



The functional response of a freshwater benthic community to cadmium pollution

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ABSTRACT

Theory predicts that in freshwater communities under chemical stress secondary production will decrease while the rate of biomass turnover (P/B) will increase. However, this concept has never been tested on organisms of smaller size (bacteria, protozoans, small metazoans), although they form the basis of the heterotrophic food web. The present work describes the results of a 7-month microcosm study, in which the effects of low and high toxic stress on an entire sediment community were examined, with cadmium (Cd) as the model pollutant (50 and 400 mg Cd kg⁻¹ dry sediment). While metazoans and protozoans generally followed the expected trend, in bacteria both production and P/B decreased under Cd stress. These observations provide new insights into the functioning of freshwater ecosystems and demonstrate the functional consequences of toxicants on biological systems.

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1. Introduction

The field of ecotoxicology has rapidly evolved over the last decades and much information has been gained about effects of many contaminants on organisms of freshwater ecosystems. However, this information has largely been derived from the study of community structure than of function (Carlisle and Clements, 2003). Structural measures generally deal with the abundance, biomass and diversity of organisms, whereas functional parameters are usually employed at the community level and involve process rates such as those of energy flow, the cycling of matter and secondary production (Buffagni and Comin, 2000). Indeed, it is well established that secondary production is one of the major pathways of energy flow throughout ecosystems (Stead et al., 2005; Strayer and Likens, 1986; Waters, 1977). Consequently, production has long been discussed in the literature as a functional measure in ecotoxicology (Benke and Huryn, 2010; Cairns et al., 1992; Newman and Clements, 2008); until recently, however, this endpoint was only rarely included in biological assessments (Newman and Clements, 2008). Basically, it has been reported that secondary production of freshwater communities decreased under toxic stress (Carlisle and Clements, 2003; Lugthart and Wallace, 1992). The corresponding ratio of biomass turnover (production/biomass, P/B) was predicted to increase (Margalef, 1975; Odum, 1985) that was later confirmed in field studies (Lugthart and Wallace, 1992;

Woodcock and Huryn, 2007). Nonetheless, this information was almost exclusively obtained by studying macrobenthic communities, typically those in polluted streams. By contrast, taxa of smaller size have remained essentially unconsidered even though bacteria, protozoans and small metazoans (collectively referred to as meiofauna) are of major relevance in ecology and ecotoxicology for several reasons: First, freshwater sediments are a site of high biological activity, communities are often very diverse with high abundances (Giere, 2009; Rundle et al., 2002). Second, these communities represent the basis of energy flow throughout the heterotrophic food web and thus play a key role in ecosystem structure and function (Hakenkamp et al., 2002; Stead et al., 2005). Third, bacteria, protozoans and most benthic metazoan taxa exhibit endobenthic development and short generation times, both of which render them of great importance for ecotoxicological investigations as these organisms are essentially exposed to pollution during their entire life span (Traunspurger and Drews, 1996). Despite their ecological importance, functional responses (secondary production, P/B rate) of these taxa to toxic stress have never been considered in a comprehensive approach, neither in freshwater nor in marine ecosystems. This may be due, at least in part, to the labor-intensive efforts required to estimate the secondary production of the numerous inhabitants of benthic communities (bacteria, flagellates, ciliates, nematodes, rotifers, copepods, oligochaetes, among others). Such efforts are further complicated by the absence of a standard method applicable to all taxa but see Rigler and Downing (1984) for a critical discussion. The methodical implementation of this study can be well realized in microcosms. Although these model ecosystems have been criticized for their inadequate representation of natural communities

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Table 1

Cohort production intervals of metazoan taxa (CPI) in the control (CN) and the treatment-specific fits of these values under low (LC) and high (HC) Cd concentration.

Taxon	Used in this study for	Treatment-specific development time [days]			Source of CPI
		CPI			
		CN	LC	HC	
<i>Aeolosoma viride</i>	Aeolosomatids (Oligochaetes)	68.6	147.5 ^a	182.5 ^a	Falconi et al. (2006)
Oligochaeta	Other oligochaetes	205.3	441.0 ^a	546.1 ^a	Size-frequency histograms
Microturbellaria	Platyhelminthes	49	105.3 ^a	130.3 ^a	Kolasa (2000)
<i>Alona costata</i>	Cladocerans	140	203.4 ^b	311.2 ^b	Dole-Olivier et al. (2000)
Megacyclops	Cyclopoids	137	199.0 ^b	304.4 ^b	Dole-Olivier et al. (2000)
Bryocamptus	Harpacticoids	243	353.0 ^b	539.9 ^b	Dole-Olivier et al. (2000)
<i>Notodromas monacha</i>	Ostracods	28	40.7 ^b	62.2 ^b	Dole-Olivier et al. (2000)
Monogononta	Monogonate rotifers	8.5	8.5 ^c	10.4 ^c	Ricci and Balsamo (2000)
Bdelloidea	Bdelloid rotifers	36.8	36.8 ^c	45.2 ^c	Ricci (1983)
Nematoda	Nematodes	2.1–169.3	(×1) ^c	(×1.23) ^c	Individually calculated by Vranken et al. (1986)

The CPI was multiplied by a treatment-specific factor obtained from the footnotes.

^a Size-frequency histograms of oligochaetes.^b *Daphnia magna* bioassay.^c *Caenorhabditis elegans* bioassay.

and low in environmental realism (Carpenter, 1996), they facilitate the testing of biological hypothesis by allowing the manipulation of a single parameter (Höss et al., 2004). Therefore, in the present work, we carried out a microcosm study over a period of seven months using freshwater sediment, examining the effects of toxic stress in which cadmium (Cd) served as the model pollutant. Specifically, our objectives were 1) to quantify the effects of low and high Cd impact on secondary production and the P/B ratio of bacteria, protozoans and metazoans, and 2) to assess the functional importance of each of these three taxa under Cd impact by comparing their individual P/B rates. We assume that the production will decline and the P/B ratio increase under Cd impact in the course of time.

2. Materials and methods

2.1. Microcosm set-up

The secondary production of the benthic community was studied in indoor microcosms over seven months with monthly sampling at eight occasions (T0–T7; T0: initial value, T1–T7: experimental time). The temperature was maintained at 20 °C under a 12:12 h light:dark regime. To increase the diversity of the benthic community, sediment was obtained from two lakes, Schöhsee (mesotrophic) and Löptiner See (polytrophic), both of which are situated in Schleswig-Holstein, Germany (Schöhsee: 54° 9' N, 10° 26' E; Löptiner See: 54° 10' N, 10° 13' E). Sediment was taken from the surface (upper 5 cm) of the littoral zone of the two lakes; it had a mean loss on ignition (LOI) value of 1.14% (SD = 0.34, $n = 12$). The sediments were gently mixed immediately after sampling and equal amounts were then placed in grey 15-L polypropylene boxes (37 × 27 × 21 cm; $L \times W \times H$) to a depth of 3 cm. The overlying water column was adjusted to a depth of 10 cm (10 L) with artificial freshwater (pH: 7.5, phosphate: 152.1 $\mu\text{g L}^{-1}$, nitrate: 0.2 mg L^{-1}).

2.2. Application and chemical analysis of cadmium

The overlying water was skimmed to sediment surface level after which a 1-L aqueous Cd solution (as $\text{CdCl}_2 \cdot 1\text{H}_2\text{O}$, dissolved in deionized water) was added to final nominal low (LC) and high (HC) concentrations of 50 and 400 mg kg^{-1} dry sediment, respectively. For the control, 1 L of deionized water was added. Five replicates were set up for the control and each of the two Cd concentrations (=15 microcosms). The Cd-spiked sediments were gently mixed using a large plastic comb. Skimmed water was refilled up to 10 cm one day later. For Cd analysis, sediment and pore water samples were collected. Pore water was defined as water filtered through a 0.45- μm Millipore membrane (Carignan et al., 1985).

2.3. Analysis of abundance and biomass

Bacterial abundance was determined by direct counts using the DAPI method of Porter and Feig (1980), considering Schallenberg et al. (1989). Protozoans (ciliates and flagellates) were counted following the method of Gasol (1993) and divided into four size classes. Bacterial biomass was calculated after Bratbak and Dundas (1984) and protozoan biomass after Finlay (1978). Metazoans encompassed the following

taxa: nematodes (species level), ostracods, bdelloid and monogonate rotifers, aeolosomatid and non-aeolosomatid oligochaetes, cyclopoid and harpacticoid copepods, platyhelminthes and cladocerans. Metazoan biomass was calculated using taxon-specific methods. For a detailed description and the sampling procedure of the benthic community, please refer to Faupel et al. (2011).

2.4. Estimating secondary production of bacteria and protozoans

Bacterial production was measured using the tritium thymidine incorporation (TTI) technique described by Findlay (1993). For each replicate, five aliquots of sediment were taken at randomly selected positions in the microcosm. One mL of this mixed sediment and 2 mL of filtered site water (0.2 μm , Nuclepore) were then transferred to test tubes. The sediment slurries were incubated with 20 $\mu\text{Ci } ^3\text{H}$ -methyl-thymidine (^3H -TdR, specific activity: 75 Ci mmol^{-1}) for one hour. Zero-time controls were run by killing samples with formalin immediately after the addition of ^3H -TdR to the sediment. The rate of ^3H -TdR incorporation into DNA was calculated from $I = \text{dpm SA}^{-1}$ where I is the incorporation rate ($\text{nmol } ^3\text{H}$ -TdR mL^{-1} sediment min^{-1}), dpm the disintegrations per minute (mL^{-1} sediment min^{-1}), and SA the specific activity of the added thymidine (dpm nmol^{-1}). Production was calculated using a conversion factor of 10^{18} cells mol^{-1} (Fuhrman and Azam, 1982). Isotope dilution in the sediment was estimated by the method of Pollard and Moriarty (1984). Production of ciliates and flagellates was indirectly estimated from biomass data using the method of Calkorskaja as cited in Finlay (1978), in which the generation time as a function of temperature and cell size is taken into account. Yearly P/B ratios were obtained from annual production and mean biomass values for the period.

2.5. Estimating secondary production of metazoans

Production of rotifers, cladocerans, copepods, ostracods, platyhelminthes and oligochaetes was estimated using the size-frequency method (Benke, 1979; Hamilton, 1969; Hynes and Coleman, 1968). As taxa differ in their individual development times, the data were corrected by the cohort production interval (CPI), obtained from literature values, determined under conditions devoid of chemical stress and at temperatures as close as possible to that of the current set-up (20 °C). Since delayed somatic growth is a commonly observed response to sublethal toxicant doses (Sullivan et al., 1983), the CPIs were multiplied by a treatment-specific factor, as described below: To determine the developmental delay for crustaceans, a chronic *Daphnia magna* bioassay was conducted according to OECD Guideline 211 (OECD, 1998). Body dimensions were measured daily (from day 3 on) and the individual biomass increase determined at concentrations consistent with those of the LC and HC pore water concentrations (see section 3.1), with four replicates including five daphnia each. In this bioassay, the length achieved by *D. magna* after 14 days (2.44 mm = 100%) in the control was achieved under LC conditions after 20.34 days (=145.3%) and under HC conditions after 31.11 days (=222.3%; both according to pore water analysis, see 3.1). These results were subsequently used to correct the CPIs of crustaceans (see Table 1). Nematode production was estimated using the method of Vranken et al. (1986), which takes into account species-specific egg-to-egg development in relation to body size and temperature. A 96-hour *Caenorhabditis elegans* bioassay was conducted with same Cd concentrations and by following the standard ISO guideline (ISO 2010), using three replicates of 10 individuals each that were sampled every 12 h to trace cohorts. These relative values of the treatment-specific developmental delays were multiplied by the CPIs of nematodes and rotifers of the microcosm communities. According to the *C. elegans*

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