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The effects of the psychiatric drug carbamazepine on freshwater invertebrate communities and ecosystem dynamics

Amanda L. Jarvis, Melody J. Bernot *, Randall J. Bernot

Department of Biology, Ball State University, Muncie, IN, USA

HIGHLIGHTS

- A mesocosm experiment quantified carbamazepine effects on aquatic ecosystems.
- Invertebrate diversity increased with carbamazepine but effects varied with taxa.
- Carbamazepine indirectly altered organic matter, primary production, and nutrients.
- Carbamazepine may alter freshwater community structure and ecosystem dynamics.

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ABSTRACT

Freshwater ecosystems are persistently exposed to pharmaceutical pollutants, including carbamazepine. Despite the ubiquity and recalcitrance of carbamazepine, the effects of this pharmaceutical on freshwater ecosystems and communities are unclear. To better understand how carbamazepine influences the invertebrate community and ecosystem dynamics in freshwaters, we conducted a mesocosm experiment utilizing environmentally relevant concentrations of carbamazepine (200 and 2000 ng/L). Mesocosms were populated with four gastropod taxa (*Elimia*, *Physa*, *Lymnaea* and *Helisoma*), zooplankton, filamentous algae and phytoplankton. After a 31 d experimental duration, structural equation modeling (SEM) was used to relate changes in the community structure and ecosystem dynamics to carbamazepine exposure. Invertebrate diversity increased in the presence of carbamazepine. Additionally, carbamazepine altered the biomass of *Helisoma* and *Elimia*, induced a decline in *Daphnia pulex* abundance and shifted the zooplankton community toward copepod dominance. Lastly, carbamazepine decreased the decomposition of organic matter and indirectly altered primary production and dissolved nutrient concentrations. Changes in the invertebrate community occurred through both direct (i.e., exposure to carbamazepine) and indirect pathways (i.e., changes in food resource availability). These data indicate that carbamazepine may alter freshwater community structure and ecosystem dynamics and could have profound effects on natural systems.

1. Introduction

Freshwater ecosystems are continually exposed to anthropogenic stressors including urban and sub-urban development, climate change, and point (e.g, WWTP effluent) and non-point source pollution (e.g., overland flow, septic leakage; Halpern et al., 2008; Hughes et al., 2013). Globally, only a fraction of freshwater ecosystems remain relatively pristine (Palmer et al., 2009; Vörösmarty et al., 2010). Multiple studies have demonstrated how alterations in biodiversity and community structure influence ecosystem dynamics (e.g., Tilman et al., 1997; Hooper and Vitousek, 1997; Downing and Leibold, 2002; Steiner et al., 2005). However, many of these studies assessed relatively pristine ecosystems, despite their rare occurrence. Anthropogenic stressors have the potential to profoundly alter both ecosystem dynamics and community structure (Jonsson et al., 2002; Relyea, 2005; Muñoz et al., 2009; McMahon et al., 2012; Liess et al., 2013; Dolciotti et al., 2014). Therefore, further research is needed to quantify how common anthropogenic stressors influence biodiversity and ecosystem dynamics (Relyea and Hoverman, 2006; Clements and Rohr, 2009; Rosi-Marshall and Royer, 2012).

A wide range of pollutants regularly enter surface waters (Murray et al., 2010). Previous studies have identified the source, fate and effects of many pollutants, including pesticides (Relyea, 2005), industrial compounds (Runck, 2007), heavy metals (Clements et al., 2013) and nutrients (Bernot et al., 2006), yet little is known about pharmaceuticals (Halling-Sørensen et al., 1998; Larsson et al., 1999; Rosi-Marshall and Royer, 2012). Unlike other pollutants, pharmaceuticals are biologically active and elicit responses from organisms across taxonomic groups (Brun et al., 2006). Pharmaceutical compounds are frequently detected

* Corresponding author.
E-mail address: mjbernot@bsu.edu (M.J. Bernot).

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in urban and agriculturally dominated ecosystems due to both human and veterinary uses and subsequent movement into aquatic environments (Veach and Bernot, 2011; Bunch and Bernot, 2011; Hughes et al., 2013). Currently, studies that focus on the influence of pharmaceuticals on ecosystems are needed (Rosi-Marshall and Royer, 2012; Hughes et al., 2013).

Frequently detected pharmaceutical pollutants span a number of different chemical classes, including painkillers, psychiatric drugs and antibiotics and are detected across environmental matrices inclusive of water, sediment and organismal tissue (e.g., Kolpin et al., 2002; Du et al., 2012; Gelsleichter and Szabo, 2013; Hughes et al., 2013). The psychiatric drug carbamazepine is one of the most frequently detected pharmaceutical compounds in freshwater ecosystems of North America, Europe and Asia. Carbamazepine is an anti-epilepsy drug, which is also used to treat bipolar disorder, depression and addiction (Neppe et al., 1988). Carbamazepine reduces the firing of neurons by blocking sodium channels (Porter and Meldrum, 2012). Concentrations of carbamazepine sampled from freshwaters worldwide range from 0.5 ng/L to 11,561 ng/L across rivers, streams, and lakes (e.g., Loos et al., 2009; Ferguson et al., 2013; Bernot et al., 2013) with a detection frequency of 85% among study sites (Hughes et al., 2013). Removal of carbamazepine through abiotic and biotic degradation pathways is minimal in natural ecosystems (5–26%; Miao et al., 2005). Thus, carbamazepine is considered recalcitrant in freshwater ecosystems (half-life 82 d; Lam et al., 2004). With high usage rates (1014 tons per year; Zhang et al., 2008) and limited removal, urbanized freshwater ecosystems are persistently exposed to this pollutant.

Carbamazepine is not lethal to freshwater organisms at environmentally relevant concentrations (LC50 > 4 mg/L in *Lumbriculus variegatus* and *Chironomus riparius*; Nentwig et al., 2004). However, chronic effects from exposure to carbamazepine at environmentally relevant concentrations include altered behavior, reduced immune response, and changes in growth and fecundity (Quinn et al., 2008; Gust et al., 2013; Brandão et al., 2013; Lamichhane et al., 2013). For example, Martin-Diaz et al. (2009) determined that environmentally relevant concentrations of carbamazepine reduced cyclic AMP (cAMP) levels and protein kinase A (PKA) activities in *Mytilus galloprovincialis* (Mediterranean mussel). Though many studies have demonstrated that carbamazepine poses little acute risk to freshwater organisms, laboratory estimates of toxicity may underestimate the sensitivity of freshwater organisms in natural settings (Buchwalter et al., 2007; Clements et al., 2013). Freshwater organisms may experience sub-lethal effects, such as changes in behavior, mating success, immuno-competence and development. These sub-lethal effects could alter community structure and diversity of freshwater ecosystems (Bernot and Turner, 2001). Therefore, more information is needed to determine how carbamazepine influences freshwater community structure and ecosystem dynamics (Hughes et al., 2013).

The objectives of this study were to determine how environmentally relevant concentrations of carbamazepine influence the invertebrate community and freshwater ecosystem dynamics. We hypothesized that carbamazepine would reduce the diversity of freshwater invertebrates and change the invertebrate community, which would alter ecosystem characteristics such as primary production, decomposition and dissolved nutrient concentrations (Fig. 1).

2. Materials and methods

2.1. Experimental design

A mesocosm experiment was conducted to quantify the effects of carbamazepine on freshwater invertebrate biodiversity and ecosystem dynamics. Mesocosms (75 L HDPE circular containers; 56.5 cm height; 56.5 cm diameter) were maintained at a Ball State University field station (Hults farm) located in Albany, Indiana (40°18′12″N, 85°13′52″W). Each mesocosm contained 41.5 L of well water (physicochemical characteristics, N = 31: pH 8.2 ± 0.09; dissolved oxygen 6.5 ± 0.7 mg/L; temperature 25.6 ± 0.92 °C; nitrate (NO_3) 0.15 ± 0.21 mg/L; phosphate (PO_4) 43.1 ± 57.5 μg/L), which was added 27 d prior to the introduction of organisms to allow for environmental equilibration. Mesocosms were covered with a fiberglass screen (mesh size: 1 mm) and were exposed to natural elements and light cycles from 8 June 2013 to 11 July 2013 as they were incubated in situ for 31 d following completion of experimental set-up. Each mesocosm received one of four treatments (water and methanol controls and carbamazepine treatments of 200 and 2000 ng/L) with four replicates each (N = 16 total mesocosms). Because carbamazepine treatments used methanol as a solvent, both water and methanol controls were employed to ensure any effects observed could be attributed to carbamazepine as opposed to methanol. Treatment concentrations were selected to represent environmentally-relevant concentrations previously measured in central Indiana freshwaters (Veach and Bernot, 2011; Bernot et al., 2013; Ferguson et al., 2013).

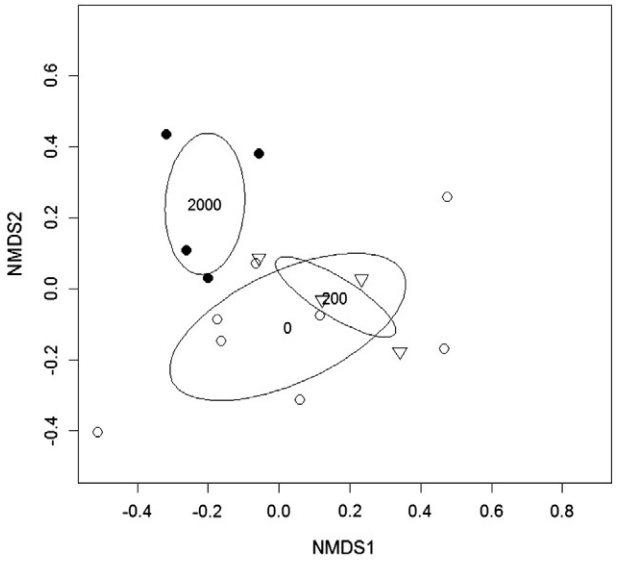

Fig. 1. Non-metric multidimensional scaling (nMDS) ordination of the invertebrate communities in outdoor mesocosms exposed to 0, 200, and 2000 ng/L of carbamazepine in the summer of 2013. Ellipses represent ellipses of standard deviations of their weighted averages.

2.2. Mesocosm substrates

Mesh bags (mesh size: 1 mm; dimensions: 14 × 10 cm) containing 20 g of leaf litter were added to each mesocosm to provide nutrition and refuge. Leaf litter was collected from a local pond (40°20′12″N, 85°13′41″W) then dried and weighed prior to addition to mesh bags. Additionally, homogenized sediment collected from a local pond (40°20′12″N, 85°13′41″W) was equally distributed among mesocosms (~300 cm^3 of sediment per mesocosm). Both the leaf litter and sediment were added to each mesocosm 27 d prior to introduction of organisms.

2.3. Experimental treatments

Carbamazepine treatments reflected environmentally relevant concentrations measured in surface waters (Loos et al., 2009; Hughes et al., 2013; Ferguson et al., 2013) at 200 and 2000 ng/L in addition to water and methanol controls. Carbamazepine (5H-dibenz[b,f]azepine-5-carboxamide; CAS no 298-46-4) and methanol (HPLC grade) were obtained from Sigma-Aldrich (Milwaukee, WI). A stock solution of 2 mg/mL was prepared by dissolving 0.5 g of carbamazepine in 250 mL of pure methanol (>99%), as pure carbamazepine is insoluble in water (17.7 mg/L; Syracuse Physprop Database, 2003).