CHAPTER 28

Microscale Toxicity Testing With Rotifers

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ABSTRACT

Toxicity tests are one of the essential tools for evaluating the effects of anthropogenic stress in aquatic ecosystems. Methods using microscale organisms have improved the speed, simplicity, and reduced the cost of making toxicity measurements. The size, ecology, and life cycle of rotifers makes them well suited for microscale assays and toxicity assessment. In the last five years the use of rotifers in routine toxicity assessment has dramatically increased, due primarily to the widespread availability of rotifer cysts and standardized protocols for performing tests. Development of new endpoints for rapid toxicity measurements are described, as well as their application to evaluating effluents, sediment and soil elutriates, and sediment pore waters. Most work has used 24-h acute tests with brachionid rotifers, but a standardized 2-d population growth test and a 7-d full life cycle test are available for estimating chronic toxicity. The chronic toxicity of several compounds has been evaluated and acute/chronic ratios of 0.9 to 33 reported. Behavioral assays of swimming are described that are based on computer tracking of rotifer movement. Methods for

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measuring ingestion rates are described using fluorescent microspheres and image analysis of single rotifers. Several substrates that are metabolized to fluorescent products have been used to measure *in vivo* enzyme activity. The reduction of enzyme activity with increasing toxicant exposure has been quantified in single rotifers with image analysis or in populations with a fluorometer. Results from changes in rotifer stress protein gene expression as a result of toxicant exposure are summarized. The need to relate biological effects at one level of organization to those at higher levels is discussed. Understanding how effects in population level processes like growth, predation, and competition translate into changes in community structure is especially critical. Several areas where rotifers could contribute to the understanding of how toxicants modify aquatic ecosystems are described.

I. INTRODUCTION

As the impact of environmental pollution and public awareness about it has increased, the need to monitor and control the release of toxic substances has grown. Measuring biological responses in test organisms is a key element in monitoring pollution effects. Conventional aquatic toxicity tests such as the internationally accepted methods with algae, cladocerans, and fish[1] have well-recognized limitations and are not sufficient in number or type for routine toxicity testing.[2-4] Some major drawbacks of conventional fish toxicity tests are: 1) problems in culturing test organisms — availability and response variability, 2) the need for large test volumes, 3) large amount of bench space required, and 4) the high costs of executing the tests. This has promoted the development of microbiotests which are rapid, small-volume toxicity tests with microscale organisms.[2,5] Although they are designed to be rapid, inexpensive, and simple, most of these screening assays attempt to retain high ecological relevancy. Recently, some advances in cell biology have been adapted for use in microbiotests, improving test speed, cost, and sensitivity.[6] Because of this, microbiotests can be used to assess toxic effects with more species. Risk assessments based on the toxic responses of several phylogenetically diverse species are more reliable for predicting real-world risks.

In this chapter, we describe how one group of microscopic animals, the rotifers, can be used for aquatic toxicity testing on a microscale. We describe features of their biology that enable them to be confined and manipulated in microvolumes and how this can be done while making ecologically relevant measurements.

II. ASPECTS OF ROTIFER BIOLOGY

A. Ecology

Rotifers play an important role in the ecological processes of many aquatic communities.[7,8] As suspension feeders, many species of planktonic rotifers influence algal species composition through selective grazing. Individual rotifers typically clear 1 to 10 µL/animal/h,[9,10] so that the rotifer zooplankton can collectively filter very large water volumes daily. They consume >10 times their body weight of dry mass per day, assimilating 20 to 80% of the energy.[11] Since rotifer populations can reach densities of >10/mL, rotifers make substantial amounts of biomass available to higher trophic levels.[12] Indeed, in oligotrophic and mixotrophic lakes, rotifers are probably the most important link between the pico- and nanoplankton and the macrozooplankton.[13] In eutrophic lakes, the importance of rotifers in the microbial loop is probably diminished as protozoans account for more of the bactivory.[14] Rotifers also often compete with cladocerans and copepods for phytoplankton in the 2 to 18 µm size range. Along with crustaceans, rotifers contribute substantially to nutrient recycling,[15,16] further influencing phytoplankton composition.

Because they reproduce rapidly, rotifers can account for >50% of zooplankton production.[17] This biomass is utilized by several predators including other rotifers like *Asplanchna*,[18] copepods,[19] insect larvae,[20] and fish.[21] Rotifers are often the first food of many larval fish, a fact that has led to their extensive use in aquaculture.[22,23]

B. Phylogeny

Rotifers are members of the animal phylum Rotifera, one of several phyla of lower invertebrates.[24] Recent phylogenetic analyses suggest that the closest relatives of the Rotifera are the Acanthocephala and Nematoda.[25,26] Rotifers are phylogenetically distinct from the other major zooplankton groups. Other major zooplankters such as the Cladocera and Copepoda are sister lineages in the phylum Arthropoda, whereas ciliates are classified in the kingdom Protista. This phylogenetic distinction suggests that the response of rotifers to xenobiotics may be quite different at the whole-organism level from that of cladocerans, copepods, and ciliates. Consequently, water quality criteria based on the responses of cladocerans or fish may or may not be protective of rotifers. As rotifers play a major functional role and represent an important element of biological diversity in aquatic environments, it could be argued that rotifers should be considered separately in the analysis of ecotoxicological effects.

C. Distribution

The phylum Rotifera is comprised of about 3000 described species, most of which are freshwater zooplankters in the class Monogononta.[26] These species are found in all major habitats from soils, mosses, and sediments, to freshwater and marine zooplankton.[24] Many species have broad geographic distributions; some are even pan-global. It is possible, therefore, to select species as ecotoxicological models that represent a particular habitat worldwide. For example, the planktonic species *Brachionus calyciflorus* is found in lentic freshwater habitats on all continents, and it is an ecologically significant component of many natural water bodies.[26] Thus, ecotoxicological results can be globally compared with high ecological relevancy.

D. Life Cycle

The rotifer life cycle is based on cyclical parthenogenesis where asexual reproduction predominates with occasional periods of sexual reproduction[24] (Figure 28.1). Asexual females produce genetically identical daughters via mitosis and are employed exclusively in ecotoxicology. For toxicity testing, these females are obtained from stock cultures or by hatching dormant embryos called cysts.[27] It is these cysts, which are described below, that give rotifers and other taxa producing dormant stages a special advantage in ecotoxicological studies.[3,28,29] Cysts are the product of sexual reproduction between ephemeral males and sexual females. To our knowledge, male rotifers have not been used in ecotoxicological studies, but there has been some recent work on the response of sexual female reproduction to toxicants.[30] Snell and Carmona[30] found that the rate of sexual female production, the initial step in sexual reproduction, is more reduced by pentachlorophenol, chlorpyrifos, cadmium, and naphthol than the rate of asexual female production. Fertilization rate and males produced per female were unaffected by the four chemicals at the concentrations tested.

E. Rotifer Size and Its Consequences

Rotifers are among the smallest metazoans, ranging in size from 50 to 1500 μm.[24] Their small size provides several advantages for use in ecotoxicological investigations. For example, typical exposure volumes for effluent or sediment pore water tests with rotifers range from 300 μL to a

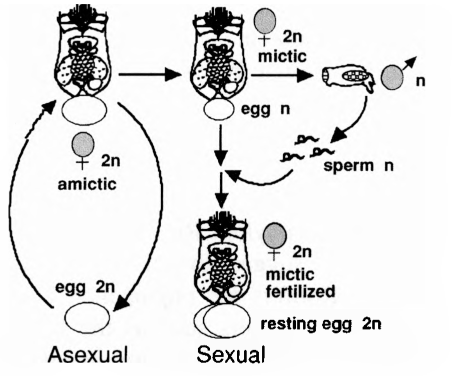

Figure 28.1 The rotifer life cycle.

few mLs depending on type of test. The ability to use small sample volumes can considerably reduce costs when working with pore water, solid waste elutriates, or when conducting toxicity identification evaluations.[31] Small size also permits a relatively large number of rotifers to be exposed in each treatment, increasing the power of statistical tests. Although small, rotifers are large enough so that single individuals can be easily transferred using standard plastic micropipets at 10 to 20× magnification using a dissecting microscope.

Rotifers also have one of the shortest generation times of any animal so that population growth rates can be measured in just a few days.[32,33] The population dynamics of rotifers are similar to bacteria, and some microbiological techniques are applicable to rotifers, such as growth in chemostats.[34] Test chambers developed for the mass microbiological market like multiwell microplates are well suited for rotifer experiments. This is important since many automated instruments are based on the microplate format.

The small size of rotifers allows them to be grown in continuous culture in chemostats. In fact, they are the only metazoan that can be grown in such cultures.[34] Other zooplankters like cladocerans have high enough growth rates for continuous cultures. However, they have a relatively long time to first reproduction, resulting in long residence times and time delays. These life cycle traits require very large culture vessels with small flow rates, all of which combine to prevent successful continuous culture. Chemostat culture of rotifers permits different kinds of experimental designs for toxicity analyses such as bioaccumulation studies and long-term, low level continuous exposures.

For example, the application of chemostats in toxicity studies is described for *B. calyciflorus*.[35] In these cultures, there were no detectable adverse effects at 0.02 m*M* and 0.06 m*M* lead after a 25-day exposure. The lethal effects of lead were detected at 0.6 m*M* and sublethal effects at 0.2 m*M*. At 0.2 m*M*, rotifers were washed out at a rate equal to the dilution rate, indicating cessation of growth, but no excess mortality. The use of continuous cultures allowed for accurate determination of lethal and sublethal thresholds of lead toxicity in exposures lasting 10 to 12 generations.

III. ROTIFERS IN AQUATIC TOXICOLOGY

A. History

Although rotifers have been used for experimental toxicity studies since the 1970s, it has been only in the last five years that they have been used routinely in toxicity testing.[27] The vast majority of these studies have used species in the genus *Brachionus*. This strong reliance on *Brachionus* has been more a matter of convenience rather than being based on any scientific rationale. It is not known how sensitive brachionids are to environmental contaminants as compared to other rotifer species.

The Brachionidae is certainly a large family that is distributed widely and abundant in many water bodies. However, other rotifer families, like the Asplanchnidae, Euchlanidae, Lecanidae, and Synchaetidae as well as the bdelloids, also have many species. The evaluation of the comparative sensitivity of some species in these families should therefore be recognized as an important research need.

Most studies on rotifers in aquatic toxicology have used mortality as an endpoint in one- or two-day exposures. A standardized protocol has been approved by ASTM[36] for estimating acute toxicity using the freshwater *B. calyciflorus* or the marine *B. plicatilis*. In addition to the rotifer acute tests, a variety of toxicity tests based on sublethal endpoints has also been developed.[27]

Perhaps the most significant recent advance in the use of rotifers in toxicity assessment is the controlled production of cysts.[28,29] Rotifer cysts, which can be stored for years, can be hatched in about 24 h by placing the dried cysts in water at 25°C in light. The neonates emerge synchronously and can be used in a variety of toxicity tests. As emphasized by several authors, eliminating the need to culture test animals results in significant savings in time, simplicity, and overall cost, while increasing the standardization and reproducibility of toxicity tests.[3] All labs can use the same strain and start a test with animals in the same physiological condition.[2,5] Rotifers and a few species of anostracan crustaceans are the only animals for which cysts currently are available commercially for toxicity testing. Recent advances in the controlled production of cladoceran ephippia suggest that these resting stages also will be available soon, according to G. Persoone of Belgium.

B. Recent Advances in Rotifer Toxicity Testing

The use of rotifers in ecotoxicity tests has increased in the past five years (Figure 28.2). Although rotifers have been used frequently in fundamental toxicological work, most test methods have not progressed past initial development. Only the acute (mortality) toxicity test with the freshwater rotifer *Brachionus calyciflorus* and, to a lesser extent its marine counterpart *B. plicatilis*, has been extensively evaluated as a routine toxicity screening tool. A review of recent advances in ecotoxicological research with rotifers, organized according to endpoints, is given below.

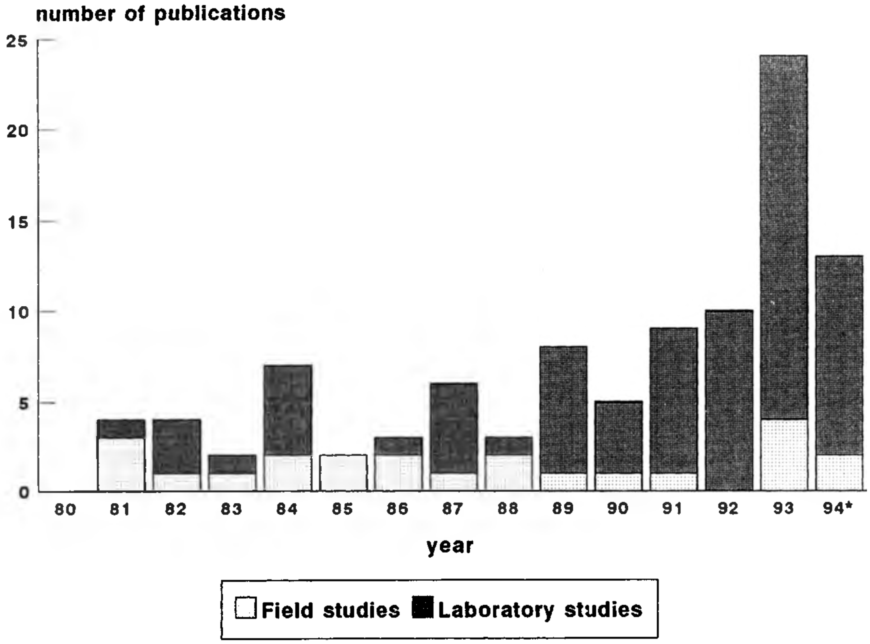

Figure 28.2 Temporal trends in the use of rotifers for ecotoxicological purposes. (Data from Poltox I database (Silverplate International). (*): period 1 January 1994 -31 March 1995.)

1. Mortality Assays

Standard protocols for performing a 24-h LC_{50} test with *B. rubens* (freshwater) and *B. plicatilis* (marine) were first described in 1989.[28,29] The freshwater species was, for reasons of convenience, replaced by *B. calyciflorus*.[37] These rotifer assays, which use dormant stages to obtain test organisms, have been incorporated in toxicity testing kits (Toxkits) and commercialized as the Rotoxkit F and Rotoxkit M tests.[3] An extensive review of the methodology, advantages, and limitations of these and other cyst-based toxicity tests is given in Chapter 30 in this volume.[38]

Several authors have reported on the use of acute rotifer tests in ecotoxicological studies. Acute (cyst-based) toxicity tests with *B. calyciflorus* and *B. plicatilis* were used as part of a battery of screening assays to determine the toxicity of the 50 priority chemicals of the Multicentre Evaluation of *in vitro* Cytotoxicity (MEIC) program in Europe. In a series of papers, Calleja and co-workers reported on the predictive power of these screening tests for evaluating human toxicity of various classes of chemicals.[39-42] Persoone et al.[31] have assessed the potential use and sensitivity of the *B. calyciflorus* test in a toxicity monitoring study of 398 environmental samples including effluents, sediment pore waters, solid waste elutriates, and monitoring wells. The rotifer assay was at least as sensitive as the acute *Daphnia magna* test for 62% of the samples. The Microtox® test was in 68% of the cases more sensitive than *B. calyciflorus*. However, this study also demonstrated that, for most samples, the rotifer assay gave nonredundant data. Similar evaluations of the freshwater rotifer test for screening environmental wastes have been reported.[43,44]

Recently, Keddy et al.[45] critically evaluated various whole-organism bioassays for the assessment of soil, freshwater sediment, and freshwater quality for their application in the Canadian National Contaminated Sites Remediation Program.[46] Of the 25 toxicity tests considered for freshwater quality assessment, three tests (the 72-h algal growth inhibition test with *Selenastrum capricornutum*, the 48-h survival test with *Daphnia magna*, and the 5- to 15-min bacterial test with *Photobacterium phosphoreum*, now named *Vibrio fischeri*) were selected for the recommended screening battery. The 24-h acute toxicity test with *B. calyciflorus* was suggested for the augmented screening battery. It was further recommended that a definitive testing battery should consist of the three above-mentioned screening tests plus an acute fish test. Also recommended was inclusion of the acute rotifer assay in the augmented definitive battery.

An in-depth analysis of the potential use of microbiotests was performed by Willemsen et al.[5] In their conclusions, these authors considered both the marine and freshwater rotifer test as practical and easy tests, exceptionally well documented and completely standardized. In comparison with the freshwater microbiotest with *Thamnocephalus platyurus*, the average sensitivity of the freshwater rotifer test for chemicals and environmental samples scored somewhat lower. A similar critical evaluation of the scientific and economic aspects of various conventional and alternative toxicity assays, including the *B. calyciflorus* test, is given by de Zwart.[47]

2. Reproduction Assays

A number of short-term tests to estimate chronic toxicity with rotifers, primarily *B. calyciflorus*, have been described.[32,33,48] In the simplest of these tests, the number of rotifers in a population in log-phase growth is counted at two time points and population growth rate (r) is estimated. For example, Janssen[48] and Janssen et al.[33] compared the sensitivity of a 72-h *B. calyciflorus* reproductive test used to estimate r with that of the 24-h LC_{50} test for copper, pentachlorophenol (PCP), 3,4 dichloroaniline (DCA), and lindane. The resulting acute to chronic ratios (A/C) were 1.2 and 12.4 for copper and 3,4 dichloroaniline, respectively. Using a 48-h toxicant exposure, a similar comparison was made by Snell and Moffat[32] for 11 chemicals. The A/C ratios ranged from 0.9 (2,4 dichlorophenoxyacetic acid) to 33 (chlorpyrifos). Such ratios are within acceptable limits as found for other species.

Because of their short generation time and lifespan, rotifers are ideally suited for life cycle toxicity testing. Under ecologically relevant conditions (25°C), *B. calyciflorus* typically produces, depending on the food availability, between 5 and 18 offspring during its lifespan which ranges from 5 to 7 days.[33,48] In life cycle toxicity tests, isolated neonates (<2 h old) are exposed to the test medium containing algal food in multiwell plates. Every 12 or 24 hours, the number of attached eggs, offspring, and mortality is recorded. Test medium is renewed daily, and the assay is terminated when the last rotifer dies, usually after 6 to 10 days. From these observations, survival and fertility tables are constructed and the following demographic parameters calculated: intrinsic rate of natural increase (r_m), net-reproductive rate, generation time, and life expectancy. Based on a series of life table experiments with Cu, PCP, DCA, and lindane, these authors showed that 95% of r_m calculated from an entire life table was reached after only 4 days and concluded that a life cycle test with rotifers can be conducted in one work week without loss of information or sensitivity. Comparison of the threshold toxicity levels obtained for the above-mentioned chemicals in life cycle tests with those of 3-day population growth tests showed that both tests have similar sensitivity.[33] The latter test, which only requires the initial setup and the final scoring of the number of rotifers after 2 or 3 days, could thus be considered a simple alternative to the more labor-intensive life cycle tests. Using life table techniques, the effect of methyl parathion, diazinon, and endosulfan on the demographic parameters of *B. calyciflorus* also was studied by Fernandez-Casalderrey et al.,[49-51] again demonstrating the usefulness of rotifers for ecotoxicological investigations of the entire life cycle.

3. *Behavioral–Physiological Assays*

A number of rapid toxicity tests based on behavioral endpoints such as swimming and feeding has been described.[27] The swimming behavior of *B. calyciflorus* exposed to Cu, PCP, DCA, and lindane, for periods ranging from 5 min to 5 h was studied by Janssen et al.[52] For the 3 of the 4 chemicals tested, the 3-h EC_{50}s based on swimming activity were lower than the 24-h LC_{50}s obtained in mortality tests. This assay, based on visual observation of individual rotifers swimming over a grid, is labor intensive and requires at least 3 h to complete. Recent developments in computer-aided video image capture have made it possible to acquire these data automatically. Charoy et al.[53] and Charoy and Janssen,[54] for example, have used computer-assisted data acquisition for tracking the swimming behavior of *B. calyciflorus* exposed to Cu, PCP, and DCA. After exposures of 5 min to 6 h, juvenile rotifers were placed individually in a microscale chamber and their swimming path recorded and digitized using a PC. From these data the average swimming speed, sinuosity, and the duration of swimming were calculated. In general, results similar to those of Janssen et al.[52] were obtained. Because rotifer swimming behavior responds rapidly and is a sensitive indicator of toxicity, automated data acquisition should expand the usefulness of behavioral endpoints in toxicity assessment.

Ingestion is an ecologically important behavior that has direct effects on growth and reproduction. The feeding activity of rotifers can easily be assessed by allowing a small population to feed on a known quantity of unicellular algae for a specified time and then quantifying the number of algal cells remaining at the end of the test. A 5-h toxicity screening test based on the feeding of *B. calyciflorus* was described by Janssen,[48] Janssen et al.,[55] and Ferrando et al.[56] Similar techniques were used to assess the effects of methyl parathion on the ingestion rate of *B. calyciflorus* and *Daphnia magna*.[51]

Juchelka and Snell[57,58] used fluorescently labeled 2 μm microspheres rather than algae to assess the effects of toxicants on the ingestion rate of *B. calyciflorus* and *B. plicatilis*. Since these rotifers are relatively nonselective suspension feeders,[59] they ingest more than a hundred of these microspheres in five minutes. Ingestion rate was quantified using image analysis by measuring the intensity of fluorescence in the gut of a single rotifer and dividing by the intensity of a single microsphere. For the 10 chemicals tested, these authors found that the ratio of NOECs based on 48-h reproductive rate (r) to those of the 1-h ingestion assay ranged from 0.13 to 1.0.

4. Biochemical and Molecular Assays

The use of *in vivo* enzyme activity as an endpoint in toxicity tests with invertebrates was first introduced for rapid toxicity screening with *Daphnia magna*.[6] This type of assay uses commercially available substrates that are initially nonfluorescent, but yield fluorescent products when cleaved by endogenous enzymes within the test animals. These probes of intracellular enzyme activity have been used as sublethal endpoints to assess toxicity in *B. calyciflorus*[60] and *B. plicatilis*.[61] *In vivo* esterase activity has proven especially useful, and it decreases in a concentration-dependent manner with toxicant exposure. The size of rotifers has allowed these enzyme reactions to be quantified in small rotifer populations in 200 μL using a 96-well microplate and an automated fluorometer or in single rotifers using an image analysis system.

Based on the rationale that the expression of certain classes of genes might be increased or decreased upon toxicant exposure, genes coding for stress proteins have proven useful for assessing toxicity in some aquatic species.[62] The expression of these genes in the rotifer *B. plicatilis* has been investigated[63,64] and techniques for visualizing stress protein abundance on Western blots with antibodies have been described. The abundance of a 58 kD stress protein (SP58) was increased fourfold upon exposure to a sublethal concentration of copper. The maximum SP58 increase occurred at a concentration of 5% of the copper 24-h LC_{50}, suggesting that stress protein abundance may be quite a sensitive endpoint. Although a similar response was detected for tributyl tin, several other toxicants had no effect on SP58 abundance. Antibody quantitation of stress protein abundance in rotifers needs more research before it can be widely applied for toxicity assessment. This technique, however, has good potential for automated microscale testing with existing instruments capable of making densitometric measurements.

Success in using SP gene mRNA abundance as an indicator of stress in *B. plicatilis* also has been reported.[64] The mRNA abundance was detected in dot blots using oligonucleotide probes generated using the polymerase chain reaction. This approach has potential for identifying particular genes involved in stressor-specific responses that could be useful as biomarkers.

5. Microcosm Assays

The small size of zooplankton and phytoplankton make it possible to construct a "realistic" aquatic community on a microscale in the laboratory. A three-liter standard laboratory microcosm was described by Taub[65] which is composed of rotifers (the bdelloid *Philodina*), algae, and crustaceans, all obtained from laboratory cultures. These microcosms are small enough so that sufficient replicates can be set up to allow a rigorous design and statistical analysis of the data. The microscale nature of this gnotobiotic community also allows more plant and animal species to be included in the microcosm than is possible with larger organisms. Exposure periods are typically 60 days, during which population density of each species is measured at regular intervals. This experimental system has the advantage of maintaining a considerable fraction of the ecological complexity of aquatic communities, such as competitive and predator-prey interactions. These interactions could well be more sensitive and/or more ecologically relevant measures of toxicity than traditional endpoints used in single-species tests, as stated many times by John Cairns and colleagues.[66] A similar microcosm has been described by Sugiura[67] that uses several species of bacteria, ciliates, algae, and the rotifers *Philodina* and *Lepadella*.

IV. RELATING EFFECTS ON DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

Although the ultimate goal of ecotoxicological testing is to predict the ecological effects of chemicals and other stressors, currently methods are inadequate to reach that goal.[66,68-70] The scientific basis for the development and application of toxicity tests is limited because most basic scientific questions about the extrapolation from one level of biological organization to another remain unanswered. As yet, insufficient research effort has been directed toward understanding how environmental stress affects biological systems at different levels and how adverse effects at one level affect higher levels.[71] Because of their size and life cycle, rotifers can be useful as models to study the effects of xenobiotics at multiple levels of biological organization.

Some examples of research on the effects of xenobiotics on different levels of organization have been reported. Relationships between different levels like swimming and feeding behavior, and individual life history parameters and population dynamics of *B. calyciflorus* exposed to several toxicants were investigated by Janssen et al.[55] and Janssen.[48] A comparison of toxicant concentration thresholds found for the various test endpoints is given in Table 28.1. The relationship between ingestion rate and reproduction was also studied by Juchelka and Snell.[57] Using the same species, they examined the effect of chemicals on feeding behavior (ingestion rate) and how this subsequently affected reproduction at the population level. In the ingestion test, rotifers were exposed to pentachlorophenol, chromium, chlorpyrifos, or naphthol for 30 min at 25°C. Ingestion rate was estimated by feeding rotifers fluorescently labeled beads and quantifying with image analysis gut fluorescence intensity in single females. The reproductive test exposed rotifers for 48 h and population growth rate (r) was measured. For three of the four toxicants, the lowest observed effect concentration (LOEC) was identical for ingestion rate and reproductive tests. For naphthol, the ingestion LOEC (1.2 mg/L) was actually lower than the reproductive LOEC (2.4 mg/L). These results suggest that the rotifer 30-min ingestion test is a good predictor of the 48-h reproduction test LOEC, pointing to the strong relationship between ingestion and reproduction.

Rotifers and other small zooplankters like cladocerans and copepods are ideal for the experimental analysis of the relationship between endpoints. Future research along these lines, for example, how changes in stress protein gene expression or esterase enzyme activity affect reproduction, should be encouraged. A mechanistic understanding of toxicity effects requires such knowledge and will allow for the development of more appropriate biomarkers.

V. FUTURE APPLICATIONS

In most freshwater planktonic habitats, several rotifer species are present as part of the herbivore guild. In temperate and tropical lakes, there are typically 5 to 10 rotifer species, depending on the season.[72] This author further reported that the number of planktonic rotifer species resident in a lake over an extended period of time may be as high as 20 to 35, with the percentage of the most abundant species (dominance) ranging from 40 to 70%. Two symptoms of rotifer communities under stress have been noted: decrease in the number of species and an increase in dominance. A comparison of the differential sensitivities of rotifer species might help explain the patterns of dominance that are observed in natural rotifer assemblages exposed to anthropogenic impacts.[73-77]

An unexplored area of aquatic toxicology is whether species interactions like predator–prey relationships are disrupted at lower toxicant concentrations than single species survival and reproduction. This is important to know since water quality criteria are based exclusively on single species tests. The predator–prey interaction between the rotifers *Brachionus calyciflorus* and *Asplanchna girodi*[18] is an excellent model for examining the effect of toxicants above the single species level. The existing database of toxicant effects on the reproductive rate of *B. calyciflorus*[32,33] permit single species effects to be compared to predator–prey interactions. It would be an important result if the predator–prey interaction was disrupted at toxicant concentrations below that affecting reproduction in either species. The small size of rotifers allows for the experimental analysis of this type of problem quickly and inexpensively with rigorous experimental designs.

The use of rotifer behavior for toxicity assessment is becoming easier with computer-assisted data acquisition. Motion analysis systems for tracking swimming behavior in many animals simultaneously

Table 28.1 **Comparison of Threshold Toxicity Levels for Cu and Pentachlorophenol (PCP) Obtained with *Brachionus calyciflorus* in Toxicity Tests with Various Endpoints**

	Acute Test	Swimming Activity Test			Feeding Activity Test			Short Chronic Test		Life Table Studies		Population Studies	
Criterion	Mortality	Swimming activity			Ingestion			Population growth		Population growth		Carrying capacity	
Exposure period	24 hours	3 hours			5 hours			3 days		10 days		28 days	
Endpoint	LC_{50}	EC_{50}	LOEC	NOEC	EC_{50}	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC
Cu (μg/L)	26	15	12	6	32	20	12	5	2.5	5	2.5	10	2.5
PCP (mg/L)	0.92	2.1	1.0	0.5	1.85	1.0	0.5	0.8	0.4	0.4	0.2	0.4	0.1

Data from references 33, 48, 52, 55, and 56. See text for explanation.

are now available.[78] Calculations of swimming speed, direction, turning frequency, and other parameters can be made automatically in a few seconds in 500 μL in multiwell plates. Image analysis also is finding applications in aquatic toxicology and has been used to quantitate the intensity of fluorescent signals in individual rotifers. Fluorescent markers have been used to measure the effects of toxicants on ingestion rate and *in vivo* enzyme activity in single individuals. Such applications are likely to expand as image analysis systems continue to decrease in price and software improves for automated image recognition.

As aquatic toxicology matures as a science, more emphasis is being placed on understanding the mechanisms of toxicity. As microscopic animals, rotifers are excellent experimental systems for investigating the relationship between molecular and cellular events and survival and reproduction at the whole-organism level. Better understanding of the effects of xenobiotics on biological systems will permit the identification of enzymes playing a regulatory role in the stress response. Similarly, regulation of the expression of specific genes could be implicated in the response to toxicant exposure. Knowledge of these stress responses will permit the design of specific molecular probes to track these endpoints and to use them in toxicity assessment. Automated measurements on small animals will surely play a central role in these advances in measuring toxicity.

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