Science of the Total Environment 484 (2014) 102–113

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Isotopic niche metrics as indicators of toxic stress in two freshwater snails

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HIGHLIGHTS

- Isotopic niche metrics tested as ecological risk assessment endpoints for chemicals
- Chemical exposure affect the isotopic niche of Lymnaeid gastropods
- Lymnaeids exposed to thiram or hydrocarbons showed trophic niche expansion
- Thiram caused a long term trophic niche contraction
- Non-lethal effects of chemicals can be assessed using isotopic niche metrics

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 21 February 2014
Accepted 2 March 2014
Available online 29 March 2014

Keywords:
Thiram
Petroleum distillate
Freshwater snails
Artificial streams
Pond mesocosms
Functional endpoints

ABSTRACT

Descriptors of trophic niche and of food web structure and function have been suggested as integrative and sensitive endpoints of toxicant effects. In the present study, carbon and nitrogen stable isotope signatures were used to assess the effects of the dithiocarbamate fungicide thiram (35 and 170 μg/L nominal concentrations) and of a petroleum distillate (0.01, 0.4, 2 and 20 mg/L nominal loadings as Hydrocarbon Emulsion or Hydrocarbon Water Accommodated Fraction) on the trophic niche of two freshwater gastropods in artificial streams (*Radix peregra*) and ponds (*Lymnaea stagnalis*). Results were analyzed using classical univariate statistical methods and recently proposed uni- and multivariate metrics of the realized trophic niche of species. The trophic niche metrics were highly sensitive to both types of chemicals, but exposure resulted in different response patterns according to the nature of the tested compound. Thiram clearly affected gastropod trophic niche leading to a change in the food resources used and resulting in trophic niche expansion (i.e., increase of diversity of used resources, especially dead animals) or trophic niche contraction (i.e., decrease of diversity of used resources) across time. Both gastropod taxa exposed to hydrocarbons showed a clear trophic niche expansion. Trophic niche metrics therefore provide a promising way of investigating non-lethal effects of exposure to organic chemicals on aquatic invertebrates, and subsequent disturbances in food webs.

1. Introduction

During the last decades, the number of studies focusing on the effects of toxicants on ecological processes has regularly increased, showing that these compounds may directly or indirectly affect organisms and populations in positive or negative ways, with functional consequences at different organization levels (Fleeger et al., 2003; Relyea and Hoverman, 2006). Taking into account such effects is a challenging task for Ecological Risk Assessment (ERA) of chemicals at the community level. The diversity of experimental systems used for high-tier ERA provides a large range of test conditions but this complicates result interpretation and analysis of the consequences of chemical-induced changes. In particular, investigation of long-term direct and indirect effects on aquatic taxa raised potential differences which may be due to the type of experimental systems (van den Brink, 2006). Thus, there is an interest in comparing the effects of chemical exposure between different types of artificial ecosystems because of their different functioning which may lead to differences in chemical fate, taxa composition and biological traits, and ecological processes, as recently reviewed by Brock (2013). Most of the currently used biological metrics applicable at the community level may be measured in any system but they usually buffer the weak signals. Therefore, increasing the number and diversity of endpoints, especially those dealing with ecological processes such as trophic relationships or food web properties, has been advocated to obtain a more comprehensive overview of toxicant effects (Relyea and Hoverman, 2006; Clements and Rohr, 2009). Toxic compounds may change trophic relationships within food webs (Eby et al., 2006).

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http://dx.doi.org/10.1016/j.scitotenv.2014.03.005
0048-9697/

Conversely, food web characteristics may have an influence on the fate and effects of toxic substances (Hebert and Weseloh, 2006). Therefore, descriptors of trophic relationships between species and of food web structure and function (e.g., redundancy, nutrient cycling or energy fluxes) have been suggested as potential integrative and sensitive endpoints of toxicant effects (Post, 2002; Clements and Rohr, 2009).

Identification of trophic niche features is usually carried out using, alone or in combination, the analysis of gut content or faeces, or the analysis of the stable isotope signature of consumer tissues (Layer et al., 2013). Gut content or faeces analysis provides information about the current or recent food bolus (from a few hours to a few days; Rudnick and Resh, 2005), but identification of food items may be difficult and highly time consuming (Pinnegar and Polunin, 2000). Moreover, gut content analysis only provides a snapshot of ingestion, with no indication on the assimilated fraction. Conversely, stable isotope analysis of tissues provides information on the assimilated part of bolus (Pinnegar and Polunin, 2000; Fry, 2006). It provides a time-integrated vision of matter fluxes across food webs and ecosystems, enabling between-ecosystem comparisons (Perga and Gerdeaux, 2004), including between control and toxicant-exposed ecosystems (Caquet, 2006). Basically, carbon stable isotope signature ($\delta^{13}C$) of an organism provides information on the diversity of the exploited carbon sources whereas nitrogen stable isotope signature ($\delta^{15}N$) reflects its trophic level, i.e., the number of food levels between itself and the basis of the food web (DeNiro and Epstein, 1981; Fry, 2006).

Stable isotope signature of biological tissues is driven mainly by mixing and fractionation processes (Fry, 2006). It also heavily depends on the physiological activity of the organisms (Bearhop et al., 2002) and on environmental conditions such as water flow (Finlay et al., 1999; Trudeau and Rasmussen, 2003) or season (McCutchan and Lewis, 2001; Cloern et al., 2002). Such variations may complicate inter-site or inter-date comparisons. To overcome this difficulty and take into account possible influence of environmental factors on the stable isotope signature of a given species, it is necessary to standardize the results using a suitable baseline (Vander Zanden and Rasmussen, 1999; Post, 2002). This baseline is given by the data obtained for another species or group of species located as close as possible to the bottom of the food web, such as phytoplankton, periphyton, detritus, or strictly herbivorous organisms (Cabana and Rasmussen, 1996; Matthews and Mazumder, 2003; Smyntek et al., 2012).

For a long time, results of $\delta^{13}C$ and $\delta^{15}N$ analysis have been presented as biplots and analyzed using classical univariate statistical tests applied to mean signature values. Various quantitative metrics have recently been proposed to characterize the trophic niche of a species or group of species in bivariate (e.g., $\delta^{13}C/\delta^{15}N$) space (Layman et al., 2007a). They have been used in a number of studies (e.g., Layman et al., 2007b, 2012; Quevedo et al., 2009) and recently completed through the implementation of bivariate approaches that provide a quantitative description of how patterns of isotopic signature change in response to spatial and temporal gradients (Turner et al., 2010). However, the main problem of most metrics is their high sensitivity to sample size and outliers. Jackson et al. (2011) recently suggested to tackle this problem using multivariate Bayesian models to estimate trophic niche metrics (Parnell et al., 2010).

Until now, stable isotopes have mostly been used in ecotoxicological studies dealing with the fate of contaminants across food webs (Kiriluk et al., 1995; Banas et al., 2009). Only one study highlighted the effects of experimental pesticide exposure on the trophic niche of an aquatic invertebrate predator following changes in the structure of planktonic food web in pond mesocosms (Caquet, 2006) applying classical parametric statistical tests to mean $\delta^{13}C$ and $\delta^{15}N$ values. The aim of the present study was to use stable isotope signature and isotopic niche metrics (used as proxies of the realized trophic niche), for the analysis of the effects of two organic chemicals, the dithiocarbamate fungicide thiram and the hydrocarbon fraction of a petroleum distillate, on the trophic niche of freshwater gastropods in experimental aquatic ecosystems (stream and pond mesocosms). These two chemicals, as well as mesocosm hydrological functioning, may affect the availability of food resources for the snails. They may also be incorporated into food webs following uptake by e.g., heterotrophic microorganisms, leading to a change in the stable isotope signature of consumers, especially for hydrocarbons that are naturally depleted in carbon stable isotopes. The analysis focussed on two species of lymnaeid snails, *Radix peregra* and *Lymnaea stagnalis* for streams and ponds, respectively. Since these two species are mainly grazers (Tachet et al., 2010) and the isotopic signature of their food resources are highly sensitive to disturbances (Sanz-Lazáro et al., 2011), biofilm stable isotope signature was used as the baseline to standardize snail signatures.

There is no available information on the response of recently proposed trophic niche metrics to toxic stressors. Defining a priori hypothesis on the direction and amplitude of responses of the various metrics in the presence of toxicants is therefore not a straightforward task. The literature devoted to the assessment of toxicants in aquatic experimental systems clearly shows that, although some direct effect may reasonably be forecasted, many unpredictable indirect effects may also occur. Characteristics of the tested compounds (e.g., half-life time, bioaccumulation) and of the test systems (e.g., size, hydrodynamics) further complicate the problem. Although consequences of exposure to chemicals on trophic niche metrics appear to be hardly predictable, some hypothesis can be elaborated. First, trophic niche metrics should be mostly relevant for species with some plasticity in the food resources they may use. If a toxicant causes a significant decrease in the abundance of a given food item, such a consumer may increase its use of another resource that was or not already exploited. The consequence on isotope metrics will depend on the nature of this alternative resource. If its signature is close to the signature of the initially exploited resource, the isotopic metrics will not significantly change. Conversely, if its signature is different, an increase in the dispersion of the individual signatures may be detected using appropriate metrics such as Total Area (TA; Layman et al., 2007a) or Standard Ellipse Area (SEA_B; Jackson et al., 2011). If the alternative resource is exploited only by some individuals, Centroid Distance (CD; Layman et al., 2007a) values should probably increase. Opposite predictions can be made if the toxicant reduces the availability of a resource with a signature differing from these of other exploited resources. Finally, if changes in the array of available food resources affect the corresponding array of isotopic signatures, a shift of the whole isotopic niche could be observed that may be detected through comparisons of trophic niche location or trajectory across time (INL and IND, respectively) between treated and control systems (Turner et al., 2010).

The results presented in this paper are part of a larger study aiming at assessing responses of various endpoints and biological metrics that may be applied in higher-tier studies in ERA (Bayona et al., in review-a, b and c). Throughout the study, emphasis was put on comparisons between lotic and lentic mesocosms, in order to identify similarities and discrepancies in the pattern of responses to organic chemicals used as study compounds in the present context.

2. Materials and methods

2.1. Chemicals and treatments

Thiram is a dithiocarbamate fungicide commonly used in orchard crops against fungal pathogens (such as the apple scab). Treatments were performed using a commercial form (Ordoval®, 80% thiram, WG form, Compo Expert, Münster, Germany). Nominal exposure concentrations (35 and 170 μg/L) were defined from drift curves used in pesticide registration procedure. They correspond to the estimated amounts of thiram that could drift towards surface water under two treatment scenarios for orchards. Ordoval® treatment solutions were prepared using distilled water, taking into account the percentage of thiram in the commercial product. Fungicide stock solutions were prepared in 200 L polyurethane barrels, to ensure a continuous injection into the streams. The same initial solution was used for the whole treatment period (September 28 to October 19, 2010) in streams whereas freshly prepared treatment solutions were used for application to the ponds.