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Workshop on rotifers in ecotoxicology

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Abstract The aim of the workshop on rotifers in ecotoxicology was to stimulate discussions on new developments in the field. Discussions about the use of biomolecular tools indicate that gene expression analysis with rotifers should be available in the next few years. Such analyses will be a great asset as they enable ecotoxicologists to study molecular mechanisms of toxicity. Rotifers also appear as useful tools in the risk assessment of pharmaceuticals and their metabolites that find their way into aquatic ecosystems because their sensitivity to some of these substances is higher than that of cladocerans and algae. The nature and extent of the impact of potential endocrine disruptors on aquatic invertebrates is another poorly resolved issue for which rotifers are a promising tool. Indeed, rotifers seem to be particularly sensitive to androgenic and anti-antiandrogenic substances, whereas copepods and cladocerans are typically more affected by estrogens and juvenile hormone-like compounds. Besides their usefulness in these emerging fields of aquatic ecotoxicology, it was emphasized that research with rotifers on basic issues like, e.g., toxicant interference with predation, competition, or interspecific and interclonal variation in ecotoxicological tests is still needed.

Keywords Ecotoxicology · Gene expression · Endocrine disruption

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Advances in Rotifer Research

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Introduction

The workshop on rotifers in ecotoxicology was aimed at stimulating exchange of ideas and discussions about advances in methods for studying ecotoxicology of rotifers. The general discussion was mainly focussed on the use of biomolecular tools and the use of rotifers in emerging areas of concern, i.e., to assess the impact of pharmaceuticals and endocrine disrupting substances on aquatic organisms. The overview of the discussions here concern two main topics and other issues that participants identified as areas that need to be addressed in the future.

New ecotoxicology methods using rotifers

Snell introduced this topic by describing the rapidly advancing rotifer nucleic acid sequencing projects currently underway under the guidance of David

Mark Welch. These include cDNA library sequencing of *Brachionus plicatilis*, to be followed in near future by partial genome sequencing. A parallel research effort in the named aspects in bdelloids is also in progress. These genetic advances are making it possible to construct microarrays of rotifer stress genes for expression analysis as has been done in many other animals.

The use of a quantitative analysis of rotifer stress gene expression to assess toxic responses stimulated much discussion. Rick Hochberg posed a question about the phylogenetic conservation of stress genes and whether we can expect stress genes isolated from one rotifer species to be conserved in other species. Peter Starkweather described the gene expression work currently being done on *Daphnia pulex* which found a group of genes up-regulated after exposure to cadmium. As a result of gene sequencing efforts in *Daphnia*, there are now more than 20,000 oligos representing most of the transcriptome available for microarray studies in ecotoxicology. The work on *Daphnia* is clearly "leading the way" for zooplankton. It was asked if sequences have been BLASTed in rotifers and reference was made to the collection of ESTs presented by Suga et al. at this meeting, and to the growing availability of such data Wheelbase (http://www.jbpc.mbl.edu/wheelbase/). To Alan Tunacliffe's question as to if libraries of stressor genes are built, Snell replied that libraries of such genes for rotifers do not exist yet, but that work with other animals (nematodes, *Drosophila*) should facilitate the effort.

Ramesh Gulati stressed the need for adequate explanation in the introduction of works on genomics so that readers not acquainted with the field can comprehend the work. Manuel Yúfera asked if proteomics are being used with rotifers; Snell specified that although proteins arrays are indeed useful, they are not yet available for rotifers.

Rotifers as tool for the assessment of pharmaceuticals and endocrine disruptors

Joaquim-Justo began the discussion by describing how human and animal pharmaceuticals and their metabolites find their way into wastewater or sewer systems. The efficiency of sewage treatment plants to remove these substances is variable (Kolodziej et al., 2003; Cargouet et al., 2004) and many pharmaceuticals and their metabolites have been detected in aquatic environments at concentrations where effects are expected (ng l^{-1} to μg l^{-1}) (Heberer, 2002; Pickering et al., 2003; Kolodziej et al., 2003). The most commonly encountered such substances are analgesics, antibiotics, anti-epileptics, anti-inflammatories, β-blockers, β_2-sypathomimetics, psychiatric drugs, and lipid regulators (Jones et al., 2001). To determine the impact of these chemical compounds on aquatic ecosystems, toxicity studies have been conducted using representative organisms from different trophic levels. Rotifers have been shown to be among sensitive primary consumers, more sensitive to some chemicals than cladocerans (Okay et al., 2005; Isidori et al., 2005).

Certain pharmaceuticals reaching the aquatic ecosystems interfere with the endocrine system of aquatic animals and have been shown to significantly affect, especially, fish at very low concentrations (Kime et al., 1995; Jobling et al., 1996). The other, more classical environmental contaminants, such as pesticides, have also been shown to interfere with the endocrine signaling in aquatic animals (Guillette et al., 1996; Mathiessen & Gibbs, 1998). Studies by Snell and his collaborators (Snell & Carmona, 1995; Gallardo et al., 1997; Preston et al., 2000) indicate that rotifers may be affected by chemicals via endocrine disruption. For example, Preston et al. (2000) observed depression of fertilization rate in *Brachionus calyciflorus* upon exposure to nonylphenol (estrogen agonist), testosterone (androgen agonist) and flutamide (androgen antagonist). The effects of this last chemical on sexual reproduction were significant at concentrations two orders of magnitude lower than the asexual no observed effect concentration (NOEC). Data presented at this symposium by Dingman, Joaquim-Justo, and Snell confirm the impact of other anti-androgens on the sexual reproduction of *B. calyciflorus* at concentrations one to two orders of magnitude lower than asexual NOECs. These results are consistent with an endocrine disruption mechanism, although they do not constitute a proof of this mode of action in rotifers.

Discussion on the commonness of environmental estrogens from natural sources like plants was initiated by Jim Green. Bob Wallace raised the question of bioconcentration and biomagnification of endocrine disruptors; Ramesh Gulati emphasized on the lack of our knowledge about the modes of entry of these chemicals—are these chemicals assimilated from the ingested food or are they directly taken up from water? Joaquim-Justo reported that accumulation patterns of substances of apparent concern for rotifers like, nonylphenol, testosterone, and fenitrothion have not been studied in planktonic animals. Two studies on the accumulation of estrone and other steroid estrogens in algae and daphnids, however, indicate that uptake of these molecules occurs mainly from the water. Bioconcentration factors (BCF) measured are low (≤ 326) and biomagnification is considered as unlikely in invertebrates (Lai et al., 2002; Gomes et al., 2004).

Judith Rios-Arana suggested that impacts of estrogens in natural rotifer populations were to be expected considering that most rotifer populations consist of females. Helen Marcial noted that 17β-estradiol is a common environmental contaminant, although it has no known effect on reproduction of *B. plicatilis* at fairly high concentrations. Likewise, 17α-ethinylestradiol and nonylphenol, two estrogenic contaminants frequently reported in surface waters, were found to have little effect on sexual reproduction of *B. calyciflorus* up to concentrations quite close to asexual NOEC (Radix et al., 2002). In contrast, androgens affected sexual reproduction at concentrations two orders of magnitude lower than the asexual NOEC (Preston et al., 2000). These results and those presented at this meeting by Dingman et al., indicate that sexual reproduction in brachionids is more sensitive to disruption by androgens than by estrogens.

Jim Green suggested a complementary approach to clarify the impact of estrogens on rotifers by using estrogen inhibitors on females. Helen Marcial further emphasized that resting-egg hatchability might be a useful endpoint. Indeed, in *B. plicatilis*, Marcial et al. (2005) have shown this endpoint to be particularly sensitive to pesticide exposure. However, Joaquim-Justo (personal observation) could not corroborate this finding in *B. calyciflorus*.

Bill Birky inquired whether the objective of using rotifers in determining the impact of endocrine disruptors (EDs) is to develop tests based on sentinel species aimed at detecting EDs in natural waters or to understand their ecotoxicological impact on rotifers and aquatic ecosystems. Joaquim-Justo responded by saying that both objectives are important and worthy of investigation. Endocrine disruptors (ED) have only recently been recognized as a potential problem in aquatic ecosystems. Knowledge of the endocrine systems of aquatic invertebrates is mainly based on arthropods: it is important to clarify ED responses in other groups such as rotifers since they are important among freshwater zooplankton. Such information can also be usefully exploited to develop biomarkers of exposure to androgens, given the apparent sensitivity of sexual reproduction of rotifers to several androgens.

Henri Dumont raised the question of difference in magnitude and type of response from short-pulse exposures rather than continuous exposure and the persistence of these substances in the environment (most putative EDs are short lived in aquatic environments). He said that in chydorids an exposure to the androgen methyl farnesoate elicits male production and that this might be a good strategy for rotifers as well. Stretching his argument further Dumont suggested to test bdelloids to examine if males, which have never been observed, are induced!

Helen Marcial emphasized the relevance of exposure time citing her own observations on the differential impact of diazinon on resting egg production and hatchability of *B. plicatilis* according to the developmental stage at which exposure occurred (Marcial & Hagiwara, results presented in this meeting). Bob Wallace questioned whether there is dose reciprocity in toxicant exposures (duration vs. intensity of exposure). Except for the work of Marcial and co-workers, there are so far no published data on this aspect of endocrine disruption in planktonic invertebrates.

Jorge Ciros brought the focus to effect of EDs on community dynamics as few studies have addressed the impact of EDs on planktonic communities. There is some evidence that planktonic communities are sensitive to nonylphenol and 17α-ethinylestradiol, this latter compound is known to affect only copepod densities (Hense et al., 2004), whereas nonylphenol led to lower abundance of all zooplankters (Severin et al., 2003). It should be kept in mind that the effects observed might be mediated through non-endocrine mechanisms and may be a general toxic response.

Gregor Fussman asked whether or not clonal variation needs to be taken into account in ED responses. Joaquim-Justo replied that although this aspect has not been addressed so far, her own results with *B. calyciflorus* and *B. plicatilis* reported in Marcial et al. (2005) revealed considerable interspecific differences in response to fenitrothion.

Ramesh Gulati asked which zooplankter would be best to assess ED: cladocerans, copepods, or rotifers? Joaquim-Justo replied that results from single-species laboratory experiments indicate that each group shows a specific sensitivity to each of the different EDs. In copepods, estrogen effects on developmental stages are observed at concentrations two orders of magnitude lower than the NOEC for acute toxicity (Marcial et al., 2003). Notably, considerable differences in sensitivity to estrogens are found in the literature, with effects often being observed at concentrations close to acute toxicity levels (Bechmann, 1999), or being absent (Andersen et al., 2001; Breitholtz & Bengtsson, 2001). Effects have also been observed with androgens and methoprene (insecticide, insect juvenile hormone agonist) but at high concentrations (Marcial et al., 2002). In cladocerans, androstenedione and testerone have been shown to affect the development of secondary sexual characteristics (Olmstead & LeBlanc, 2000) and 4-nonylphenol to decrease fecundity, with no effect on survival (Baldwin et al., 1997). The strongest impacts have been observed with methoprene, the insect juvenile hormone, methylfarnesoate, the crustacean juvenile hormone, and the insecticide pyriproxyfen, a juvenile hormone analog (Olmstead & LeBlanc, 2000, 2001, 2003). All these substances drastically increase the proportion of male offspring relative to females. Dieldrin, in contrast, was shown to decrease the proportion of male offspring in the population (Dodson et al., 1999). Even though juvenile hormone, estrogens and other vertebrate hormones have been shown to impact rotifers, androgen agonists and antagonists seem to have the strongest impact (Gallardo et al., 1997; Preston et al., 2000). Rotifers therefore seem to be a good candidate for the study of endocrine disruption by androgens.

Alejandro Pérez-Legaspi wanted to know if toxicokinetic studies of EDs with rotifers exist. Joaquim-Justo replied in affirmative for cladocerans, giving examples of studies on testosterone metabolism in *Daphnia* (Baldwin et al., 1997; Le Blanc & Mc Lahan, 2000) and toxicokinetics of putative EDs in molluscs. However, to her knowledge, such works are not available for rotifers.

Needs in rotifer ecotoxicological studies

While S.S.S. Sarma expressed the need to develop indicator species for ecotoxicological studies in tropical waters, S. Nandini stressed the importance of studying littoral taxa in ecotoxicology and questioned the representativeness of *B. calyciflorus* and *B. plicatilis* of all rotifers. They are both found only in the pelagic zone of lakes and the comparative sensitivity of littoral rotifers is unknown. A noteworthy exception is the work on *Lecane* by Roberto Rico-Martinez. Littoral species are associated with sediments and organic matter that are known to accumulate/adsorb contaminants. Thus, the use of littoral species would permit toxic effects to be estimated in these habitats.

Ramesh Gulati underscored the importance of studies on ecological interactions in ecotoxicology as they deal with processes like feeding on assimilation of toxic materials before the toxic effects become manifest. Atsushi Hagiwara described how daphnids are known to display escape behavior when exposed to contaminants and asked if rotifers exhibited similar behavior. There was a general consensus that no such studies have been conducted with rotifer thus far, and that it would be interesting to determine whether rotifers can detect contaminants. Terry Snell noted that, upon toxicant exposure, rotifers typically slow down their swimming speed and eventually sink. Henri Dumont and Peter Starkweather recalled the work of Janssen and Charroy and co-workers on the impact of toxicants on swimming behavior of *B. calyciflorus*. Swimming speed, sinuosity of swimming path and periods of activity were either not affected or more generally depressed proportional to dose of Cu, pentachlophenol, 3-4 dichloroaniline and to a lesser extent to lindane. Only sinuosity of swimming was increased with some chemicals (Janssen et al., 1994; Charoy et al., 1995).

Brian Dingman argued for the importance of exploring the impact of toxicants on rotifer predator-prey interactions. Studies by Irene Van der Stap and co-workers have provided a well-characterized rotifer predator-prey system for ecotoxicological investigations. In the same line of reasoning, Bill Birky suggested that Rick Hochberg's neurobiological studies might be useful to examine the impact of toxicants.

Conclusion

The workshop has been a good opportunity to outline new research fields in which rotifers are useful tools for investigations in ecotoxicology. Discussions about the use of biomolecular tools indicate that gene expression analysis with rotifers should be available in the next few years. Such analyses will be a great asset as they enable ecotoxicologists to study molecular mechanisms of toxicity. Rotifers also appear as useful tools in the risk assessment of pharmaceuticals and their metabolites that find their way into aquatic ecosystems because their sensitivity to some of these substances is higher than that of cladocerans and algae. The nature and extent of the impact of potential endocrine disruptors on aquatic invertebrates is another poorly resolved issue for which rotifers are a promising tool. Indeed, rotifers seem to be particularly sensitive to androgenic and anti-antiandrogenic substances, whereas copepods and cladocerans are typically more affected by estrogens and juvenile hormone like compounds.

Although rotifers are very useful in many types of ecotoxicological studies and are definitely a promising tool in emerging fields of aquatic ecotoxicology, certain basic issues still need to be clarified, as pointed out by several workshop participants. For example, the representativeness of the most commonly used rotifer species in ecotoxicological tests is questionable for tropical and littoral waters. Studies based on species more typical of these environments should be pursued. Toxicant interference with ecological interactions like predation and competition still need more investigation, as do the impact of interspecific and interclonal variation in ecotoxicological tests.

Research directed along the lines suggested in this workshop should both improve use of rotifers in ecotoxicological studies and help reveal new issues of concern for aquatic ecosystems.

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