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Short-term disturbance of a grazer has long-term effects on bacterial communities—Relevance of trophic interactions for recovery from pesticide effects

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ABSTRACT

Little is known about the transfer of pesticide effects from higher trophic levels to bacterial communities by grazing. We investigated the effects of pulse exposure to the pyrethroid Fenvalerate on a grazer–prey system that comprised populations of *Daphnia magna* and bacterial communities. We observed the abundance and population size structure of *D. magna* by image analysis. Aquatic bacteria were monitored with regard to abundance (by cell staining) and community structure (by a 16S ribosomal RNA fingerprinting method). Shortly after exposure (2 days), the abundance of *D. magna* decreased. In contrast, the abundance of bacteria increased; in particular fast-growing bacteria proliferated, which changed the bacterial community structure. Long after pulse exposure (26 days), the size structure of *D. magna* was still affected and dominated by a cohort of small individuals. This cohort of small *D. magna* grazed actively on bacteria, which resulted in low bacterial abundance and low percentage of fast-growing bacteria. We identified grazing pressure as an important mediator for translating long-term pesticide effects from a grazer population on its prey. Hence, bacterial communities are potentially affected throughout the period that their grazers show pesticide effects concerning abundance or population size structure. Owing to interspecific interactions, the recovery of one species can only be assessed by considering its community context.

1. Introduction

Pesticides are widely used in agriculture and can have negative effects on non-target organisms such as bacterial communities. Investigations of pesticide effects on bacteria have dealt mainly with the short-term disturbance of endpoints, such as growth, enzyme activity, species diversity, and community structure (DeLorenzo et al., 2001; Widenfalk et al., 2008; Cycon and Piotrowska-Seget, 2009; Lew et al., 2009). However, little is known about the long-term effects of pesticides on bacteria, including those on trophic interactions in aquatic ecosystems.

Aquatic bacteria are known to serve as an important food source for a wide range of grazers (Hall and Meyer, 1998; Langenheder and Jurgens, 2001). Despite the importance of such grazer–prey relationships, indirect pesticide effects between grazers and bacterial communities have rarely been studied. Friberg-Jensen et al. (2003) have shown that bacteria proliferated when the abundance of grazing crustaceans was seriously reduced by an application of cypermethrin. In addition, low bacterial abundances were observed in association with high densities of ciliates and small flagellates 45 h after exposure to atrazine (DeLorenzo et al., 1999a). The aforementioned studies focussed on short-term effects of pesticides. Populations of grazers and prey were thereby monitored by integrating endpoints. Integrating endpoints are simple measures that ignore the structure of a system, such as the total abundance or biomass of a population system (Liess and Foit, 2010). These integrating endpoints were often found to recover within one or two generation times after exposure (Sherratt et al., 1999; Barnthouse, 2004). In contrast, longer recovery times were observed repeatedly for differentiating endpoints that focus on the structure of a system. For example, long recovery times were observed for the disturbed size structure of population systems (Driskell et al., 2001; Johnston and Keough, 2005) and species composition of community systems (Liess et al., 2008; Pesce et al., 2008).

We investigated the long-term effects of pesticides on bacterial communities in the presence of trophic interactions. For this investigation, we exposed a grazer–prey system, which comprised the cladoceran *Daphnia magna* and bacterial communities, to pulses of the pyrethroid Fenvalerate. We monitored two differentiating

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endpoints, the population size structure of *D. magna* and the community structure of bacteria, in order to focus on the long-term effects of a pesticide pulse on the grazer and its prey.

2. Methods

2.1. Test system

Populations of *D. magna* and bacterial communities were cultured in cylindrical glass vessels (Harzkristall, Derenburg, Germany) that contained 4.5 L of Elendt M7 medium (OECD, 1997). The communities were initiated with 30 neonates of the grazer *D. magna* 37 days before disturbance. *D. magna*, clone B (Bayer, Monheim, Germany), were fed daily with a suspension of batch-cultured green algae (*Desmodesmus subspicatus*). The quantity of food given daily was 1.1×10^5 cells/mL (0.45 mg C/L). The bacterial cell density in this non-sterile open system accounted for 0.4×10^6 cells/L (standard error = 0.024×10^6 cells/L, $n = 20$; 1 day before disturbance). The base of each glass vessel contained 500 g of washed aquarium gravel (diameter, 1–2 mm) as a support for the bacteria to promote self-purification of the test system. A clear glass plate was placed on top of each vessel to prevent excessive evaporation. The studies were performed at 20 °C. The photoperiod was controlled (16:8 h light:dark), and lighting was provided by a 70 W, cool-white fluorescent tube situated 10 cm above the test vessels. The water used was aerated three times a day for 15 min via silicone tubing (immersed 14 cm below the surface of the water; diameter, 4 mm; tapered ending, 0.5 mm). Every week, 75% of the water in the test vessels was replaced via silicone tubing capped with a 200 μm nylon mesh to prevent the loss of daphnids. To minimize a possible effect of water replacement, bacterial communities were in general monitored after a minimum recovery time of 4 days after water change. A detailed description of the test system can be found in Liess and Foit (2010).

2.2. Short-term disturbance

The grazer–prey systems of *D. magna* and bacterial communities were exposed to pulses of the pyrethroid Fenvalerate at the following nominal concentrations: 0 μg/L (control; six replicates), 0.8 μg/L (five replicates), 1 μg/L (four replicates), and 3 μg/L (three replicates). The measured exposure concentrations were, on average, 22% less than the nominal concentrations and were given as nominal values. On day 7 after exposure, we replaced 80% of the water with fresh, uncontaminated Elendt M7 medium. A previous investigation with an identical test design demonstrated a temporary reduction of the nominal concentration of 3.2 μg/L Fenvalerate to 77, 26, 18, 11, and 4% at 1, 24, 48, 96, and 144 h after exposure, respectively (Liess et al., 2006). An additional treatment (five replicates) did not involve exposure to Fenvalerate but rather consisted of a mechanical removal of *D. magna* at the same time when Fenvalerate was applied in the other treatments. This treatment allowed us to observe the effects caused by a reduction in the density of daphnids in the absence of any delayed effects of the pesticide Fenvalerate. For this purpose, 50% of the population of *D. magna* were mechanically removed, killed by boiling in water, and then returned to the test systems to mimic conditions of pesticide exposure. This treatment is referred to as mechanical$_{50\%}$. The treatments mechanical$_{50\%}$ and 1 μg/L Fenvalerate had similar acute effects on the abundance of *D. magna*. This similarity in the short-term effects of the two treatments allowed a direct comparison of the following recovery processes. A detailed description of the short-term disturbance of *D. magna* can be found in Liess and Foit (2010).

2.3. Monitoring of D. magna populations

The abundance and size structure of *D. magna* were recorded three times a week by image analysis. Algorithms for automatic detection were implemented in the public domain software *ImageJ* (Rasband, 1997–2009). In contrast to traditional subsampling, image analysis is non-invasive, quick, and enables frequent recording of whole populations. Individuals of *D. magna* were divided into three classes in terms of body size: neonates and juveniles (size class I, 0.8–2.3 mm), small adults (size class II, 2.3–2.8 mm), and large adults (size class III, >2.8 mm). Comparison of the results of the image analysis with manual measurements of abundance and body size showed a high degree of agreement between the methods. Correlations for abundance were determined as follows: log image analysis counts = 0.14 + 0.91 log manual counts ($r^2 = 0.999$, $p < 0.001$). Correlations for body size were determined as follows: log image surface area = 1.65 + 1.77 log manual length ($r^2 = 0.991$, $p < 0.001$) (Liess et al., 2006). The total biomass of populations and that of each of the different size classes were calculated as the sum of individual dry weights (W; μg), which were estimated on the basis of detected body lengths (L; mm). For *D. magna* we used the relationship $W = 1.5 \times 10^{-8}\, L^{2.84}$ (Dumont et al., 1975). A detailed description of the image analysis of *D. magna* can be found in Liess et al. (2006).

2.4. Monitoring of bacterial abundance

The total abundance of bacteria was monitored on days −1, 2, 5, 13, and 26 after disturbance by image analysis of stained bacteria cells. The water in all the test vessels was sampled. A volume of 10 mL was filtered through an isopore polycarbonate filter with pores that measured 0.2 μm in diameter (Millipore, Schwalbach, Germany). We used black filter paper as a dark background to improve the clarity of the images of bright cells. Samples were fixed with paraformaldehyde (4% final concentration) and stained with 4′-6-diamidino-2-phenylindole (DAPI; 2 μg/mL for 5 min in the dark). We randomly photographed ten spots per filter paper. Photos were taken using a Sony 3 CCD camera coupled to an Axioskop fluorescence microscope (Zeiss, Göttingen, Germany). Each image showed an area of the filter paper equivalent to 0.003 mm^2 (63 μm × 47 μm). The selected sample volume of 10 mL in combination with an average concentration of bacteria of 500–1000 cells/mL and an effective filter diameter of 41 mm minimized the overlap of cells in the images.

The bacterial cells were detected automatically using *ImageJ* (Rasband, 1997–2009). The bright bacterial cells were separated from the dark background of the filter paper by applying an adaptive threshold algorithm. The adaptive threshold was conducted by subtracting a smoothed version of the image (mean convolution filter, radius = 7) from the original image; relevant details are subsequently detected by setting a threshold (greyscale difference > 8). Objects that were in contact were separated by watershedding algorithms. The bacterial cells that were detected were counted automatically ($\text{Cells}_{\text{Detected}}$). Comparison of the results obtained by image analysis with manual counts of cell densities ($\text{Cells}_{\text{Counted}}$) for 50 randomly selected images showed a linear relationship and high degree of agreement between the two methods. The regression equation was $\text{Cells}_{\text{Counted}} = 1.01 \times \text{Cells}_{\text{Detected}}$ ($r^2 = 0.9998$, $p < 0.001$). To calculate the bacterial abundance of one sample, we averaged the detected cell densities of the ten photos that had been taken of each filter paper. In addition, we measured the length of the first five DAPI-stained cells in the upper left corner of each image and determined the mean cell size to be 0.6 μm in length (standard deviation ± 0.28 μm, $n = 250$).