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REVIEW PAPER

The use of rotifers as test species in the aquatic effect assessment of pesticides in the tropics

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Abstract The present study aimed at evaluating the suitability of rotifers as standard invertebrate test species for the aquatic effect assessments of pesticides, with special emphasis to tropical settings. This was done by weighing rotifers against the criteria that are traditionally used for this end. Rotifers are easy to maintain and culture in the laboratory and their (biological) response to chemical stressors like pesticides is well known. As abundant organisms in aquatic ecosystems, they play a key role in energy flow and nutrient cycling. Although they are often considered to have a low sensitivity to pesticides, a sensitivity analysis conducted in this study revealed that they may be more sensitive than the standard invertebrate test species *Daphnia magna* to fungicides. In addition, few toxicity data were available for rotifers other than *Brachionus calyciflorus* and these data were almost exclusively acute (EC_{50}) toxicity values. Subsequently, the sensitivity of other rotifers as well as the chronic sensitivity, bioaccumulation potential, and possible role in biomagnification of pesticides in aquatic foodwebs remains largely unknown. Given their greater diversity and ecological role in tropical freshwaters as compared to temperate freshwaters, the use of rotifers in tropical risk assessments and immediate research needs are discussed.

Keywords Rotifers · Tropics · Aquatic ecotoxicology · Ecological risk assessment · Pesticides

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Introduction

Ecological risk assessments involve a comparison of the (predicted or measured) exposure level of a chemical with the probability to cause toxic effects at that level. Initial or lower-tier toxicity assessments are traditionally conducted by testing a concentration series of the test compound in single-species tests as to establish toxicity threshold values [e.g., concentration of half-maximal response (EC_{50}), no observed effect concentration (NOEC)]. These tests are conducted with a limited number of standard test species selected as surrogates for the sensitivity of other organisms that may exist in natural ecosystems. In Europe, for example, the first-tier prospective effect assessment of pesticides for aquatic organisms in edge-of-field surface waters is based on toxicity tests using test organisms representing different trophic levels, for which there are internationally standardized protocols, such as the fish *Oncorhynchus mykiss*, the algae *Pseudokirchneriella subcapitata*, the macrophyte *Lemna* spp., and invertebrates. Regarding invertebrates, testing *Daphnia* sp. (preferably *D. magna*) is always required but additional invertebrate tests with the saltwater crustacean *Americamysis bahia*, the insect *Chironomus* sp., and/or the oligochaete *Lumbriculus* sp. may also be required depending on the type and mode-of-action of the pesticide of concern (European Food Safety Authority, EFSA, 2013). Single-species tests with rotifers are hence not required in this first-tier effect assessment, although they may be included in higher-tier assessments (e.g., species sensitivity distributions) and rotifers are generally also well represented in model ecosystem studies (EFSA, 2013).

Ecotoxicological research into the fate and side effects of agrochemicals on aquatic ecosystems surrounding agricultural fields has focused almost exclusively on temperate countries. Hence, aquatic risk assessments in tropical countries often rely on temperate toxicity data, even though the fate and effects of pesticides may be different between climatic regions (e.g., Lacher & Goldstein, 1997; Kwok et al., 2007; Daam & Van den Brink, 2010). Given the enormous natural variability in the structure and function of freshwater communities, it is reasonable to question whether the set of standard test species generally used in temperate regions is appropriate for tropical ecosystems. For example, the standard invertebrate test species *D. magna* does not exist in tropical freshwater ecosystems. Subsequently, other invertebrate taxa well represented in tropical edge-of-field waterbodies could be more suitable candidates as surrogates for invertebrate communities in tropical risk assessment schemes. In this regard, the need to develop indicator species belonging to the Phylum Rotifera for ecotoxicological studies in tropical waters has previously been expressed (e.g., Snell & Joaquim-Justo, 2007).

The aim of the present study was to evaluate the suitability of rotifers to serve as standard invertebrate test species in tropical aquatic effect assessments. This was done by weighing rotifers against the (adapted) criteria for the selection of suitable organisms in single-species toxicity testing by Rand et al. (1995) and Van Leeuwen (1995): they should (1) be easy to keep in the laboratory or can reproduce and be cultured under laboratory conditions, (2) have adequate background information on their biology and response to toxicity available as reference database, (3) be valuable in terms of economics, ecology (e.g., key organism), and/or recreation, (4) be native to (or representative of) the ecosystem under study, and (5) be sensitive to a wide range of compounds. Each of these criteria is briefly evaluated in the following sections, followed by an overall conclusion on the use of rotifers as test organisms in tropical effect assessments based on the assessment of these criteria.

Ease to keep and culture in the laboratory

Numerous studies have indicated that the biological attributes of rotifers readily recommend them as test organisms (e.g., Snell & Janssen, 1995; Snell & Joaquim-Justo, 2007; Dahms et al., 2011). Their small size, high fecundity, and short life cycle mean that several rotifer species have been successfully grown in the laboratory (Snell & Joaquim-Justo, 2007). In addition, because of their breeding strategies and widespread dispersion in the form of resistant eggs or by zoochoric transport, rotifers enjoy great ecological success and are hence readily available (Ruppert & Barnes, 1994). Despite these traits, only two protocols have so far been standardized for toxicity tests performed with rotifers, namely the American Society for Testing and Materials, ASTM (1991) protocol for acute testing, and the Snell (1998) protocol for chronic toxicity with the freshwater rotifer *B. calyciflorus* and the brackish-water species *B. plicatilis.*

Background information on biology and response to toxicity

The Phylum Rotifera has about 2030 currently known species and is classified into three main groups: Seisonidae, Bdelloidea, and Monogononta (Segers, 2007). Rotifers are small metazoans, unsegmented, with bilateral symmetry, and generally between 100 and 1,000 μm long, although, some species can reach 3,000 μm (Edmondson, 1959). Their biology and taxonomy are generally well known and detailed identification keys have long been developed (e.g., Edmondson, 1959; Ruttner-Kolisko, 1974; Koste, 1978).

There has been an increasing number of studies with rotifers aiming to assess the effects of pesticides on their life cycles and intrinsic population growth rates, as well as mortality, reproduction, delayed development, morphological alterations, amictic female production, resting egg production, probability of extinction, feeding, swimming activity, and in vivo enzyme activity (Table 1). Besides the great variety in responses to toxicity that have been evaluated in these studies, it has also been shown that the biological responses of rotifers to toxicants like pesticides are reproducible (Suga et al., 2007).

Valuable in terms of economics and/or ecology

As generalist organisms, rotifers consume a variety of food items in their natural habitats—debris, bacteria, and algae—and are subject to significant predation (Barnes et al., 2001). They have a key role in energy flow and nutrient cycling in aquatic ecosystems, because they act with remarkable efficiency in the conversion of much of the primary production of algae and bacteria into low secondary consumers like insect larvae and fish (Nogrady et al., 1993). In Lake Nakuru, for example, the rotifers *Brachionus dimidiatus* and *B. plicatilis*, even though not especially significant in terms of biomass, had the highest production rates (1.7 kJ m^{-3} day^{-1}) out of all invertebrates evaluated (Vareschi & Jacobs, 1984). Doohan (1973) presented an energy budget for adults of the brackish-water rotifer *B. plicatilis*: at 20°C, the hourly consumption of *Dunaliella salina* by an individual rotifer was found to be 333 ± 93 cal, with a measured assimilation rate of 64 ± 10 cal h^{-1}.

Despite their small size, they contribute significantly to secondary production in aquatic systems because of the large population sizes that can be attained due to their rapid growth, short life cycle and, sometimes, parthenogenetic reproduction (Snell & Janssen, 1995; Wallace et al., 2006). For example, Rothhaupt (1990) showed that the rotifers *B. calyciflorus* and *B. rubens* had maximal growth rates slightly below 0.8 day^{-1}, a value that is comparable to that of most protozoan species. Since they are important prey organisms for fish and shrimps that may in turn be used for human consumption, rotifers are indirectly also important in economic terms.

Native to (or representative of) the ecosystem under study

According to Fernando (1994), the major difference in fish and zooplankton between tropical and temperate freshwaters is the predominance of Rotifera and herbivorous fish in tropical freshwater ecosystems versus Crustacea and non-herbivorous fish in their temperate counterparts (Fig. 1). Although rotifers were historically considered as cosmopolitan, a study into the world's distribution of rotifers by De Ridder (1981) indicated that although 52% are indeed cosmopolitan, the remaining 48% have a more or less limited distributional area of which 7% are endemic in some areas. In this regard, rotifer diversity has been reported to be highest in the (sub)tropics, with rotifer biodiversity hotspots in northeast North America, tropical South America, Southeast Asia, Australia, and Lake Baikal (Segers, 2008). However, as pointed out by Fontaneto et al. (2012), it should be taken into account that sampling intensity influences the results of diversity geographical distribution masking the actual distributional patterns, and that therefore this factor should be carefully considered before drawing conclusions from distributional analysis. Also, García-Morales & Elías-Gutiérrez (2013) called attention for the need of additional taxonomic molecular studies, such as DNA barcoding, throughout the world in order to understand the processes that drive global patterns of rotifer diversity.

Given the above, it may be deduced that rotifers and crustaceans are the dominant and keystone zooplanktonic invertebrates in tropical and temperate freshwaters, respectively. In addition, due to year-round predation by the great diversity of fish and invertebrate predators and increased metabolic costs with increasing temperatures, large cladocerans like *Daphnia* have been reported to be practically absent in the tropics (e.g., Dumont, 1994; Fernando, 1994). The ecological relevance of using toxicity data derived from tests with daphnids in tropical aquatic risk assessments is therefore highly disputable (Daam & Van den Brink, 2011; Allinson et al., 2011).

Table 1 Endpoints commonly analyzed when using rotifers as test organisms for pesticide toxicity assessments

Rotifer species	Pesticides (type: chemical class, common name)	Endpoints	References
Brachionus calyciflorus	Insecticide, organochlorine: dieldrin	Amitic females production	Huang et al. (2013)
Brachionus calyciflorus	Herbicide, carbamate: molinate and thiobencarb	Intrinsic population growth rate	Ferrando et al. (1999)
Brachionus calyciflorus	Fungicide, dicarboximide: vinclozolin	Morphological alterations	Alvarado-Flores et al. (2015)
Brachionus calyciflorus	Insecticide, organochlorine: endosulfan	Mortality	Fernandez-Casalderrey et al. (1992)
Brachionus calyciflorus	Insecticide, organophosphate: chlorpyrifos	Reproduction	Snell & Carmona (1994)
Brachionus calyciflorus	Insecticide, organophosphate: dimethoate	Swimming behavior	Guo et al. (2012)
Brachionus plicatilis	Insecticide/fungicide, organochlorine: pentachlorophenol	Enzyme activity	Moffat & Snell (1995)
Brachionus plicatilis	Insecticide, organophosphate: chlorpyrifos	Feeding	Juchelka & Snell (1995)
Brachionus plicatilis	Insecticide, organophosphate: diazinon	Resting egg production	Marcial & Hagiwara (2007)
Brachionus rubens	Insecticide/fungicide, organochlorine: pentachlorophenol	Life cycle	Halbach et al. (1983)
Brachionus sp.	Insecticide, triazine	Delayed development	Rioboo et al. (2007)
Lecane quadridentata	Insecticide, carbamate: carbaryl Insecticide, organophosphate: methylparathion	Probability of extinction	Pérez-Legaspi et al. (2010)
Asplanchna girodi *Euchlanis dilatata* *Keratella cochlearis* *Lecane quadridentata* *Lepadella patella* *Philodina acuticornis* *Plationus patulus* *Trichocerca pusilla*	Insecticide/fungicide, organochlorine: pentachlorophenol	Mortality and enzyme activity	McDaniel & Snell (1999)

Sensitivity

Rotifers have been shown to be among sensitive primary consumers, more sensitive to some chemicals than cladocerans (Snell & Joaquim-Justo, 2007; Dahms et al., 2011). On the other hand, rotifers are generally considered to have low sensitivity to pesticides and have frequently shown increased abundances in temperate model ecosystem studies evaluating insecticides as a result of decreased competition for food through the death of sensitive crustacean taxa (e.g., Brock et al., 2000; Hanazato, 2001). To evaluate this further, freshwater laboratory toxicity data for rotifers were compiled from the United States Environmental Protection Agency (US-EPA) ECOTOX database (http://cfpub.epa.gov/ecotox/) and compared with those for *D. magna* originating from the same source. Only data for compounds classified in the Alan Wood Compendium (http://www.alanwood.net/pesticides/) as insecticides or fungicides were

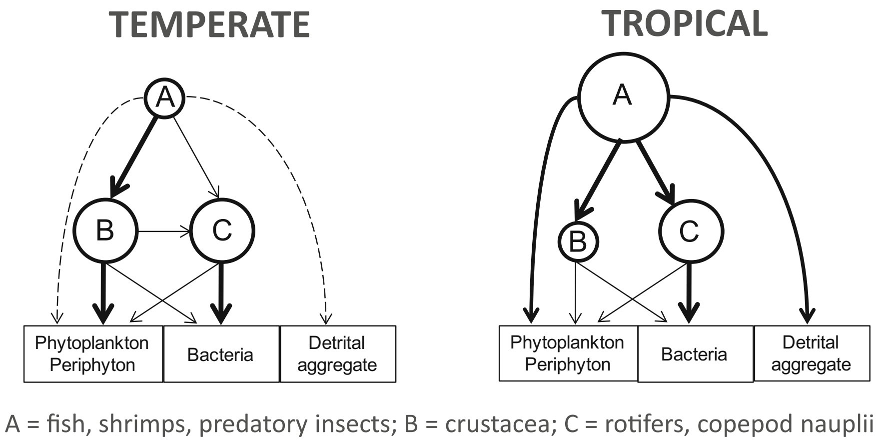

Fig. 1 Main freshwater ecosystem components and their food interrelationships in temperate versus tropical freshwaters. Sizes of the *circles* indicate the relative size of the ecosystem components, the thickness of the *arrow* the strength of the interaction between them, while its direction points to the consumer → food relationship. *Source* modified from Fernando (1994) and Daam & Vanden Brink (2011)

analyzed further since a greater sensitivity of rotifers may be expected for these pesticide types. To allow comparison of toxicity data between taxa, only data fulfilling the selection criteria provided in Table 2 were used. The geometric mean was calculated if more than one toxicity value was available for a given species and compound. Rotifer short-term toxicity data were obtained for 12 insecticides, but only almost exclusively for *B. calyciflorus* (see Supplementary Material Table S1). Besides carbaryl, for which the toxicity value of *B. calyciflorus* was approximately five times lower than that of *D. magna*, the latter species was one to four orders of magnitude more sensitive than *B. calyciflorus*.

Short-term fungicide data (see Supplementary Material Table S2), however, indicated that *B. calyciflorus* had similar toxicity to copper sulfate, greater sensitivity to pentachlorophenol (PCP; 2 times) and fluazinam (113 times), although it appears eight times less sensitive to vinclozolin than *D. magna*. The few fungicide toxicity data obtained for rotifer taxa other than *B. calyciflorus* showed a similar pattern. *B. rubens* showed a similar sensitivity as *D. magna* (and *B. calyciflorus*) to copper sulfate, whereas *Philodina acuticornis* was 60 times less sensitive to this compound but 33 more sensitive to chlorothalonil than *D. magna* (no data available for *B. calyciflorus*). Most rotifer toxicity data were obtained for PCP, and the sensitivity distribution of rotifer taxa and *D. magna* for this compound is provided in Fig. 2. *D. magna* as well as *B. calyciflorus* appears relatively tolerant to this compound, with several rotifers showing an up to two orders of magnitude greater sensitivity (Fig. 2). The suitability of the commonly tested *B. calyciflorus* as sole test species for the sensibility of all rotifers has indeed often been disputed (e.g., McDaniel & Snell, 1999; Wallace et al., 2006; Moreira et al., 2015). Locally available taxa other than *B. calyciflorus* are seldom considered but may show a greater sensitivity to chemical stress (e.g., Gama-Flores et al., 2004). In

Table 2 Selection criteria for short-term and long-term toxicity test data

	Short-term toxicity tests	Long-term toxicity tests
Endpoint	EC_{50}, LC_{50}	NOEC, EC_{10}, EC_{10}-20/2, MATC/$\sqrt{2}$
Test parameters	Mortality, immobilization	Growth, feeding, reproduction, development, mortality, or immobilization
Test duration (*d*)	1–7	>2

Adapted from Van den Brink et al. (2006) and EC (2011)

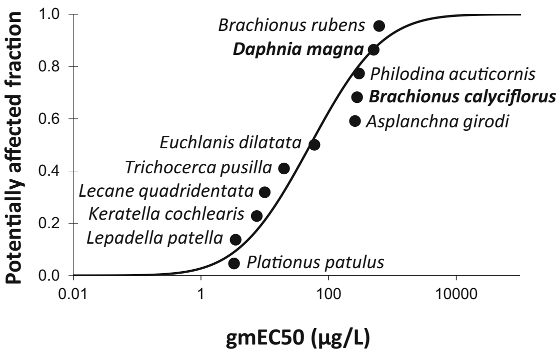

Fig. 2 Species sensitivity distribution constructed with EC_{50} data for pentachlorophenol (PCP) of rotifers and *D. magna*

line with this, the tropical rotifer *Keratella tropica* was noted to be the most sensitive zooplankton species to carbendazim in an outdoor model ecosystem study conducted in Thailand (Daam et al., 2010). In the same study, *B. calyciflorus* appeared to be among the most tolerant zooplankton taxa and increased in numbers even when exposed to the highest carbendazim concentration tested (Daam et al., 2010). These authors further noted that the temperate species *K. quadrata* was among the most affected zooplankton taxa in model ecosystem studies evaluating carbendazim conducted by Slijkerman et al. (2004) and Van den Brink et al. (2000). In a microcosm study evaluating the fungicide triphenyltin acetate, *K. quadrata* was also the most sensitive zooplankton taxon and showed a quick negative response to the fungicide (Roessink et al., 2006). Another *Keratella* species, *K. cochlearis*, was the most sensitive taxon of six rotifer taxa tested to the fungicide PCP as determined by swimming behavior and vulnerability to predation (Preston et al., 1999). Apparently, the genus *Keratella* includes the most susceptible representatives of rotifers and often even the zooplankton community to fungicides (Daam et al., 2010).

The greater diversity and limited distribution of rotifers in the tropics as discussed in the previous section also imply that the number of potential sensitive species that could be present and hence affected by pollutants is also greater in tropical freshwaters. In addition, several studies have shown that rotifer taxa previously considered to be cosmopolitan can be complexes of sibling species, including *B. calyciflorus* (e.g., Gilbert & Walsh, 2005). Different strains of the same species as well as experimental conditions (e.g., temperature) may influence sensitivity levels of rotifers (e.g., Serrano et al., 1986; Snell et al., 1991). Subsequently, it is imperative to test locally derived rotifers in tropical toxicity tests to obtain an accurate assessment of their true sensitivity (Moreira et al., 2015).

The toxicity values for rotifers available in the US-EPA database were almost exclusively short-term EC_{50} values. Besides NOEC values of the insecticide lindane for *Brachionus angularis* and *B. rubens*, NOEC values were exclusively encountered for *B. calyciflorus* and only for five insecticides and two fungicides (see Supplementary Material Tables S3, S4, respectively). This limited availability of chronic rotifer toxicity data has previously been noted by Preston & Snell (2001) and Marcial et al. (2005). These authors discussed that because only a portion of the rotifer life cycle is investigated in the acute EC_{50} rotifer toxicity tests, the true vulnerability of rotifer life cycles to toxicants is often underestimated. Especially fertilization appears to be a sensitive chronic endpoint, as demonstrated by Preston et al. (2000) for a number of potential endocrine disruptors including pesticides. Since pesticides are generally applied with a high frequency in the tropics (Daam & Van den Brink, 2010a, b; Sanchez-Bayo & Hyne, 2011), a chronic (pulsed) exposure and hence chronic toxicity tests may be especially relevant for tropical toxicity testing.

Besides a limited availability of chronic toxicity data, data on the bioconcentration and bioaccumulation potential of insecticides and fungicides were completely lacking in the US-EPA database. Given the prominent role that rotifers play in tropical aquatic foodwebs (Fig. 1), bioconcentration and bioaccumulation studies are relevant to assess the potential of pesticide biomagnification by rotifers. Sarma et al. (1998), for example, showed that rotifer prey (*B. calyciflorus*) exposed to sublethal methyl parathion concentrations for 2 h had a significant negative effect on the population growth of its predator (*Asplanchna sieboldi*). The great importance of the trophic pathway (food ingestion) and the great potential of pollutants to accumulate through the aquatic trophic food chain (algae, zooplankton, fish) have previously been demonstrated for metals (e.g., Dobbs et al., 1996; Alvarado-Flores, 2012) and polychlorinated biphenyls (e.g., Joaquim-Justo et al., 1995). For example, exposure to lead resulted in deposited granules of this metal in the mastax and vitellarium of *B. calyciflorus* (Alvarado-Flores et al., 2012), and *Lecane quadridentata* showed adverse effects on growth rate after lead bioaccumulation (Hernández-Flores & Rico-Martínez, 2006).

General remarks and conclusions

For the reasons discussed above, the use of rotifers as test organisms in ecotoxicology has been recognized for some time (e.g., Snell & Janssen, 1995; Gama-Flores et al., 2004; Snell & Joaquim-Justo, 2007). US-EPA (2001) also stressed the need for conducting more studies with rotifer taxa other than *B. calyciflorus* to allow comparing the intra- and inter-species differences in the sensitivities of rotifers to environmental pollutants. Besides acute toxicity testing, such studies should also include chronic evaluations and studies into bioaccumulation and biomagnification.

From the above, it may also be concluded that given their role and diversity in tropical freshwater ecosystems, this great potential of rotifers as test species holds especially true for the tropics (e.g., Snell & Joaquim-Justo, 2007). In a review paper by Organization for Economic Co-operation and Development as far back as 1998, rotifers were already identified as important test species to be included in warm freshwater environments. Future research efforts should shed light on (i) the acute and chronic sensitivity of local taxa, (ii) the bioaccumulation potential and possible role in biomagnification through the aquatic foodweb, and (iii) candidates to be used as tropical test species surrogates for *B. calyciflorus*.

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