11 | 12.96 | 4.34 | **<0.001** |
| Atr, low | Atr, high | 30.083 | 27.66 | 32.51 | 12.39 | **<0.001** |
| Cu, low | Cu, high | 3.260 | 0.83 | 5.69 | 1.34 | 0.666 |
| EFA | Control | Atr, low | 2.143 | 1.30 | 2.99 | 8.00 | **<0.001** |
| Atr, high | 3.191 | 2.34 | 4.04 | 12.08 | **<0.001** |
| Cu, low | -0.046 | -0.89 | 0.80 | -0.17 | 1.000 |
| Cu, high | 1.372 | 0.52 | 2.22 | 5.12 | **<0.001** |
| Atr, low | Atr, high | 0.317 | 0.13 | 0.51 | 5.72 | **<0.001** |
| Cu, low | Cu, high | 0.305 | 0.11 | 0.50 | 4.91 | **<0.001** |

**Table 1:** Pairwise comparisons of biomass and EFA production in diatom communities as estimated by generalised least squares model fits. 'Process' indicates to which response variable models were fitted (biomass or EFA production). 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicates which treatments are compared. 'Est' indicates the difference in biomass (in 103 mm3/ml), and EFA (in µg/mm3) production between the compared treatments as estimated by generalized least squares models fitted to untransformed biomass and Box-Cox transformed EFA data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Effect | Com | Treatment | Com | Treatment | Est | lwr | upr | Z | P |
| SE | Syn | Control | Syn | Atr, low | 2.695 | 1.85 | 3.54 | 10.06 | **<0.001** |
| Atr, high | 2.746 | 1.90 | 3.60 | 10.25 | **<0.001** |
| Cu, low | -0.173 | -1.02 | 0.67 | -0.65 | 0.999 |
| Cu, high | -0.381 | -1.23 | 0.47 | -1.42 | 0.921 |
| CD | Exp | Control | Syn | Control | -0.110 | -0.96 | 0.74 | 0.41 | 1.000 |
| Atr, low | Atr, low | 0.442 | -0.41 | 1.29 | 1.65 | 0.823 |
| Atr, high | Atr, high | -0.554 | -1.40 | 0.29 | -2.07 | 0.550 |
| Cu, low | Cu, low | -0.237 | -1.08 | 0.61 | 0.89 | 0.997 |
| Cu, high | Cu, high | -1.863 | -2.71 | -1.02 | -6.95 | **<0.001** |

**Table 2:** Pairwise comparisons of EFA production in treatments of experimental and synthetic diatom communities as estimated by generalised least squares model fits. 'Effect' indicates which type of stress is analyzed: selective stress effects ('SE': comparison of synthetic communities reflecting the community structure under control and stress conditions), context-dependent stress effects ('CD': comparison of stressed and synthetic communities of the same community structure). 'Com' ('Exp': Experimental and 'Syn': Synthetic) and 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicate which communities and treatments are compared. 'Est' indicates the difference in EFA production between the compared treatments as estimated by generalized least squares models fitted to Box-Cox transformed EFA data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Stressor | Quality | Model | Slope | s.e. | T | P | AIC | Log lik. | Validity | LR | P LR |
| Atrazine | EFA | 1 | 0.059 | 0.008 | 7.38 | **0.0002** | 28.46 | -11.23 | Yes | 0.69 | 0.41 |
| 2 | 0.060 | 0.007 | 9.06 | **<0.0001** | 29.77 | -10.89 | Yes |
| Comp | 1 | 0.045 | 0.008 | 5.88 | **0.0006** | 31.69 | -12.85 | Yes | 0.07 | 0.79 |
| 2 | 0.045 | 0.008 | 5.64 | **0.0008** | 33.62 | -12.81 | Yes |
| Copper | EFA | 1 | 0.137 | 0.021 | 6.57 | **0.0003** | 35.21 | -14.61 | Yes | 3.64 | 0.06 |
| 2 | 0.133 | 0.014 | 9.17 | **<0.0001** | 33.57 | -12.79 | Yes |
| Comp | 1 | 0.204 | 0.126 | 1.62 | 0.1486 | 44.82 | -19.41 | No | 0.75 | 0.39 |
| 2 | 0.071 | 0.150 | 0.48 | 0.6494 | 46.07 | -19.03 | Yes |

**Table 3:** Results of generalized least squared models predicting copepod EFA content from diet quality (diatom EFA production) and diatom community structure. 'Stressor' denotes which diet the copepods were offered: diatom communities exposed to copper or atrazine. 'Quality' indicates the predictor: diet quality in terms of diatom community composition or EFA production. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) variance structure. 'Slope' indicates the relation between predictors and copepod EFA content, i.e. the effect of diatom diet quality and community structure on copepod EFA content. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values are statistically significant. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood . 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If ‘no’, models were refitted (‘Model 2’) with a variance structure allowing the residuals to change with the predictor. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

**Figure legends**

**Fig. 1:** Total biomass production per treatment and biomass of the component species for diatom communities grown in control, low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') treatments. Significant differences in biomass production from the control are indicated with asterisks (\*).

**Fig. 2:** Atrazine and copper effects on diatom diet quality (EFA production). Experimental communities were grown in control, low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') treatments. Synthetic communities were computed from unstressed monocultures in the same community structure observed at each stress level. Significant differences in EFA production indicating selective and context-dependent stress effects are indicated with asterisks (\*). \*C indicates pairwise differences among stressed and synthetic communities of the same structure (context-dependent stress effects). \*S indicates pairwise differences among control and stress treatments within the synthetic communities (selective stress effects).

**Fig. 3:** Community structure and diet quality (EFA production per unit biomass) of diatom communities exposed to stress, and EFA content of *Microarthridion littorale* after 10d of feeding on the respective diets, visualised as percent similarity to the corresponding control. The similarity of diatom community structure is calculated as the Bray-Curtis similarity to the control mean (see methods section). The similarity of diatom and copepod EFA content is calculated as fraction percentage of the EFA concentration in treatment *i* and the mean (µ) EFA concentration in the corresponding control c: (EFA*i*/µEFAC)x100. Diatom communities were exposed to low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') concentrations, copepods were not exposed to any of the stressors.

**Figures**

Figure 1



Figure 2



Figure 3

