



## **International Conference on Rotifers**

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## Abstracts



## Rotifer species from the deep sea

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This is the first time rotifers are reported from the deep sea. Rotifers of the taxon *Lecane* were present in several samples from the Antarctic and the Pacific. The samples from the Antarctic were obtained during the Andeep I - Expedition in January and February 2002 in depth of 2274-2917 m and from the southern crest of the Hydrate Ridge of the coast of Oregon, Cascadia convergent margin during RV Sonne cruise 143-2 in August 1999 in a water depth of about 780m and in a basin adjacent to Hydrate Ridge in 2304 m depth. The sediment samples were fixed in 10% formalin. The rotifers were extracted using LUDOX AM floating technique.

The Pacific samples contained two presumably new species of *Lecane*. One of these species resembles *Lecane luna* the other one *Lecane (Monostyla) arcuata*, which are known from limnetic habitats. The species, which resembles *L. luna*, was also present in the samples of the Antarctic. Both *Lecane* species from the deep sea of the Pacific settle in high abundance in anoxic and highly sulphidic sediments associated with outcropping gas hydrates (GH)! They co-occur with sulphide oxidising bacteria *Thioploca* sp.

## **Revisiting seasonality of rotifer populations of Lough Neagh, Northern Ireland: A time series analysis approach**

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Long runs of seasonal rotifer population data allow analysis of seasonal occurrence using mathematical tools. The application of Fourier analysis to a 15year data set describes seasonality in simple mathematical terms. This facilitates comparison of population expression with potential population driving variables and provides a basic modelling tool. Results show that annual patterns of occurrence and density are strongly influenced by maximum and minimum environmental temperature. The data set also represents a powerful case for the collection of long term limnological data sets.

## Temporal changes in the rotifer community from a stressed wetland, "Las Tablas de Daimiel" National Park, Spain

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The National Park "Las Tablas de Daimiel" is an eutrophic wetland in a semi-arid region in the Iberian Peninsula. Water inputs to the wetland are irregular and hydrological fluctuations are strong. Physico-chemical variables of the water (conductivity, light penetration, nutrient concentration) show pronounced variations. Plankton community from the wetland has been studied since 1996. We present the zooplankton data (densities and biomass) at one station, focusing on temporal changes. Rotifers were the most diverse group and they dominated the zooplankton densities, especially during summer. The rotifer numbers fluctuated seasonally and among years, an average of around 50 species was found each year. Two genera characteristic from shallow waters, *Lecane* and *Brachionus*, showed the highest specific richness. The most abundant rotifer species also fluctuated among years and seasons; they belong to genera as *Synchaeta*, *Brachionus* and *Keratella*. Another aspect of studies is directed to check the fitness of zooplankton data with a general model designed for the plankton dynamics of eutrophic semiarid wetlands.

**The phylogenetic relationships of *Squatinella* Bory de St. Vincent, 1826 and *Bryceella* Remane, 1929 (Rotifera)**

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*Squatinella* (Colurellidae) and *Bryceella* (Proalidae) were examined morphologically. An extended phylogenetic analysis of the taxa Colurellidae and Proalidae especially of *Squatinella* and *Bryceella* lead to a sistergroup-relationship hypothesis between the last two taxa, although *Squatinella* and *Bryceella* belong to different families in the traditional system. Besides, our results confirm findings of authors of the 19th century who already mentioned a close relationship of the species of *Squatinella* and *Bryceella*.

Additionally, the work revealed an urgent necessity for a standardized method of describing taxa and establishing adequate synonymy combined with a detailed re-examination of all involved taxa.



## Several methods for improving status mass culture of rotifer strains

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We studied if manipulating the salinity (4-40 ppt) and addition of gamma aminobutyric (GABA) at 50 mg.l<sup>-1</sup>, are useful in stabilising the status of mass cultures strains (Tokyo, Russia and Australia) of *Brachionus plicatilis*. The cultures were exposed to stressful environment: by increasing the concentration of free ammonia (2.4 mg.l<sup>-1</sup>), contamination with protozoa *Euplotes* sp. (10 cells.ml<sup>-1</sup>) and addition of methyl cellulose to increase the culture water viscosity to 15 cp. Reproduction and enzyme activity were measured (esterase, phospholipase and glucosidase) to determine the effects of treatments. All tests were conducted at 25°C and rotifers fed *N. oculata* at 7x10<sup>6</sup> cells.ml<sup>-1</sup>. Tokyo strain was most resistant against the stressors, and culturing at low salinity was least stressful for rotifers. Combined effects of stressors significantly suppressed the lifespan and caused a fast decline in fecundity and glucosidase activity compared with those of controls (p<0.01). In contrast, reproductive output and glucosidase of GABA treated rotifers were significantly higher than those of controls.

## **Influence of biotic and abiotic factors on psammic rotifers in artificial reservoirs and natural lakes (Poland)**

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Psammic rotifers of lakes have not been intensively studied in man-made reservoirs of Upper Silesia. Such reservoirs are usually formed due to flooding sand works by polluted waters from rivers. They are characterized by large sandy beaches. Psammic rotifers were studied in three artificial reservoirs from spring to autumn 2002, as well as in three natural lakes in the West Lake District in Poland. These lakes were used as reference waterbodies. Samples were collected in three zones of sandy beach: one metre from the shoreline (euarenal), near the shoreline (hygroarenal) and from sub-surface (hydroarenal). The relationship between abundance and the species composition of psammic algae and rotifers was investigated. Also, the influence of substratum texture on the abundance and composition of rotifers in the psammon was studied. The structure of rotifer communities in the man-made reservoirs and natural lakes differed. However, their qualitative composition of rotifers was similar. The rotifer community in the psammon of the highly polluted "Dzierzno Duze" reservoir differs significantly from the communities in the other reservoirs. This reservoir is supplied by water from the Klodnica River, which carries municipal and industrial wastewater. The differences in substratum texture of the studied reservoirs were small. The influence of algae on rotifer communities is uncertain.

## **Speciation and selection without sex.**

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Basic evolutionary genetic theory predicts that asexual organisms should be able to speciate, but it is not clear whether they form species as readily as sexual organisms. We sequenced the mitochondrial *cox1* gene from 125 bdelloids collected from a variety of habitats in the US and Italy. Sites ranged from sea level to 12,000 feet, and include still and moving permanent and temporary waters, moss, and dust. The animals fall into six genera; only a few have been identified to morphological species. Phylogenetic analysis shows that 110 animals are clustered in 19 clades, and these clades meet several criteria for species: 1) Between-clade sequence differences are 4 times the within-clade differences, indicating that these clades are reciprocally monophyletic and thus have been evolving independently of each other for a long time. 2) Independent evolution implies that they are permanently allopatric and/or are adapted to different niches. In favor of the latter interpretation, many clades are obviously different in morphology, and no clade contains members of more than one morphological genus. We tested two clades for temperature tolerance, finding a consistent significant difference. Moreover, there is only a weak correlation between geographic distance and the sequence difference between pairs of isolates, consistent with the ability of dried bdelloids to disperse by wind. We also asked whether bdelloids have accumulated a high load of detrimental mutations, as predicted by evolutionary theory. We calculated the ratio of nonsynonymous substitutions to synonymous substitutions for our *cox1* sequences, and also for a smaller set of mitochondrial *cob* gene sequences. The ratio is  $\ll 1$  in both cases, showing that these genes are under strong purifying selection in the asexual bdelloids. This agrees with the results of Mark Welch and Meselson (2001 PNAS 98:6720) for nuclear genes.

## Trophic relationships among rotifers, phytoplankton and bacterioplankton in the Corumbá reservoir, Goiás State, Brazil

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The objective of this study was to evaluate the trophic relationships among rotifers, phytoplankton and bacterioplankton, based on investigating the relative abundance of these communities. Sampling was done at fourteen stations in the reservoir, including its arms and tributaries, during dry and rainy seasons. The highest rotifer abundance was found in the dry season, mainly in the upper and intermediate stretches of the reservoir. *Brachionus calyciflorus*, *Polyarthra vulgaris*, *Keratella tropica*, *K. cochlearis*, *K. americana* and *Pompholyx complanata* were the most abundant species. The densities of *B. calyciflorus* and bacteria were significantly correlated, based on both spatial and temporal variations. On the other hand, there was an inverse relationship between *Polyarthra vulgaris* and bacteria. Diatoms formed an important part of the diet of *Keratella americana*. The results of PCA support the direct and indirect relationships between rotifers and their food resources. We conclude that different rotifer populations participate in microbial and macrobial food webs.

## **Diversity and abundance of rotifers in different waters in the Upper Paraná River floodplain (Paraná State -Mato Grosso do Sul State, Brazil)**

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We propose that the diversity ( , and specific diversities) and abundance of rotifers in the spatial and temporal variations in the Upper Paraná River floodplain are due to the heterogeneity of environments and hydrological level fluctuations of the main river. The structure and dynamics of rotifer assemblages were investigated in samples collected during rainy period (February, 2000) and dry period (August, 2000) in 36 different waters, including 13 closed lagoons, 12 open lagoons, 4 channels, 4 backwaters and 3 rivers. The influence of food availability (phytoplankton biomass) for the rotifer diversity and abundance was also investigated. Surface water samples were collected in the pelagic region using a motorized water-pump and plankton net (70-mm mesh). Among the 106 taxa identified, the three main families that were encountered are: Brachionidae (23 taxa), Lecanidae (20 taxa) and Trichocercidae (18 taxa). The highest richness values were noted in the rivers and open lagoons, the highest abundance in the closed lagoons, and the highest specific diversity in the channels, all during the rainy period. – diversity values revealed that alteration in the composition of the rotifer community were the most striking in the channels, especially in the dry period. The ANOVA showed that only the abundance was significantly related to the spatial variability. The Pearson correlation between the abundance and chlorophyll-a concentration demonstrated that phytoplankton was an important food resource for the development of the different populations. The differences in flow characteristics, environment stability and food availability were the principal seminal factors for the structure and dynamics of the rotifer community.

## The development of a bdelloid egg: a contribution after 100 years

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The studies on embryology of the rotifers date back to about 1850. The very few studies in the field in recent years did not add significantly to the existing information. In more than hundred years a number of species have been investigated. The succession of cell divisions in vivo or through classical histology has been recorded. The descriptions reported by the different workers differ and the confusion can be ascribed either to different methods followed or to the developmental pattern that may differ among the species. The literature review on rotifer embryology reveals a common pattern of development that applies to the rotifers, including the bdelloids. Bdelloid rotifers are particularly neglected regarding studies on their embryonic development. For consistent information on the embryogenesis of bdelloids we have to refer to the study of Zelinka (1891). He accurately described the development of *Mniobia russeola*, recorded the cell divisions in vivo and tried to outline a preliminary cell lineage. Here we have applied fluorescence techniques to the eggs of another bdelloid species, *Macrotrachela quadricornifera*, overcoming, if possible, the technical difficulties caused by the resistant egg shell. We observed cytoskeleton (filamentous actin and tubulin) and nuclear DNA at a confocal laser scanning microscope. The results confirm the spiral cleavage and the long-lasting process of gastrulation, that starts as early as at the stage of about 20 blastomeres, as observed by Zelinka. We describe early events of organogenesis, such as the formation of mastax structures, and compared the results with previous studies for providing a general description of early embryogenesis of the rotifers.

## Copepods and rotifers: coexistence of predators and their prey

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Three main groups of planktonic Metazoa inhabit the limnetic zone of inland waters and compete for common food resources: rotifers, cladocerans and copepods. Besides the competition, their mutual relationships are strongly influenced by the ambiguous feeding mode of copepods: most species, at least in their later developmental stages, are efficient predators. They exhibit various hunting and feeding techniques which enable them to prey on a wide range of planktonic animals. The rotifers become often the most preferred prey.

Both cyclopoid and calanoid copepods (genera *Cyclops*, *Mesocyclops*, *Diacyclops*, *Tropocyclops*, *Diaptomus*, *Eudiaptomus*) as predators and a long array of rotifer species (genera *Synchaeta*, *Polyarthra*, *Filinia*, *Conochilus*, *Conochiloides*, *Brachionus*, *Keratella*, *Trichocerca*, *Asplanchna* and others) as a prey are reported in various studies of feeding relationships in limnetic communities. Generally, soft-bodied species are more vulnerable to the predation than the species possessing spines or external structures or loricate species. However, not only morphological but also behavioural characteristics of rotifer species on the individual (e.g. way of movement, escape reaction) and the population level (time and space distribution) are important to regulate the impact of the copepod predation. The reported predation rates are high enough to produce top-down control and often reach or even exceed the reproductive rates of the rotifer populations. These findings are discussed and related to the differences between life strategies of limnetic rotifer species with their ability to utilize quickly the seasonally changing food resources and more complicated life strategies of copepods known to include such components as are diapausing periods, cannibalistic feeding, switching to herbivorous feeding mode, etc.

## Morphometrical analysis of two rotifer species belonging to the *Brachionus plicatilis* complex

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In aquatic invertebrates occurrence of cryptic species is a more frequent phenomenon than previously thought, as shown by molecular marker analysis. The use of these techniques has revealed that six cryptic species of the *Brachionus plicatilis* complex inhabit the Iberian Peninsula. Some of these species show size differences, as they belong to different morphotypes (*L* for large, *SM* for small-medium, and *SS* for small-small). Two of the species (*B. plicatilis* sensu stricto and *B. 'Manjavacas'*, the latter not formally described) are sympatric, belong to morphotype *L*, and do not show morphological differences.

We present a biometrical analysis of several strains of *B. plicatilis* and *B. 'Manjavacas'*. A nested experimental design (species, strain, replicate culture, individual) was used. Species identification was performed restricting the analysis on a mitochondrial gene (COI) amplified by polymerase chain reaction. Nine lorica measurements were taken, and tested for differences by ANOVA. The species resulted to be different in three of their spine measurements, but not when the major measurements were compared (e.g., body length and width). According to a discriminant analysis, the best discrimination needs a single axis which combines the three significantly different spine measurements. However, when the individuals were plotted along that discriminant axis, a species overlap was found. This implies that, despite species being morphologically different, spine morphology is not a reliable criterion for the identification of these cryptic species.



## Restoration of tropical peat swamp rotifer communities after perturbation: an experimental study of recovery of rotifers from the resting egg bank

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We studied the effects of exposure conditions and duration on the diversity of rotifers hatching from sediments, for assessing the recovery potential of tropical freshwater communities after disturbance. For this, well-mixed surface sediment from Mai Khao peat swamp on Phuket Island, Thailand, was kept under different conditions (cool, 4°C) & continuous darkness: CD; ambient (32-42°C) & dark: AD; ambient & daylight conditions: AL), for different time durations (1, 2, 4, 6, 12, 18 and 24 months).

The number of species hatching from the sediment was affected significantly by treatment for both short-term (1 to 6 months) and long-term (6 to 24 months) exposure durations ( $F = 4.97$  and  $10.37$ ,  $p < 0.05$ ,  $df = 2$ , respectively), whereas significant effects of short- and long-term exposures within treatments were also present ( $F = 20.94$  and  $66.25$ ,  $p < 0.01$ ,  $df = 3$  and  $4$ , respectively). Both factors interacted significantly (short-term:  $F = 4.60$ ,  $p < 0.01$ ,  $df = 6$ ; long-term:  $F = 2.68$ ,  $p < 0.01$ ,  $df = 8$ ). No short-term effects of differences in treatment conditions on the numbers of rotifers hatching were found ( $F = 0.68$ ,  $p > 0.05$ ,  $df = 2$ ), although increasing the duration did have an effect ( $F = 6.55$ ,  $p < 0.01$ ,  $df = 3$ ). Significant effects of treatment occurred after 6 months ( $F = 14.83$ ,  $p < 0.01$ ,  $df = 2$ ), in addition to prolonged effects of duration ( $F = 42.00$ ,  $p < 0.01$ ,  $df = 4$ ). Again, both factors interacted significantly (short-term:  $F = 0.54$ ,  $p < 0.01$ ,  $df = 6$ ; long-term:  $F = 9.05$ ,  $p < 0.01$ ,  $df = 8$ ). Our results indicate that exposure time has a strong impact on the viability of resting eggs, whereas, an effect of exposure conditions appears only after six months. So, rotifer communities from sediment egg banks in disturbed peat swamps can only be effectively recovered if restoration is implemented within a relatively short period after perturbation. Rotifer distribution patterns in Spanish shallow lakes of contrasting environmental characteristics.

**Rotifer distribution patterns in Spanish shallow lakes of contrasting environmental characteristics.****José M. Conde-Porcuna & R. Morales-Baquero**

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In south of Spain the high summer temperatures and low annual precipitation (mean, 200-500 mm) play an important role on the dynamics of Spanish shallow lakes. Strong water-level fluctuations are very common and many of these shallow lakes get dried at the end of the summer. Moreover, conductivity values are very high in these water bodies. These factors affect the dynamic of zooplankton, regardless of biotic ones. This study analyses the distribution of rotifer populations within a wide range of environmental characteristics (temperature, conductivity, water level, turbidity, etc.) in 30 lakes in south Spain. Possible interactions with other zooplankton populations have also been considered. The rotifer biomass ranges from 1 to 5821  $\mu\text{g.l}^{-1}$ , and the species richness from 4 to 17. These patterns are not clearly related with the trophic conditions in the lakes. The implications of environmental characteristics on these differences in biomass and species richness are discussed.

## Comparative study on the trophal morphology of Synchaetidae (Rotifera: Monogononta)

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The monogonont rotifer family Synchaetidae actually comprises four genera: *Ploesoma*, *Polyarthra*, *Pseudoploesoma*, and *Synchaeta*. Although the genera are well-defined, taxonomy at the species level is extremely unsatisfactory in *Synchaeta* and *Polyarthra*, due to difficulties in assessing some discriminating morphological features, especially in preserved material, and the delicate nature of the trophi which are hard to be seen and interpreted correctly by light microscopy.

Trophi of the respective genera were investigated using scanning electron microscopy, and described in detail. From a phylogenetical point of view the trophi of *Pseudoploesoma* and *Ploesoma* are the most primitive, whereas they are the most specialized in *Synchaeta* and *Polyarthra*, and characterized among other things by a strong reduction of the uncinal teeth. Taxonomic confusion within *Synchaeta* and *Polyarthra* can probably be resolved to a great extent using SEM of trophal morphology.

**Study on scanning electron microscopy of trophi of  
*Testudinella* Bory de St. Vincent and *Pompholyx* Gosse  
(Rotifera: Testudinellidae)**

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In Testudinellidae (Rotifera, Monogononta, Flosculariacea) three genera are recognized, i.e. *Anchitestudinella*, *Testudinella*, and *Pompholyx*. Until now, the taxonomy of the species exclusively relied on the shape of the lorica, the shape and position of the foot opening, and the position of the lateral antennae (e.g. Kutikova, 1970; Koste, 1978). Detailed studies on the trophi, and data on their species specific morphology are lacking. I therefore initiated a comparative study on the trophi morphology of the commonly found genera *Testudinella* and *Pompholyx*, using scanning electron microscopy. The results show that, although overall similarity in trophi structure is great for the taxa studied, species specific differences are apparent. Intra- and interpopulation variation in number of uncinal teeth is provided for some species, and proved very small. It follows that trophi morphology in Testudinellidae can be used as an additional character for identification, and may be helpful in elucidating the status of several problematical taxa at the subspecific and infrasubspecific level. A comparison is made with the trophi of some other genera of Flosculariacea.

## Clear-water rotifer population declines in a reservoir

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The relative importance of factors responsible for the strong clear-water decline of rotifer populations was tested using in situ manipulative experiments. The influence of crustacean zooplankton, intraspecific competition and food limitation was experimentally altered on four occasions during the clear-water phase. The reservoir community of rotifers, without crustaceans, was exposed in 19 l transparent containers in three variants: 1) natural rotifer density, 2) artificially diluted rotifer density, 3) artificially diluted rotifer density with addition of phosphorus. A positive stimulative effect on growth of rotifers was found by crustacean removal, dilution of rotifer densities and phosphorus fertilization before clear water phase, i.e. during spring peak of rotifers. It seems that peak densities of rotifers in spring result from interrelations of intraspecific competition, food level and interaction with cladocerans. The clear-water phase experiments yielded different responses. Generally, the response of rotifer growth to experimental manipulation was significant, but not as strong as found during spring peak. We found significant differences in rotifer growth between the control and diluted treatments, however, no significant effect was found for phosphorus treatments. The population growth in experimental containers for all diluted variants was comparable with population growth of reservoir population. This indicates that food levels were probably the key factor regulating rotifer density. It is not clear why we did not succeed in improving food conditions by removal of crustaceans and addition of phosphorus.

## Seasonal dynamics and vertical stratification of planktonic rotifers in the Rimov reservoir

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Seasonal dynamics and vertical distribution of the rotifer community in the meso-eutrophic, dimictic Rimov reservoir was studied in the growing season 2000. Densities of rotifers were compared with available food sources and water temperature. Rotifer density, chlorophyll-a concentration, bacterial, flagellate and ciliate number, secchi depth and temperature were investigated every third day in spring and then at three-weeks intervals. Water samples were collected from 4-8 depths, from 0-35 meters, to study vertical distribution. The results showed significant changes of rotifer densities, influenced by seasonal changes of biotic and abiotic conditions. Chlorophyll-a concentrations significantly related to the densities of rotifers. No significant correlation between rotifer densities and other parameters were observed. Concentration of chlorophyll-a seemed to have an important effect on rotifer dynamics. Relationship between water temperature and presence or absence of particular rotifer species fitted well with other authors' investigations. Eurythermal (*Keratella cochlearis* and *Kellicottia longispina*), warm-stenothermal (*Polyarthra major*, *Trichocerca similis* and *Conochilus hippocrepis*) and cold-stenothermal (*Synchaeta lakowitziana* and *Polyarthra dolichoptera*) species. In comparison to high densities of cold-stenothermal species in early spring, warm-stenothermal species had the highest densities in summer. We observed migration of the cold-stenothermal species (*S. lakowitziana* and *P. dolichoptera*) from the warm epilimnion to the colder layer of the reservoir. To conclude, composition of the rotifer community was strongly influenced by food (chlorophyll-a concentration), as well as by temperature.

**Sexual reproductive biology of *Brachionus variabilis*  
(Rotifera: Monogononta)**

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We studied the sexual reproductive biology of the monogonont rotifer *Brachionus variabilis*. Important aspects of the study were: 1) morphological description of the male, so far unknown; 2) analysis of mating behavior; 3) an analysis of female and male life-span at 25°C; 4) morphometric characterization of the three egg types known for this species; and 5) determination of hatching percentages at 25°C. The mating behaviour of *B. variabilis* is similar to that of other brachionids. Attempted copulations lasted on average 12.4 seconds, and completed ones on average 71.4 seconds. The sites of mating attempts and copulations are similar to those of other members of the genus *Brachionus*.

## Assessment of the toxicity of the herbicide glifosate in the high and medium areas of the Lerma-Chapala watershed (Mexico)

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We determined the presence of herbicide glifosate at eleven sampling stations on four different sampling dates, along the Lerma-Chapala watershed in Central Mexico. We used the rotifer *Lecane quadridentata* and the cladoceran *Daphnia magna* as model organisms to study the toxicity present in water samples from the eleven sampling stations. *Lecane* was in general more sensitive than *Daphnia* for all samples in all stations regarding both acute and sublethal toxicity. We determine LC50 values for both glifosate and the commercial formula FAENA. Results are discussed in view of the present environmental condition of Lake Chapala.



## Genetic characterization of *Brachionus* sp. clones using the 16SrDNA and HSP60 markers with the SSCP technique

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Mass production of high quality fingerlings of marine fish in Europe depend on improvements in the techniques for producing and utilizing live food (*Brachionus plicatilis* and *Brachionus rotundiformis*). The mass culture of these rotifers is very unpredictable; periods with total mortality or reduced reproduction ('crashes') regularly occur.

Does the controlled mass culture of rotifers leads to an impoverishment in the genetic diversity of the cultured rotifers and in consequence makes the rotifer culture more susceptible to crashes?

The aims of the genetic approach in our project were: 1) genetic characterization of *Brachionus* clones, and 2) determination of genotypic diversity of cultured rotifers. Recent advances in molecular genetics have provided the opportunity to apply new, more sensitive techniques to population genetic studies.

Samples of rotifer cultures from hatcheries are separated into clones. These clones are fingerprinted using 2 markers: 1) the mitochondrial 16SrDNA gene and 2) the HSP60 (Heat Shock Protein 60) gene. The HSP60 gene is involved in stress related responses. Mutations in the 16SrDNA gene are considered neutral. This is not necessarily true for the HSP60 gene, where a certain genotype (if displaying a certain phenotype) can be easily selected for in mass cultures of *Brachionus*, probably depending on the culturing conditions.

Polymorphisms are detected by the SSCP technique (Single Stranded Conformation Polymorphism) and by DNA sequencing. Within the species *Brachionus plicatilis* and *B. rotundiformis* different genotypes (at least 7) have been found using this 16SrDNA marker in a series of *Brachionus* strains.

## **Genetic implications in the production of rotifers in commercial finfish hatcheries.**

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Success in rotifer production is still one of the major bottlenecks in larviculture. Periodic crashes are frequent and generally solved by an empirical approach.

Two hypotheses are put forward in this study: 1) Mass culture of rotifers leads to impoverishment in genetic diversity and 2) this makes the rotifer culture more susceptible to changes in the biotic or abiotic conditions.

Samples of cultures from commercial hatcheries will be separated into clones; these will be characterised using 16S rDNA gene, HSP60 gene and microsatellite markers.

Different culture types (batch, recirculation and continuous) will be compared for their effect on interclonal selection. The effect of the inoculation amount on the genotypic diversity will be examined. This will give information on the correlation between the culture method and the selection rate.

The effect of some environmental parameters (temperature, salinity and feed) on the genotypic diversity of the rotifer culture will be assessed. These will be kept constant during several culture periods on the one hand, variable within one culture period or between culture periods on the other. The rotifer clones may adapt to the new situation or they may crash.

In order to document the effect of bacterial strains on interclonal selection, mixtures of disinfected parthenogenetic eggs will be hatched and cultured

with or without addition of bacteria.

The methodologies for resting egg production/harvest for some clones will be fine-tuned. The advantage of resting eggs resides in the fact that hatcheries could maintain the genetic pool of rotifer strains by re-inoculation with old clones.

Field data on the genetic drift of rotifer cultures at different commercial hatcheries will be collected.

The results will be presented in a workshop at the end of the project.

## Short-time response of psammon communities of Rotifera to changes in their habitat

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Sand microhabitats are usually characterized as extremely unstable and unpredictable and their fauna seems to be very variable and fluctuating. The aim of the paper is to answer the questions: 1) can we observe large-scale fluctuations of psammon rotifer densities in short time intervals? 2) is the short-time response similar in different ecological groups of the communities?

Rotifer composition and density and weather conditions were investigated with very high sampling frequency (every half a day) in the hygroarenal of the eutrophic Lake Mikolajskie from 13 September to 9 October 2002.

In total, 62 species were found with only two species occurring permanently throughout the study period, i.e. *Lecane closterocerca* and *L. levistyla*. Densities of the two species as well as all remaining species and rotifers in general were extremely variable. Strong fluctuations were observed even at 12h intervals – the observed differences in rotifer numbers ranged from 3 to 900% of the initial values.

In average, psammobionts constituted only 8% and psammoxens 18% of the community numbers, whereas psammophilic rotifers decidedly dominated (74%). The strongest reaction to changing environment was observed in psammobionts, as the numbers of this group was more variable than the remaining two groups - standard deviation was 181% of its mean value. Even densities of psammoxens - thus rotifers often brought with lake waves - deviated from their mean numbers into much lower extent, i.e. 149% of their mean. The group most stable were psammophiles, thus species widely dispersed in different types of ecosystems, and well adapted to very different environmental conditions.

## **The determination of relationship between rotifers and biotic and abiotic factors by applying multivariate analysis in the Ova Stream (Ankara, Turkey)**

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Although a relatively large number of publications dealing with the fauna of Turkish Rotifera are available, only a few of these deal with river systems and zooplanktonic organisms of the Ovaçayı River have not been studied. The study area is located within the Sakarya River basin of Central Turkey. A prominent tributary to the Sakarya River is the Ankara Stream, an important surface water resource for the city of Ankara. The Ankara Stream encompasses a total watershed drainage area of ca. 7140 km<sup>2</sup> within the Sakarya River basin. Ovaçayı River is one of the tributaries of the Ankara Stream, a perennial stream that is generally used for irrigation. The river was sampled at four sampling stations from February 2001 on 3-monthly basis. We identified 25 rotifer species in 2001 and 17 species in 2002. Population densities of rotifers were calculated (ind.m<sup>-3</sup>) and the relationship with physico-chemical parameters were determined applying by CCA analysis. Also diversity index for each sampling was calculated. The diversity index differed between the stations and sampling times. The CCA results show that some of the rotifer genera (*Keratella*, *Notholca*) showed negative correlation with the increasing concentration of the chemical parameters and water temperature but the other genera (*Brachionus*, *Mytilina*, *Colurella*, *Testudinella*) were positively correlated with temperature.

## **The rotifer fauna of Euphrates River Basin**

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In Turkey, limnological studies were begun by Vavra in Sarı Lake in 1905, and three species of rotifers, one of the copepods and three of cladocerans were identified. More than eighty years later, Dumont and Ridder (1987) observed 78 rotifer species from 19 different water bodies and Segers et al. 1992, documented 91 rotifer species from 21 different localities. Until now 174 rotifer species have been identified from Turkey. Although a lot of water bodies were located in the River Euphrates basin, their limnological and biological parameters were not investigated. This present study was carried out at 19 localities of Euphrates river basin during 2001 and 2002 on three-monthly basis. The rotifer composition and zoogeographic distribution are discussed.

## Rotifer fauna reflecting the trophic state of the dam reservoir Snjeznica (Bosnia and Herzegovina)

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Dam reservoir Snjeznica was built in 1984 as a water source for the region of Teocak. To examine the trophic state of the reservoir for the first time, we carried out limnological studies that included analyses of the plankton and benthos communities, physico-chemical parameters of water and bacteria. The study that was carried out in 1999 (September, October), 2000 (March, June, August, November) and 2001 (March, May, September) revealed that rotifer fauna is an important part of the zooplankton densities in the reservoir. The majority of the rotifer species were the ultra nanoplankton filtrators (i.e. *Filinia longiseta*, *Kellicottia longispina*) and the microfiltrators-sedimentators, among which *Keratella cochlearis*, *K. cochlearis* var. *tecta* and *K. quadrata* were constantly present. Generally, densities and structure of rotifer communities indicate that the Snjeznica reservoir is mesotrophic during colder months, and eutrophic during the spring and summer periods. Although concentrations of chlorophyll a do not exceed  $10 \mu\text{g}\cdot\text{l}^{-1}$  rotifer numbers are not so low as is indicated by abundance in May of species *Keratella cochlearis* ( $844 \text{ ind}\cdot\text{l}^{-1}$  and  $1022 \text{ ind}\cdot\text{l}^{-1}$  at 2-m and 10-m depth, respectively) and its form *tecta* ( $466 \text{ ind}\cdot\text{l}^{-1}$  at 5-m depth in June) as well as macrofiltrator species, e.g. *Polyarthra vulgaris* ( $2574 \text{ ind}\cdot\text{l}^{-1}$  at 10-m depth in May).

## Morphology and phylogeny of *Floscularia* Cuvier, 1789 (Rotifera)

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The taxon *Floscularia* was analysed morphologically and phylogenetically. *F. melicerta* (Ehrenberg, 1832), *F. ringens* (Linné, 1758), *Ptygura cephaloceros* S.-Wright, 1957 var. *grande* Koste, 1970 and *Limnias melicerta* Weisse, 1848 were examined in detail light microscopically. We standardized the morphological descriptions. The matrix was completed on critical examination of characteristics from the literature. We have analysed the phylogenetic relationships of *Floscularia* cladistically in PAUP 4.0 with *Octotrocha speciosa* Thorpe, 1893, *Limnias melicerta* and *Ptygura cephaloceros* var. *grande* as outgroups. On the basis of the hypothesized phylogenetic system of *Floscularia* we have reconstructed the evolution of the tube making within this taxon. During the evolution of *Floscularia* functional and morphological changes did occur: the stem species of *Floscularia* form only gelatinous tubes. Within *Floscularia* a change to a gelatinous tube with faecal pellets and then a change to a gelatinous tube with detritus pellets obtained from the rotatory organ occur.



## Combined effects of food concentration and temperature on the competition among four species of *Brachionus* (Rotifera)

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We evaluated the effect of algal food density ( $1.5 \times 10^6$ ,  $3.0 \times 10^6$  and  $4.5 \times 10^6$  cells  $\text{ml}^{-1}$  of *Chlorella*) and temperature ( $21^\circ\text{C}$  and  $28^\circ\text{C}$ ) on competition among the rotifers *Brachionus calyciflorus*, *B. havanaensis*, *B. patulus* and *B. rubens*, based on population growth experiments for 24 days. The growth experiments were conducted for each of the four rotifer species (i.e. controls) and these species in mixture in equal proportions (i.e. under competition). In controls, regardless of the species, at any given temperature, rotifer densities increased with increasing food concentration. However, at all the 3 food levels higher population abundances were observed at the lower temperature. In all controls, *B. havanaensis* reached the highest population density compared with the other species. In treatments with mixed rotifer species, the competitive outcome varied depending on food level and temperature. In general, high temperature and high food concentrations, favoured numerical dominance of *B. havanaensis* over the other 3 species. At all food concentrations and at both temperatures, *B. calyciflorus* was either reduced to low abundance or completely eliminated in most test jars of mixed rotifer cultures. For any given species, peak population density and the rate of population growth were higher in controls than under competition.

**Effect of algal (*Chlorella vulgaris* and *Scenedesmus acutus*) mixture on the life table demography of *Brachionus calyciflorus* and *B. patulus* (Rotifera: Brachionidae)**

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Life history strategies of zooplankton evaluated through life table demographic approach are helpful to understand the ecological characteristics of species. In the present work, we tested the effect of two algal diets (*Chlorella vulgaris* and *Scenedesmus acutus*), offered alone or in mixture (equal proportion and 75% or 25% of either alga), on the life table study of two rotifer species. Based on the life table experiments, we derived selected life history variables (age-specific survivorship, life expectancy and stable age of distribution, gross reproductive rate, net reproductive rate, generation time and the rate of population increase) at an algal density ( $1 \times 10^6$  cells ml<sup>-1</sup> of *Chlorella*, or its equivalent in terms of biomass for *Scenedesmus*). *B. patulus* survived better when fed on *Chlorella* than on *Scenedesmus*. The average lifespan, life expectancy at birth and generation time decreased with increasing proportion of *Scenedesmus* in the medium. Data were compared with those of *B. calyciflorus* obtained under similar test conditions. The role of mixed algal diet on the life history variables of chosen rotifer species is emphasized.

## Do jaws of rotifers grow after hatching?

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A recent study on trophi size of *Macrotrachela quadricornifera* (Bdelloidea, Philodinidae) suggested that the trophi of new born individuals do not grow after hatching, while the body volume increases more than 400% in the first five days of life. We decided to extend this kind of observations to other rotifer species. Measurements of trophi of some bdelloid and monogonont rotifers at different ages have been carried out on scanning electron microscope pictures.

**Bdelloids:** measurements of the trophi of *Rotaria macrura*, *R. neptunoida* and *R. tardigrada* (Philodinidae) show that trophi do not change (neither in size nor in shape) after hatching.

**Monogononts:** measurements of the trophi of *Cupelopagis vorax* (Atrouchidae), *Dicranophorus epicharis*, *D. forcipatus* (Dicranophoridae), *Floscularia ringens* (Flosculariidae), and *Notommata glyphura* (Notommatidae), show no changes, while trophi of *Asplanchna priodonta* (Asplanchnidae) seem to change after hatching.

We hypothesize that, for each species, the size of the masticatory apparatus is related to the size of the food items, and both are independent from the animal size. This fits species that are suspensivorous and feed by filtering (like *Macrotrachela quadricornifera*, *Rotaria macrura*, *R. neptunoida*, *R. tardigrada* and *Floscularia ringens*) or species that are predators (*Cupelopagis vorax*, *Dicranophorus epicharis*, *D. forcipatus* and *Notommata glyphura*). The different sizes found in the trophi of *Asplanchna priodonta* indicate that the structure can grow. This might be related to the very wide range of food that the species can collect and feed on: *A. priodonta* is opportunistic and can feed on different food at different ages.

## On the phylogenetic position of Rotifera: have we come any further?

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Rotifers are bilateral symmetric animals belonging to Protostomia. The ultrastructure of the rotiferan trophi suggests that they belong to the Gnathifera, and ultrastructural similarities between the integuments and spermatozoa as well as molecular evidence strongly suggest that rotifers and the parasitic acanthocephalans are closely related and form the clade Syndermata. Here we discuss the phylogenetic position of rotifers with regard to the gnathiferan groups. Originally, Gnathifera only included the hermaphroditic Gnathostomulida and the Syndermata. The synapomorphy supporting Gnathifera is the presence of pharyngeal hard parts such as jaws and trophi with similar ultrastructure. The newly discovered Micrognathozoa possess such jaws and is a strong candidate for inclusion in Gnathifera because their cellular integument also has an apical intracytoplasmic lamina as is the case in the Syndermata. However, Gnathifera might include other taxa such as the commensalistic Myzostomida and the Cyclophora. Traditionally, Myzostomida has been included in the annelids but recent studies regard them either as sister group to the Acanthocephala or as sister group to the Cyclophora. Whether Cyclophora belong to Gnathifera is still uncertain. Some analyses based on a single molecular sequence of 18S RNA and total evidence point towards a close relationship between Cyclophora and Syndermata. Other cladistic studies of the metazoans using morphological characters or total evidence suggest a sister group relationship between Cyclophora and Entoprocta. More molecular and morphological data and an improved sampling of taxa are obviously needed to elucidate the phylogenetic position of the rotifers and identify which phyla belong to Gnathifera.

## The functional response of *Brachionus calyciflorus*: resource or consumer dependence?

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The uptake of resources from the environment is a vital process of all organisms. Many experimental studies have revealed that the rate at which this process occurs depends critically on the resource concentration, a relationship called "functional response". However, whether the concentration of the consumer normally affects the functional response has been the subject of a longstanding, predominantly theoretical debate in ecology. Here we present an experimental test between the alternative hypotheses that, among planktonic organisms, food uptake depends either only on the resource concentration or on both the resource and the consumer concentration. We measured the uptake of radioactively labeled, unicellular green algae (*Monoraphidium minutum* = resource) by the rotifer *Brachionus calyciflorus* (= consumer) for varying combinations of resource and consumer concentrations. We found the food uptake by *Brachionus* to depend on the algal concentration; the relationship was best described by a „Holling type 3“ functional response. We detected significant consumer effects on the functional response only at extraordinarily high *Brachionus* densities ( $> 100 \text{ ml}^{-1}$ ), which by far exceed concentrations normally encountered in the field. We conclude that consumer dependent food uptake by planktonic invertebrates is a phenomenon that can occur under extreme laboratory conditions but probably plays a minor role in natural environments.

## Biodiversity of pelagic rotifer plankton in stratified lakes with different human impacts

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Four lakes in the Vitebsk region of Belarus with similar genesis type and different human impacts were studied from June 2001 to July 2002. These were Lake S.Volos (area 1.21 km<sup>2</sup>, max. depth 40.4 m), Lake Dolgoe (area 2.6 km<sup>2</sup>, max depth 53.6 m), Lake N.Volos (area 4.21 km<sup>2</sup>, max. depth 29.2 m) and Lake Kruglik (area 0.4 km<sup>2</sup>, max. depth 31.5 m). The lakes were found to be thermally stratified from the end of May to September, with the oxygen content of the deeper waters varied with depth. The near bottom oxygen saturation values recorded were: Lake S.Volos – 58.7% in 2001 and 33.7% in 2002; Lake Dolgoe - 57,0% in 2001 and 40,0% in 2002; Lake N.Volos - 26% in 2001 and 13% in 2002 and Lake Kruglik – 5.8% in 2001 and anoxia in 2002.

Rotifer biodiversity at each site was estimated by comparing species richness, density and diversity indices on the stratified vertical layers of the water column at the deepest station. Species richness ranged irregularly between 18 and 25. The number of species in individual layers of the stratified water column in Lake Kruglik closely and significantly correlated with temperature ( $R=0.8985$ ,  $n=18$ ,  $P=0.00000$ ) and oxygen concentration ( $R=0.8669$ ,  $n=18$ ,  $P= 0.00003$ ). In the other lakes, these correlations became weaker with decreasing human impact. The index of spatial niche overlap ("diffuse competition") was greatest in Lake Kruglik ( $0.48\pm 0.025 - 0.62\pm 0.022$ ) and least in Lake S. Volos ( $0.31\pm 0.023 - 0.35\pm 0.027$ ). Hypoxia in the hypolimnion was found to reduce the diversity of spatial niches within the stratified water column. Nevertheless, species richness was similar in both Lake Kruglik and Lake S. Volos (2001 - 20, 2002 - 18). It is suggested that the conservation of species richness within lakes with a high human impact results from niche non-overlapping along trophic vector.

**Interactions among copper toxicity, temperature and salinity levels on the population dynamics of *Brachionus rotundiformis* (Rotifera)**

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Toxicants and ecological factors (temperature, food level and salinity) can interact causing both mortality and reduced reproduction in organisms. Copper (Cu) compounds are commonly used for eliminating nuisance algal blooms in aquaculture ponds. However, at certain concentrations, Cu is toxic to rotifers. *Brachionus rotundiformis* occurs in waterbodies in a range of levels of salinity, temperature and trophic conditions. Seasonal changes in these variables may affect sensitivity of the rotifers to the heavy metals. In the present work, we evaluated the combined effects of salt concentrations (2.5 and 5.0 g.l<sup>-1</sup> of NaCl), Cu levels (0, 0.031, 0.062, 0.125, 0.250 mg.l<sup>-1</sup> as CuCl<sub>2</sub>) and temperatures (20° and 25° C) on the population growth of *B. rotundiformis*, using *Chlorella* as the algal food (at 0.5x10<sup>6</sup> cells.ml<sup>-1</sup>). Regardless of temperature and salinity, an increase in the Cu concentration in the medium resulted in decreased population growth of *B. rotundiformis*. In general, rotifers in controls (free from Cu in the medium) showed higher population growth rates and peak population abundances at 5 g.l<sup>-1</sup> of salinity. In treatments containing Cu, rotifer performed better at lower salinity (2.5 g.l<sup>-1</sup>) levels. Regardless of toxicant concentration and salinity, higher population abundances of *B. rotundiformis* were observed at the lower temperature.

## Patterns in *Brachionus plicatilis* complex resting egg banks: density and viability

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Diapausing resting eggs have been recognized as an important part of the life cycle of rotifers due to their impact on population dynamics and evolution. However, the study of rotifer resting eggs in the natural environment has received much less attention than the study of active populations in the water column. Most of the information on resting stages that is available comes from other aquatic invertebrates. Here, we present a quantitative study of the resting egg bank of the *Brachionus plicatilis* species complex in 15 ponds in eastern Spain. The density and viability of these eggs were investigated at different sediment depths. Resting eggs were classified according to those of their morphological features that were believed to be related to their viability (e.g. shell integrity, embryo size).

Only eggs showing a light, brown multinuclear embryo filling more than 75% of the S2 layer-delimited space were found to be viable. At the sampling sites where healthy-looking resting eggs were detected, densities ranged from approximately 1 to 142 eggs per cm<sup>2</sup>, and the density of hatchlings from those eggs ranged from approximately 1 to 26 individuals per cm<sup>2</sup>. Almost all hatchlings (approx. 96%) were obtained from eggs in the upper 4 cm of sediment, and most (approx. 78%) of the viable resting eggs were found in the upper 2 cm sediment, but hatchlings were recorded even from the deepest (10 cm) sediment layer studied.

Most of the time, the density of total (healthy and unhealthy-looking) resting eggs did not show a uniform or a declining distribution with sediment depth. Our results suggest that rotifer populations inhabiting ponds and small lakes have high among-year variation in resting egg production. When compared to large lakes or estuarine populations, pond populations seem to have a younger resting egg bank. This might be due to higher hatching rates because the conditions that promote hatching (e.g. light) are more likely to be found in pond sediments. We hypothesize that pond populations have less evolutionary and demographic inertia than lake populations.



**Morphological and morphometrical variations of selected species of Brachionidae: a seasonal study from lake Xochimilco (Mexico)**

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We observed different morphotypes of some species in the family Brachionidae from the plankton samples of lake Xochimilco collected during different seasons of the year 2002. We quantified the body length, width, and spine lengths (posterior and anterior spines) of *Brachionus havanensis* and *B. caudatus*, *Keratella cochlearis*, *K. tropica* and *K. americana* through seasons in which the predator *Asplanchna brightwelli* was present either in great abundance or was nearly absent. In general, spines of most of the selected rotifer species were longer during the months in which *A. brightwelli* was abundant. Relatively small spines were observed during the months in which the predator was nearly absent. Data on the body lengths of brachionid rotifers indicated wide variation in samples with and without the presence of *Asplanchna*. Morphometric data were interpreted in terms of morphological adaptations of *Brachionus* and *Keratella* in response to *Asplanchna* predation.

## Mating behavior and genetic similarity among four geographically distinct strains of *Brachionus calyciflorus*

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Mating tests among *B. calyciflorus* strains from Florida (FL), Georgia (GA), Texas (TX) and Australia showed cross-mating (circling) behavior among all strains but cross-copulation only between the Florida and Georgia strains. This pattern is consistent with the distinct morphology of the Australian strain, and correlates with the genetic similarity of the strains based on non-coding nuclear sequences (ITS region) and mitochondrial gene (COI) sequences. FL and GA strains differed in only one position of 570 bp sequenced while FL and TX showed 3.9% sequence divergence. Similar levels of genetic differentiation were found using COI sequences. Observations of mating tests led to several general findings about mating behavior. Females can play an active role in mating behavior by withdrawing the corona and interfering with copulation. Mating behavior between two strains can be asymmetric regarding retraction of the female corona and the probability or intensity of mating (circling) behavior. Cross-strain mating (circling) behavior can be consistently strong but never lead to copulation, suggesting the possibility of separate stimuli for these phenomena.

## Molecular ecology of rotifers: from population differentiation to speciation

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The advent of cost-effective molecular tools allowing the amplification of minute amounts of DNA has opened the field of Molecular Ecology for rotifers. The few studies performed to date have yielded unexpected and exciting results. Here I will review these studies and discuss their importance for the investigation of sibling species complexes, clonal structure, population structure, and phylogeography in rotifers. In the past few years I have been involved in the analysis of genetic diversification and speciation in zooplanktonic faunas focusing in the rotifer species complex *Brachionus plicatilis*. The species in this complex reproduce through cyclical parthenogenesis and disperse passively through long-lived resting eggs. The use of sequence and microsatellite variation to investigate the population genetics, phylogeographic patterns and phylogenetic relationships in this complex, in the context of a good background knowledge of its ecological characteristics, mating behaviour, and temporal population dynamics have yielded surprising insights into the processes shaping its genetic diversity. Rotifers display strong population differentiation for neutral markers, and, contrary to the perceived wisdom, deep phylogeographic structure. Persistent founding effects, but also at a local scale the impact of gene flow (Monopolisation hypothesis), are responsible for these patterns. Instances of recent long-distance transcontinental migration have been also revealed. Our data indicates that history and chance might play a major role in the genetic differentiation and speciation processes of these organisms and that migration is not as pervasive as previously thought.

## Rotifer ecology in Lake Kinneret (Israel): long-term (1969-2002) record

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More than 20 rotifer taxa were recorded in routine samples collected in Lake Kinneret (Israel) in 10 offshore (deeper than 5 m) stations, at 5-13 fixed epilimnetic depths in each station, during 1969-2002. Organisms were sorted and counted (including external egg carrying females), biomass was measured and calculated for the entire lake assemblages ( $\text{g.w.m}^{-2}$ ). Metabolic parameters (rates of grazing, respiration and production) were measured experimentally at three different temperature ranges (1=15-20°C; 2=20-24°C; and 3=24-28°C). Results were applied to the lake community in respective months (1=January-April, 2= May, November, December, and 3=June-October). The most common (by number) taxa were *Keratella*, *Synchaeta*, *Polyarthra*, *Ascomorpha*, and *Brachionus*. Rotifers comprises 7.2% of total zooplankton biomass in Lake Kinneret whilst Cladocera and Copepoda 59.3% and 33.5% respectively. Rotifers were found to be most common in the lake during rainy season. Linear and multiple regressions between rotifer biomass concentration and months and inflow discharges indicated significant relations, i.e. decline of biomass with decrease of inflows in summer months. Rotifers are the most common organisms in the Jordan River inflow. Positive relations were also indicated between rotifer densities and small bodied cladoceran concentrations (both by number). Multiannual (1969-2001) means indicated total grazing capacity of herbivorous zooplankton in Lake Kinneret as  $0.84 \text{ gC.m}^{-2}.\text{day}^{-1}$  of which 11% is due to rotifers, and total respiration was estimated as  $0.25 \text{ gC}^{-2}.\text{m}^{-2}.\text{day}^{-1}$  of which 9% is due to rotifers whilst total herbivores production is  $0.14 \text{ gC.m}^{-2}.\text{day}^{-1}$  of which 5% is contributed by rotifers. The total primary production is averaged (1969-2001) as  $1.76 \text{ gC.m}^{-2}.\text{day}^{-1}$  of which 42% is due to grazable nanophytoplankton. It is likely that rotifers, as well as all other zooplanktonic grazers, are not food limited in Lake Kinneret throughout all months. The population dynamics of Rotifera is therefore, likely to be most affected by both, input discharges and fish (*Acanthobrama* spp.) predation.

## Morphological variation in *Keratella cochlearis* (Gosse) in a backwater of the River Thames.

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The morphological variation of *Keratella cochlearis* in a Thames backwater has been studied over four years. There is a general inverse relationship between lorica length and temperature, but the annual cycle of change depends upon the rate of change of temperature, and there is considerable variation between years. There is a similar inverse relationship between posterior spine length and temperature. There is a shift in both relationships depending on whether the temperature is increasing or decreasing. As the water temperature increases from winter to summer the lorica and posterior spine are longer than they are at the same temperature as the water cools from summer to winter. This shift can be modified by anomalous temperatures, such as a late spring or a cool summer.

The *tecta* form, lacking a posterior spine, usually, but not consistently, has a longer lorica than normal spined forms in the same sample. It usually disappears from the samples at the end of November, and does not reappear until March, although with a mild autumn and winter it may persist until January before disappearing.

## Food environment for resting egg hatchlings affect mixis induction in derived clones of the rotifer *Brachionus plicatilis*

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We investigated the effect of food for stem females hatched from resting eggs and their derived clones on mixis induction in a laboratory. Russian strain *Brachionus plicatilis* resting eggs were hatched and reared both by batch (in 100 ml water) and individual culture (0.2 ml) for 20 days and 10-12 generations, respectively. Cultures were maintained at 25°C, 18 ppt salinity and total darkness. A higher percentage of mixis was observed in rotifer populations fed solely on *Nannochloropsis oculata*, by comparison with those fed on baker's yeast. In comparison to the above, mixis induction was significantly higher for treatments in which stem females received a low quality diet (baker's yeast), and subsequent generations received a higher quality diet (*N. oculata*). A similar trend was observed when stem females were starved. Starvation of stem females for 12 and 24 hours after hatching resulted in 10.2 % and 7.2 % of mixis induction in subsequent generations, respectively, while it remained at 3.6 % when *N. oculata* was consistently fed to the rotifers.

## Long-term rotifer population dynamics in Lake Washington, USA

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Throughout the world, aquatic systems are experiencing climatic change, human alterations of food webs and nutrient cycles, and introductions of exotic species. The long-term limnological data set from Lake Washington provides the opportunity to study plankton community responses to several anthropogenic disturbances as well as long-term climatic changes. Over the last four decades, lake temperature has clearly increased, nutrient loading has dramatically decreased, and the food web has been altered. Newly developing autoregressive models provide a useful set of tools for understanding complex ecological dynamics in time series data. Nutrient loading reduction directly altered phytoplankton characteristics by reducing total abundance of cyanobacteria. At the same time, human modifications of the fish community reduced predation on zooplankton grazers by *Neomysis*, a voracious invertebrate predator. Together these abiotic and biotic perturbations shifted dominance of the grazer community from *Diaptomus* to *Daphnia*, which produced further changes in phytoplankton characteristics as well as phenological changes for competing grazers such as *Conochilus*. The clear water phase in the period of *Daphnia* dominance is later in the season and much more pronounced, and correspondingly, *Conochilus* populations no longer peak during June and July. While timing of population peaks has shifted for several rotifers, rotifer biomass has been steadily increasing in Lake Washington over the past 45 years, possibly in response to warmer winters. Herbivorous crustacean zooplankton have not shown similar increases in response to warming.

## Using Amplified Fragment Length Polymorphisms (AFLP) to study genetic variability in several freshwater rotifer species

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We have investigated the potential of using Amplified Fragment Length Polymorphisms (AFLP) as a tool to measure genetic variability in eight species of freshwater rotifers: *Brachionus calyciflorus*, *B. patulus*, *Lecane bulla*, *Lecane luna*, *Lecane quadridentata*, *Philodina acuticornis odiosa*, *Rotaria neptunia*, and *Rotaria rotatoria*. We used nine combinations of oligonucleotides EcoRI/MseI (three +2/+2; three +1/+1, and three +1/+2). We observed a total of 806 amplified bands, 798 polymorphic and 8 monomorphic. The data was analyzed using cluster analysis with the UPGMA method, first within each oligonucleotide combination and finally using all nine combinations. Our best dendrogram clearly separated monogonts from digononts, and grouped the species of monogonts in the two genera. However, it grouped *R. neptunia* with *P. acuticornis odiosa* rather than with *R. rotatoria*. These results are discussed in relation to recent publications in the literature that have measured genetic variability and phylogeny among rotifer species.



## **The Frank J. Myers Rotifera collection – a first step towards an illustrated world catalog of the Rotifera?**

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The Academy of Natural Sciences of Philadelphia (ANSP) houses the world's most comprehensive Rotifera collection on microslides. A particular strength of the collection lies in the excellent preparation of specimens in life-like extended state and its balanced coverage of the phylum. The collection currently comprises 774 valid taxa, equalling a taxon coverage of 88.2% (families), 75.4% (genera), and 39.1% (species) compared to the taxon numbers currently known to occur worldwide.

A searchable taxonomic database of this major reference collection and type repository has recently been compiled and digital images were prepared of > 700 species using an increased-depth-of-field imaging system. This illustrated catalog is now published on CD-ROM and it is also accessible on the Academy's website. Almost certainly, easy access to searchable virtual collections and online catalogs will have a catalytic effect on faunistic, biogeographic and revisionary studies, and may serve as an invaluable tool for educating students and raising broader interest for "underdog" groups such as the Rotifera.

Based at the ANSP, it is our intention to extend this cataloging efforts by including other significant collections, surveys of little known geographic areas, and seeking cooperation of fellow rotiferologists to contribute relevant information, specimen and image material. Although at this stage the database is restricted to the taxonomy, other data pertinent to studies on systematics of the Rotifera will be included soon, and we hope that the Academy's website will ultimately become a global database of information on the Rotifera.

## **Measuring evolutionary rates in sexual and asexual rotifers**

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Sexual reproduction has long been proposed as a major factor explaining the formation of species and species diversity. Bdelloid rotifers are the most species rich animal clade believed to reproduce exclusively asexually, therefore their existence challenges the role of sex in species diversification. However, although bdelloid species are well defined on morphological grounds, little is known about the genetic basis of these boundaries. To test the importance of sexuality for species diversification, we compare the degree of genetic clustering between individuals within and between populations of bdelloid and monogonont rotifers. We use genealogical theory to outline expected patterns of clustering in sexuals and asexuals: the rate of adaptive evolution is identified as a key parameter determining the outcome. We are testing these ideas by reconstructing gene trees from multiple gene regions (COI and 18S) in representative bdelloid and monogonont genera. The trees will also allow independent tests of sexual and asexual status within bdelloid and monogonont genera.

**Trophic links in the lowland river Meuse (Belgium):  
importance of the predation exerted by rotifers  
on bacterio- and protozooplankton.**

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Trophic interactions within the plankton of the lowland river Meuse (Belgium) were measured in Spring and Summer (2001). Consumption of bacteria by protozoa was measured by monitoring the radioactivity disappearance of <sup>3</sup>H-thymidine labelled bacteria. The proportion of bacterivory of flagellates and ciliates was determined with fluorescently labelled bacteria (FLB). Metazooplankton bacterivory was assessed with 0.5 µm fluorescent microparticles (FMPs) and predation of metazooplankton on ciliates was measured by using natural ciliate assemblages labelled with FMPs as tracer food. Grazing of metazooplankton on flagellates was determined through in situ incubations with manipulated metazooplankton densities. Flagellates were the main bacterial consumers, their grazing being  $91 \pm 10$  % of total bacterial grazing. Protozoa grazing balanced bacterial production in early samplings but was lower in July. Predation of rotifers on heterotrophic flagellates (HF) was generally low ( $1.765 \text{ mg C.m}^{-3}.\text{d}^{-1}$ ), the higher contribution of HF in the diet of rotifers being observed when *Keratella cochlearis* was dominating. Predation of rotifers on ciliates was low in the first samples ( $0.557 \text{ mg C.m}^{-3}.\text{d}^{-1}$ ) in contrast to measurements performed in July ( $8.722 \text{ mg C.m}^{-3}.\text{d}^{-1}$ ). The proportion of protozoa in the diet of rotifers was generally low (< 30% of total carbon ingestion), except when phytoplankton biomass decreased below the incipient limiting level of the main metazooplankton. At such conditions, protozoa (mainly ciliates) constituted ca. 50% of total rotifer food. These results give evidence that microbial organisms play an important role within the plankton of the river Meuse, ciliates providing an alternative food for metazooplankton when phytoplankton becomes scarce.

## Superoxide dismutases possibly related to the ageing in rotifer

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Rotifers have been used as model organisms for studies on ageing, showing that certain environmental factors, such as low temperature and caloric restriction (CR), extend their lifespan. Several chemicals, for example vitamin E and niphedipine, also extend the rotifer lifespan. Although these findings have led to the establishment of a few ageing theories, the underlying mechanisms are still unclear. In the nematode *Caenorhabditis elegans*, which is another representative model organism, ageing is regulated by an interaction between reactive oxygen species (ROS) and antioxidant enzymes. In particular, superoxide dismutases (SODs) promote a conversion of harmful ROS to relatively moderate forms, resulting in the extension of lifespan of *C. elegans* under CR. We accordingly cloned two kinds of cDNAs encoding manganese- (Mn) and copper/zinc- (Cu/Zn) activated SODs from the rotifer *Brachionus plicatilis* by a conventional PCR. The isolated Mn SOD cDNA of *B. plicatilis* was further subjected to the rapid amplification of cDNA ends (RACE), resulting in the determination of the full nucleotide sequence. Deduced amino acid sequences of rotifer Mn and Cu/Zn SODs showed 61 and 44 % similarity, respectively, with that of *C. elegans*. The four amino acid residues which bind to Mn were conserved in rotifer, whereas the seven essential residues were located at Cu and Zn binding sites in Cu/Zn SOD. Then we investigated the mRNA expression patterns of Mn SOD using high-sensitive real-time PCR. The results obtained suggest that Mn SOD is possibly related to the ageing and lifespan of *B. plicatilis*.

## Evidence of vertical migration in the rotifer community in a dystrophic lake

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Studies were carried out in Lake Kruczy Staw, which is a small (area of 1.5 ha), relatively deep (10 m) and acidic (pH ranging from 3.7 to 5.4 during the season) lake with no inflows or outflows. Because the lake is surrounded by trees and has a low surface area to depth ratio, there is little wind induced mixing of the water column. As a result, temperature and oxygen stratification develops. Species diversity within the pelagic zooplankton is very low, only one species of Rotifera, three species of Cladocera, and one species of Calanoida were found in the lake. Ichthyofauna is also very poor, the only fish population is that of the stunted perch (*Perca fluviatilis*).

Earlier studies have revealed a strong vertical stratification of rotifer and crustacean communities during the growing season. Rotifers tend to live close to the bottom whereas cladocerans occupy the central part of water column. The purpose of these studies was to determine whether the occupation of different strata in the water column by different zooplankton populations is also observed in a diel cycle. To this end, samples were taken every four hours over a 24h period (from midday to midday) at 1-metre intervals.

The results demonstrate that both crustaceans and rotifers perform pronounced diel vertical migrations with the amplitude and speed of the rotifer migrations being even higher than those of crustaceans. However, there was no overlap between vertically migrating populations during the 24h period.

Predation is known to be one of the main factors controlling diel migration of crustaceans. Rotifers were also shown to escape predation by small-scale movements. However, the only zooplankton predator in Lake Kruczy Staw is a small stock of perch, as invertebrate predators are either scarce or absent. The observed vertical separation of the rotifer and crustacean communities, both in seasonal and diel cycles, may be a result of their strong competition for very low food resources. This conclusion is supported by the inverse relationship between zooplankton densities and food (i.e. picoplankton) densities observed during the diel cycle.

## Evolutionary dynamics of ‘the’ bdelloid and monogonont rotifer life history patterns

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Substantial differences in both life table characteristics and reproductive patterns separate bdelloid from monogonont rotifers. Bdelloids reproduce only asexually, whereas most monogononts are cyclical parthenogens. We explore some of the adaptive consequences of these life history differences using a computer model to simulate the evolutionary acquisition of new beneficial mutations. A one-locus mutation-selection regime based on the life history characteristics of bdelloids indicates that asexuals can have higher rates of evolutionary change and maintain higher levels of genetic diversity over a longer time period than obligate sexuals. These results are produced by differences in the magnitude of random genetic drift that are associated with the different types of reproduction. Cyclical parthenogens have significantly higher evolutionary rates in the single locus model than either obligate sexuals or asexuals; they also maintain the lowest levels of genetic diversity. The relative rates of evolutionary change between asexuals and sexuals in a one-locus model disappear when the modeled population is undergoing simultaneous selection for beneficial mutations at two or more loci. Using monogonont life table parameters in a two-locus simulation, both obligate sexuals and cyclical parthenogens have much higher rates of evolution than obligate asexuals. However, the asexuals still retain much higher levels of genetic variation over longer time periods than the other groups. Our results are strongly influenced by both the number of loci being evaluated and the quantitative characteristics of the life table inputs. We end with an attempt to use our model to help understand the adaptive basis of the remarkably divergent life histories found in typical bdelloid and monogonont rotifers.

## Changes in the population structure of the rotifer *Brachionus plicatilis* under controlled conditions

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Two experiments, using 4-day rotifer batch cultures, were conducted in order to investigate possible changes in the population structure of *Brachionus plicatilis* in relation to feeding regimes and rotifer densities. All rotifer populations were fed on *Tetraselmis suecica*. The first experiment comprised two treatments (3 replicates): rotifers also supplied with yeast (A populations) and rotifers also supplied with Culture Selco (B populations). The second experiment comprised three treatments (3 replicates): rotifers also supplied with yeast (C populations) and rotifers also supplied with Culture Selco (D and E populations). Rotifer densities were significantly higher in A, C and E than in the B and D populations. The offered daily amount of phytoplankton per rotifer was about 20 times higher in the second experiment compared to the first (70 cells.rotifer<sup>-1</sup>.day<sup>-1</sup>). Samples were collected daily, morning and afternoon. The population structure was described as follows: eggs, immature females, mature females without eggs, females with sac, females with one, two, three or more eggs, post-reproductive females and degenerate rotifers. Multivariate analysis discriminated A from B populations in the first experiment and C from D and E populations in the second. A and C populations were characterized by mature females without eggs, while B, D and E populations were characterized by females with 2 and 3 eggs and degenerate rotifers. A and C populations showed significant diurnal variations; females with eggs dominated in the morning, and immature rotifers and females with sac in the afternoon. B, D and E populations showed significant daily variations, the most important being an increased number of females with eggs on the second and third day. Specific growth rate did not significantly vary amongst treatments. The results indicated that the observed changes in population structure could be attributed to the quality of food, but not to the quantity of phytoplankton or the density of rotifers.

## Euryhaline *Brachionus* strains (Rotifera) in tropical habitat: morphology and allozyme patterns

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The marine rotifer *Brachionus* is a complex of sibling species. Early evidence for a multispecies complex came from the allozyme study of 67 strains by Fu et al. (1991). A cluster analysis uncovered two major clades associated with morphological and size differences (*S*, for small, and *L*, for large; afterwards called *B. rotundiformis* and *B. plicatilis*). Further investigation discovered variation within the *S* morphotype, where some very small strains (called *SS*) were found both in tropical Asia and in Spain. Based on the Spanish strain analysis, three species were described (*B. plicatilis*, *B. ibericus* and *B. rotundiformis*). However, the relationships among the Spanish species, the tropical *SS* strains, and the clusters described by Fu et al. (1995) remain unknown. In this study, allozyme data for five populations from the tropical regions and two from Spanish lagoons - one of them *B. ibericus* and the other *B. rotundiformis* - were combined with data in Fu et al. (1995). Cluster analysis based on genetic distance allowed division of the 74 strains into two major groups. One group was associated with *B. plicatilis*-like strains, and the other group was associated with *B. rotundiformis* and *B. ibericus*. This latter group was divided into two clades. One of them clustered most with the *S*-morphotype strains and the *B. ibericus* species. The other clade clustered most with the tropical (*SS*) strains and the *B. rotundiformis* Spanish species. These results show a correspondence between the species description based on Spanish strains and the allozyme groups identified in a large collection of strains.



## FMRFamide and 5-HT immunoreactivity patterns in the rotifer nervous system

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We present the first results of immuno-cytochemical (ICC) observations on serotonin (5-HT) and FMRFamide immunoreactivity (IR) patterns in the rotifer nervous system made with using confocal laser scanning microscope (CLSM). Three species of rotifers were studied: two swimming - creeping rotifers, *Platyias patulus* and *Euchlanis dilatata*, and the actively swimming, *Asplanchna herricki*. Irrespective of their systematic position and locomotory mode, the same nerve structures were found in the corona, the brain, along the ventro-lateral, longitudinal nerve cords, the mastax, and in the foot. The general number of 5-HT IR and FMRF-amide IR neurons is low (8-30), but always constant for each species. The neurons vary in size from 2.5 to 10 µm. The main differences, among the species studied, were observed in the innervation of the corona, the mastax, the foot and in the pattern of the cerebral neurons. From 4 to 8 brain neurons lie at different levels and form either an x-shaped or an arch-shaped pattern. The cerebral arches are steeply or smoothly curved, and the neurons forming the arches are distributed at regular intervals (*P. patulus* and *E. dilatata*) or concentrated at the lateral ends of the arches (*E. dilatata* and *A. herricki*). One or two pairs of neurons are localized along the longitudinal nerve cords. The number and the pattern of neurons (2-10) in the mastax region differ in the species studied. Double staining of 5-HT and FMRF-amide IR elements shows no co-localization. The FMRF-amide IR neurons and fibres lie beneath the 5-HT IR ones. The present ICC data are compared with the patterns of catecholaminergic (CAE) elements studied by Kotikova (1998). The 5-HT IR, FMRF-amide IR and CAE cerebral neurons constitute only about 8 -12 % of the general number of brain cells. (The study was supported by the Russian Basic Research Foundation grant, N02 -04-48583).

## Do rotifers of different body size selectively choose the habitat in macrophyte-dominated lakes?

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Lake Budzynskie is a typical shallow freshwater body with a well developed and differentiated macrophyte cover, which may influence the behaviour of inhabitant organisms. The research on the distribution of rotifers with different body size within various vegetated stands was carried out between 1997 and 1999, from May to October. Zooplankton samples were collected from five stations including two zones of submerged macrophytes (*Chara* and *Myriophyllum*), one zone of floating macrophyte leaves (*Potamogeton*), rush station (*Typha*) and the zone of open water surrounding the vegetation beds.

The mean Rotifera densities recorded during the three years of examination differed significantly between the particular zones of Budzynskie Lake. Furthermore, there was a positive selection of body size for a particular habitat, except for medium body lengths (120-170  $\mu\text{m}$ ). In this group species such as *Keratella cochlearis*, *Polyarthra vulgaris* and *Trichocerca similis*, which are described as limnetic representatives, were found in greatest abundance. However, in all other size categories, ranging from 45 to over 250  $\mu\text{m}$ , both stands of submerged macrophytes were characterized by much higher densities compared to other zones. Additionally, the analysis of the distribution of habitat-related groups of rotifers of different body size within each of the examined habitats indicated a positive effect in all cases.

The results show that the size structure of Rotifera communities differed in various vegetated areas of the lake. This is in accordance with differing spatial and morphological qualities of the macrophyte substratum, which can be further related to different food availability in a particular habitat as well as to the selective reduction of rotifers by predators.

## Spatial distribution patterns of related species within different macrophyte stands.

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The research on the distribution of species of the genus *Lecane* among different types of macrophytes (including rushes - *Typha angustifolia*, nymphaeids - *Potamogeton natans* and submerged macrophytes - *Chara tomentosa* and *Myriophyllum verticillatum*) in comparison with open water was carried out between 1997 and 1999 in the shallow part (approx. 1m deep) of Budzynskie Lake (the Wielkopolski National Park, Poland).

Six out of twenty *Lecane* species identified from Budzynskie Lake underwent analysis (*Lecane bulla*, *L. closterocerca*, *L. flexilis*, *L. furcata*, *L. luna* and *L. lunaris*) owing to their higher contribution to the densities of the genus *Lecane*. *L. bulla* dominated at most of the examined stations each year. Detailed seasonal analysis of densities of particular *Lecane* species in most cases revealed the replacement character of their occurrence. The sudden increase in numbers of one species caused a simultaneous decrease of another one or two species within the same macrophyte station. A distinct replacement pattern was observed for *L. bulla*, which was often replaced by *L. closterocerca*, *L. luna*, *L. lunaris*, *L. furcata*. Moreover, such a pattern was also shown by two species, such as *L. closterocerca* together with *L. luna*. At the same time, pairs of species, *L. luna* and *L. lunaris* and also *L. closterocerca* and *L. lunaris* exhibited a similar pattern of seasonal changes, not revealing their exchanged occurrence.

The nature of the seasonal distribution of species of the genus *Lecane*, replacing each other over a period of time, may be connected with the niche overlap of particular species, which results in time segregation. Exploitative competition cannot be excluded when describing such behaviour. The pattern of species replacement within a genus is an example of the competitive exclusion of closely related species.

## **Rotifers in large rivers**

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Our purpose is to give an overview about the life of rotifers in lotic systems, a topic that remains poorly investigated. Planktonic rotifers are generally less numerous in running waters than in standing water, because of the interactions among natural changes in discharge and local geomorphology which induce shifts in potamoplankton. In contrast with algal studies that have become a part of large river monitoring programmes, investigations of the role of heterotrophic potamoplankton, including bacteria, protists and metazoans have been poorly documented. The activities of the above were usually estimated either by measurements at confined conditions, or by modelling. Reviewing the example of the Middle Loire, which is among the more productive European temperate rivers, we examined the development of rotifers related to abiotic variables and trophic resources, derived from results obtained during growing seasons. Beginning in 1995, the algae and rotifers of this meandering eutrophic river were routinely monitored from the end of June to early October. For a shorter time period, several studies on the microbial loop were performed in this same river stretch, to examine the trophic relationships between predaceous and filter feeding rotifers and unicellulars. In addition to the examination of their local spatial distribution, the effects of water turbulence on the rotifers' demography and the fitness of some species was also determined. In conclusion, because running water acts as a resource as well as a constraint, particularly during flood events, the rotifers' life in a lotic system, with the advantages and disadvantages to live in, will be discussed, with special attention to the river's characteristics.

## Seasonal succession of rotifer sibling species: patterns and processes.

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The application of molecular techniques is showing that cryptic species complexes are more common than previously thought in aquatic invertebrates. *Brachionus plicatilis* is a sibling rotifer species complex composed of at least six species, many of which occur sympatrically in temporal brackish ponds in Eastern Spain. *Brachionus* species undergo temporal succession, yet long periods of seasonal overlap have been found, with animals living in narrower abiotic conditions in the field than they tolerate in laboratory experiments. This suggests that biotic factors play a role in limiting seasonal distribution. Based on laboratory experiments, differential trophic specialization, disturbance and predation have been proposed as factors mediating seasonal coexistence. In this contribution, we analyzed the factors (top-down vs. bottom-up) structuring sibling species rotifer assemblages in nature. During a winter-summer period in 1999, we performed an intensive sampling program in the south pond of Cabanes-Torreblanca Marsh (Castellón, Spain) where three species of the *B. plicatilis* complex occur (*Brachionus plicatilis*, *Brachionus ibericus* and *Brachionus rotundiformis*). *B. plicatilis* was observed from February to June, while *B. ibericus* and *B. rotundiformis* were observed from May to July. We studied the association of the rotifer distribution to 1) food quality and quantity (biomass and size of edible particles), 2) presence of invertebrate predators, and 3) abiotic conditions (salinity and temperature). The role of these factors in controlling coexistence and exclusion among these sibling species are discussed.

**Effect of some pesticides on reproduction of euryhaline rotifer  
*Brachionus plicatilis***

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Pesticides have been suspected as the major contributor to environmental pollution, however their use could not be avoided. Among aquatic animals, zooplankton are frequently used as test animals to detect anthropogenic contamination because of their sensitivity, and importance in the ecosystem. The effect of four commonly used pesticides in Japan (isoprothiolane, diazinon, fenitrothion and methoprene) on reproduction of euryhaline rotifer *Brachionus plicatilis* was investigated for 7 days. Concentrations of 3 to 7 times lower than the 24-h LC50 were tested to determine the NOEC, LOEC, EC50 on specific growth rate, mixis rate, fertilization rate and resting egg production. The 24-h acute toxicity test showed that diazinon was the most toxic among the pesticides tested (26.9 mg.l<sup>-1</sup>), followed by methoprene (31.3 mg.l<sup>-1</sup>). Isoprothiolane (64.1 mg.l<sup>-1</sup>) and fenitrothion (63.7 mg.l<sup>-1</sup>) were relatively toxic. In the chronic toxicity test, the concentration that affected the specific growth rate also affected all other parameters. The hatchability of the resting eggs produced by pesticide-exposed and unexposed mothers was also investigated. The advantage of covering the entire life cycle of rotifer in conducting ecotoxicological study as well as the effect of pesticides on hatchability of the resting eggs will be discussed.

## **The potential of eco-genomic and bioinformatic approaches to the study of rotifers**

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Rotifers are a key component of many freshwater ecosystems. However, large scale temporal or geographical surveys of rotifer species and their communities are constrained by the labor-intensive process of species identification. The growing availability of molecular tools such as the polymerase chain reaction and DNA microarrays, and the ever-decreasing cost of DNA sequencing and of computer power now provide a means of coupling traditional taxonomic identification with genomic and bioinformatic technologies. This combined approach could be used to track populations of specific rotifer species in space and time at a level of detail not possible with traditional collection, and are particularly well-suited to the study of interstitial or soil samples including resting egg banks. An additional application would be rapid surveys for rotifers in new environments, to determine if more thorough inspections are warranted. While the DNA sequence information currently available for rotifers would allow only a generalized identification of rotifers in such surveys, as the number of rotifer sequences accumulates, identification would become increasingly precise. Here we discuss techniques that can be combined with traditional systematics and present examples of how this approach can increase the power of rotifer ecological studies.

## Bayesian and maximum likelihood analyses of rotifer-acanthocephalan relationships

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Rotifera is composed of groups with unusual ultrastructural, physiological, and reproductive characters. Our ability to understand the evolution of these features is complicated by the fact that the phylogenetic relationships among the three traditional rotifer groups (Seisonidea, Monogononta, and Bdelloidea) and Acanthocephala remain unresolved. Here I present maximum likelihood and Bayesian analyses of rotifer-acanthocephalan relationships using both the protein-coding gene *hsp82* and a combined data set of *hsp82* and ribosomal small subunit (*SSU*) DNA sequences, using a variety of nucleotide-, codon- and amino acid based models of evolution. Statistical analysis of the phylogenetic support for any of the likely relationships among rotifer groups suggests that more than a combined *hsp82+SSU* data set may be needed to resolve rotifer-acanthocephalan phylogeny with any degree of certainty.



## **Changes in rotifer species composition and density along a trophic gradient in Loch Lomond, Scotland, UK.**

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Loch Lomond (56°10'N, 4°36'W) is the second largest body of freshwater in Great Britain in terms of its volume. The loch is used for public water supply and hydro-electric power generation and is of considerable economic, recreational and scientific value. As such, it has formed part of an EU funded research programme ('Eurolakes') which considers mechanisms for the integrated resource management of important deep European lakes and their catchments.

Loch Lomond (36.4 km long, 8.8 km wide) is a long, narrow lake. The northern basin is fjord-like, surrounded by a mountainous, base-poor, rocky catchment. In contrast, the southern basin is much broader shallower with a mainly lowland, base-rich, agricultural catchment. This causes a trophic gradient along the length of the loch that runs from the oligotrophic north basin to the mesotrophic south basin.

Rotifer samples were collected at monthly intervals between April and October 2002 at 3 locations along the north-south axis of the loch. The number and species composition of the rotifer community showed a marked variation along the length of the loch that reflected the trophic gradient. The implications of these findings in relation to the selection of biological indicators of ecological status under the EU Water Framework Directive are discussed.

## Novel biostabilisers from bdelloid rotifers

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Although anhydrobiosis – the state of suspended animation induced by desiccation – was first described in bdelloid rotifers by Antoni van Leeuwenhoek three hundred years ago, the phenomenon is only poorly understood at the biochemical level. In principle, however, there are enormous benefits to be gained from an improved understanding of anhydrobiosis. In the dry state, anhydrobiotic organisms can survive temperature and pressure extremes from near absolute zero to 150°C, and from vacuum to several thousand atmospheres. This degree of biostability would be beneficial if applied to fragile medicines and vaccines, many of which need constant refrigeration to maintain activity. This can be particularly problematic in developing nations where refrigeration may be difficult to achieve, for example, during vaccination programmes. One biostabiliser which has been used with pharmaceuticals is the non-reducing disaccharide of glucose, trehalose. This sugar is found in many anhydrobiotic organisms, but recently we have shown that trehalose is not present in clonal populations of bdelloid rotifers, *Philodina roseola* and *Adineta vaga*. This suggests that other biostabilisers are used by rotifers to enable them to undergo anhydrobiosis, and we are therefore attempting to discover these molecules. In our hands, slow drying of bdelloid rotifers yields greater survival than rapid drying, suggesting that biochemical adaptations are induced to improve desiccation tolerance. We therefore plan to compare the metabolome (small molecule profile), proteome and transcriptome of dehydrating bdelloid rotifers with those of control, hydrated animals to look for induction of desiccation stress adaptations. Molecules identified in this way will then be tested for their ability to protect biomolecules and cells against desiccation damage.

## Trophi structure in bdelloid rotifers

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Bdelloids exhibit a rather uniform morphology of jaws, all having a ramate trophi. The most recognizable features are the unci plates, in which teeth with different sizes (minor and Major unci teeth) vary in number. In particular the number of the Major unci teeth (M.u.t.) ranges from one to ten according to the species or genus, or both. Of known species, 38.2% have "two M.u.t.", while 22.1% have "three M.u.t."; other numbers are less frequent. Using SEM photomicrographs of trophi and literature data, we investigated the relationship between the total number of the unci teeth, the number of the M.u.t. and the size of trophi (as there is no growth of trophi structures during ontogenesis), and the habitat preference.

- A significant positive relationship was found between total number of teeth and trophi size (measured as rami length): in general large trophi have more teeth than small trophi.
- The number of M.u.t. seems to be negatively related to the trophi size: "one M.u.t." condition can be found in large trophi, while "ten M.u.t." condition is present in small trophi.
- Bdelloids that inhabit aquatic environments only have a teeth number that is significantly lower than in the bdelloids that exclusively inhabit terrestrial environments. Moreover, more than 60% of aquatic species have the "two M.u.t." condition.

It is still unclear if the differences in M.u.t. number in bdelloids from different habitats could be related to differences of food items or to phylogenetic constraints.

## Life history characteristics of *Asplanchnopus multiceps* (Rotifera) fed rotifer and cladoceran prey

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Members of Asplanchnidae are important invertebrate predators in freshwater plankton communities. While a considerable amount of published information exists on *Asplanchna* spp., relatively less is known about *Asplanchnopus*. We isolated *Asplanchnopus multiceps* from the littoral of a small river in the State of Hidalgo in Central Mexico and established a clone. The gut-content analysis of this rotifer collected in field revealed the presence of cladocerans and rotifers, and therefore we cultured this species on a mixture of littoral zooplankton (rotifers and cladocerans). We conducted population growth experiments on *A. multiceps* using six prey types (*Macrothrix triserialis*, *Alona rectangulara* and *Pleuroxus aduncus* among cladocerans and *Brachionus patulus*, *B. macracanthus* and *B. urceolaris* among rotifers). The prey species (*A. rectangulara* and *B. patulus*) on which the highest growth rates were observed were used to test the life-table demographic patterns in *A. multiceps*. All experiments were conducted in 50 ml containers with 25 ml of the medium and at three prey levels (0.5, 1.0 and 2.0 ind. ml<sup>-1</sup> for the cladocerans, and 1.0, 2.0 and 4.0 ind. ml<sup>-1</sup> for the rotifers) with four replicates for each treatment. The spines of *M. triserialis* and *B. macracanthus* were effective deterrents against *Asplanchnopus* predation since these diets resulted in low and sometimes, negative growth rates of *Asplanchnopus*. The average lifespan and net reproductive rate of *A. multiceps* on *A. rectangulara* ranged from 3.8 to 8.4 days and 2.6 to 12.2 ind. female<sup>-1</sup>, respectively; on *B. patulus* these varied from 5.0 to 9.4 days and 1.6 to 18.4 ind. female<sup>-1</sup>. The rate of population increase of *A. multiceps* ranged from 0.1 to 0.8 ind.d<sup>-1</sup>, depending on the prey type and density. The role of *A. multiceps* in structuring littoral and rotifer and cladoceran communities is emphasized.

## Culture of five freshwater rotifer species

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We isolated and cultured five species of freshwater rotifers; *Brachionus havanaensis*, *Lecane closterocerca*, *L. hamata*, *L. quadridentata*, and *Lepadella patella*. These species were fed with five different algal species; *Nannochloropsis oculata*, *Selenastrum capricornutum*, *Ankistrodesmus falcatus*, *Chlamydomonas reinhardtii* and *Stichococcus bacillaris*, at three food concentrations ( $1 \times 10^5$ ,  $1 \times 10^6$  and  $1 \times 10^7$  cells.ml<sup>-1</sup>), and at three different temperatures (20, 25 and 30° C). The best results for rotifer growth revealed: the use of *N. oculata* as food ( $r = 0.217$ ), a concentration of  $1 \times 10^6$  cells.ml<sup>-1</sup> ( $r = 0.313$ ), and a temperature of 30° C ( $r = 0.195$ ). From these experiments we choose *Lecane quadridentata*, *L. hamata* and *Lepadella patella*, to develop 2-l cultures for potential mass production, and their use as live food for larval fish. *Lepadella patella* was the rotifer species with the greatest potential for mass production in our work with maximum yield of 3,403 individuals ml<sup>-1</sup>.d<sup>-1</sup>, and a medium yield of 506 individuals ml<sup>-1</sup>.d<sup>-1</sup>, for seventeen days.

**The genetic structure of a cyclical parthenogen:  
*Brachionus plicatilis* species complex**

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Recently, an effort has been made to unify more than thirty years of population genetic studies of aquatic cyclical parthenogens in a model that takes into consideration three factors as crucial in determining their genetic structure: size of resting egg banks, length of the growing season and strength of clonal selection. Four species from the *Brachionus plicatilis* species complex will be used to assess the importance of these factors. Genotypic diversity and heterozygosity estimates obtained from allozyme data recorded in a temporal survey of four Mediterranean ponds will be analysed in the light of this unifying model. We will show that our results are concordant with the theoretical expectations of a negative correlation between genotypic diversity and the average length of the growth season. The size of resting egg banks and fluctuating changes in the selective regime should be therefore crucial in order to maintain the genetic diversity observed in our zooplankton populations. Furthermore, we will discuss to what extent the cyclically parthenogenetic life cycle together with the type of habitats these organisms inhabit can explain the patterns of within-population genetic diversity and among-population genetic differentiation observed in this system.

**Genetic analysis of *Brachionus plicatilis* strains used for aquaculture: preliminary results**

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Many marine fish hatcheries use *Brachionus plicatilis* as live food for the first feeding of young fish. Despite their importance, little is known of the genetic make up of the strains of *B. plicatilis* that are used in these aquaculture facilities. Also, it is unclear whether the process of controlled mass culture leads to an impoverishment of the genetic diversity of the rotifer stock.

Two approaches have been used to characterise the strains of *B. plicatilis* that are used in aquaculture:

- 1) analysis of the cytochrome oxidase I mitochondrial gene (both with restriction enzymes as well as with sequencing) and
- 2) analysis of the variability of nuclear microsatellites.

Preliminary results have been used to infer the species status of these strains. Additionally, the success of establishing clones of parthenogenetic *Brachionus* females has been checked. The results are discussed in the light of new bibliographic data that support the hypothesis that *Brachionus plicatilis* is a multi-species complex.

**Combined effects of algal (*Chlorella vulgaris*) food level and temperature on the demography of *Brachionus havanaensis* (Rotifera): a life table study**

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Among different species of rotifers, *B. havanaensis* is often found in freshwater bodies in Mexico. Its ability to tolerate wide ranges of food and temperature and to occur throughout the year in different freshwater ecosystems is fascinating. In the present work, we evaluated the combined effects of food ( $0.5 \times 10^6$ ,  $1.0 \times 10^6$  and  $2.0 \times 10^6$  cells.ml<sup>-1</sup>) and temperature (15° and 20°C) on the life history variables (both survivorship and reproductive-related) using life table demographic approach. Our results indicated that regardless of food density, average lifespan, life expectancy at birth and generation time were higher at the lower temperature tested. At any given temperature, gross reproductive rate, net reproductive rate and rate of population increase were higher at higher algal food density. The longest average lifespan was about 14 days (at 15°C, at  $1 \times 10^6$  cells.ml<sup>-1</sup>). The highest population growth rate (0.41 ind.d<sup>-1</sup>) was observed at 20°C under  $2 \times 10^6$  cells.ml<sup>-1</sup> of *Chlorella*. Both food density and temperature interacted in a positive way for enhancing gross and net reproductive rates and the rate of population increase.



## Identification of acetylcholinesterase receptors in several freshwater rotifer species

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We have identified acetylcholinesterase receptors in several parts of the lorica of several freshwater rotifer species. Muscarinic receptors were found in *Brachionus patulus*, *B. variabilis* (females and males), *Lecane luna*, *L. quadridentata*, and *Rotaria rotatoria*, using  $\alpha$ -bungaratoxin labeled with FITC (FluoresceinIsoThioCyanate). Similarly, nicotinic receptors were identified in *Brachionus patulus*, *B. variabilis*, *Lecane bulla*, *L. luna*, *L. quadridentata* and *Rotaria rotatoria* using  $\alpha$ -bungaratoxin-FITC. We used concentrations as low as 1.49 nM of  $\alpha$ -bungaratoxin, and 5 nM of  $\beta$ -bungaratoxin to identify the receptors localized mainly in the digestive tract area. Higher concentrations of both toxins identified further receptors along the side of the lorica. An analysis of fluorescent intensity in *Lecane quadridentata* showed that the response to  $\alpha$ -bungaratoxin increases with age from newborns to 24 h old individuals, suggesting increases in binding sites and possibly in the number of nicotinic receptors. As far as the authors know, this is the first report of the presence of acetylcholinesterase receptors in rotifers.

## Interactions between rotifers and selected phytoplankton species in submontane dam reservoirs in southern Poland.

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Allogenic and autogenic features influence the functioning of the ecosystem of dam reservoirs. Autogenic features include interactions between phyto- and zooplankton. A better recognition of the co-existence of algal and animal planktonic species is very important to the better understanding of the complete reservoir ecosystem. The relationships between chosen species of planktonic diatoms, green algae and rotifers were investigated in the three submontane reservoirs (Czorsztyn, Dobczyce, Roźnów in southern Poland). These reservoirs differ from each other in trophic status, age, shape, depth etc. The following most abundant species were chosen for these studies: *Asterionella formosa*, *Aulacoseira granulata*, *Cyclotella* spp., *Fragillaria crotonensis*, *Melosira varians*, *Navicula* sp., *Nitzschia* sp. (diatoms), *Coelastrum microporum*, *Desmodesmus eornis*, *D. quadricauda*, *Elakatothrix acuta*, *Lagerheimia* sp., *Oocystis* sp., *Pandorina morum*, *Pediastrum boryanum*, *P. duplex*, *P. simplex*, *Scenedesmus acuminatus*, *Tetraedron minimum* (green-algae), and *Anuraeopsis fissa*, *Asplanchna priodonta*, *Conochilus unicornis*, *Kellicottia longispina*, *Keratella cochlearis*, *K. cochlearis* f. *tecta*, *Keratella quadrata*, *Pompholyx sulcata*, *Polyarthra vulgaris*, *Synchaeta* sp., *S. oblonga*, *S. pectinata* (rotifers). Physico-chemical parameters were also included in the statistical analyses of the data. The most important factors influencing rotifers dynamics in the three reservoirs were: temperature and SiO<sub>2</sub>. In the oldest reservoir, Roźnów, the greatest amount of interactions among diatoms, green algae and rotifers species were noted. The relationship common to all of the reservoirs investigated was that between the density of *K. cochlearis* f. *tecta* and the total density of coccal green algae.

## Spatial distribution of rotifers in a cascade of pomeranian reservoirs (Hajka, Rosnowo) in Poland.

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The structure of rotifer communities, and their spatial distribution, were examined in two stratified reolimnetic pomeranian reservoirs, Rosnowo (7 sampling sites) and Hajka (4 sampling sites), in July 1999. The reservoirs, constructed in a cascade system, are situated on the Radew River in northern Poland. The phytoplankton community in both reservoirs was similar, the dominant group being diatoms (*Aulacoseira granulata*, *Diatoma elongatum*, *Stephanodiscus hantzschii*) with significant and spatially variable numbers of flagellates (*Dinobryon sociale*, *Cryptomonas* sp., *Chlamydomonas* sp.). In Rosnowo reservoir, 23 species of rotifers were found; in Hajka reservoir, 21 species of rotifer were found. The dominant rotifers in both reservoirs were *Keratella cochlearis* f. *tecta* and *Polyarthra vulgaris*, with *Synchaeta pectinata* of lesser importance.

At least two mechanisms were thought to affect the composition of the rotifer communities. These were 1) the impact of physico-chemical conditions, including nutrient (trophic) gradients, and 2) the effect of algal species composition on the spatial distribution of the rotifers. Statistical analyses suggested that physical and chemical parameters within different areas in the reservoirs were reflected in the rotifer community structure and spatial distribution.

## The relationship between planktonic rotifers and phytoplankton in lakes of different trophic status in the Polesie Region of Eastern Poland

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Four lakes of differing trophic status in the Polesie Region (Poland) were studied: Lakes Piaseczno (mesotrophic), Usciwierz (eutrophic), Rotcze (eutrophic) and Moszne (dystrophic). Plankton samples were collected in the spring, summer and autumn of 2001 and 2002. The study found 145 species of phytoplankton and 43 species of planktonic rotifers. The greatest species diversity in each of these groups was recorded in Lake Rotcze, the lowest in Lake Moszne. There were less planktonic rotifer species than phytoplankton species in all of the lakes studied (22 - 29 rotifer species; 31 - 47 phytoplankton species). Among the phytoplankton, the chlorophytes exhibited the greatest number of species. The greatest number of phytoplankton species occurred in the pelagic zone, whilst the greatest species richness of rotifers was found in the littoral zone. Among the planktonic rotifers, 11 species were most numerous: *Anuraeopsis fissa*, *Asplanchna priodonta*, *Kellicottia longispina*, *Keratella cochlearis*, *Keratella quadrata*, *Trichocerca capucina*, *Trichocerca similis*, *Brachionus angularis*, *Brachionus rubens*, and *Filina longiseta*. Among the phytoplankton, the highest density was reached by two species: *Aphanothece clathrata* and *Ceratium hirundinella*. Variation in phytoplankton and planktonic rotifer densities showed similar trends in all lakes irrespective of their trophic status. The pattern of dominance in the phytoplankton community differed depending on the trophic status of the lake. In Lake Piaseczno and Lake Moszne, *Aphanothece clathrata* was the dominant species, *Aphanothece clathrata* and *Closterium di-anae* predominated in Lake Rotcze and a single species, *Cyclotella glomerata*, was dominant in Lake Moszne. The rotifer community was dominated by *Keratella cochlearis* in all lakes except Lake Piaseczno where two species, *Keratella cochlearis* and *Kellicottia longispina*, were co-dominant.

## Rotifer body wall muscles observed using phalloidin fluorescence and confocal scanning laser microscopy

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The combination of phalloidin fluorescent staining of F-actin fibres with confocal scanning laser microscopy allows us to observe the complex muscular patterns in rotifers. Species with different modes of locomotion have been investigated: swimming-creeping forms with loricas (*Testudinella patina*, *Platylas patulus*, *Euchlanis dilatata*, *Brachionus quadridentatus*) and a swimming form without a lorica (*Asplanchnopus multiceps*). The species investigated differ in the position and strength of the coronal muscles and retractors, the longitudinal and circular muscles of the trunk and the retractors of the trunk and the foot. In the corona of the swimming-creeping rotifers, few muscles and short retractors occur, while in the swimming *A. multiceps*, strong semi-circular muscles occupying all the apical field are observed. In rotifers with a lorica, only 2-3 thin ring muscles occur in the trunk, whereas in *A. multiceps*, a species without a lorica, the trunk possesses six thick circular muscle bands. Significant differences among species are observed in the position and development of the trunk retractors. Their number varies from 2 to 5 pairs, and thickness from 1.6  $\mu\text{m}$  to 160  $\mu\text{m}$ . The proximal ends of the trunk retractors are connected either to the transversal muscle (*T. patina*, *E. dilatata*, *B. quadridentatus*), the thin ring muscle (*P. patulus*), or the strong postcoronal ring (*A. multiceps*). Dorso-ventral muscles, varying in number from 4 to 17, occur only in species with a lorica. The foot has 1 to 3 pairs of retractors of varying strength. The mode of locomotion causes essential differences in the patterns of the rotifer muscles. The actively swimming *A. multiceps* has prominent trunk retractors, a strong skeleton of longitudinal and ring muscles and extensively developed coronal muscles. The presence of complex muscular patterns with highly differentiated single muscles in rotifers serves as evidence of the progressive development of musculature within the Spiralia (Supported by RFBR 02-04-48583).

## Laboratory studies on the feeding and reproduction in the predatory rotifer *Asplanchnopus hyalinus*

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The predatory rotifer *Asplanchnopus hyalinus*, unlike *Asplanchna*, is generally associated with periphytonic microhabitats, and is locally seasonal and rarely abundant. Gut content examination shows that it preys predominantly on chydorid cladocerans. The rotifer was collected from a shallow lake, cloned and cultured in the laboratory using chydorids and brachionid rotifers as food. *A. hyalinus* is a voracious predator; an adult female ( $809 \pm 36.4 \mu\text{m}$ ) could consume  $17.6 \pm 2.2$  individuals of *Brachionus rubens* ( $150 \mu\text{m}$ ) in 30 min. Prey handling times were generally function of the body sizes of both prey and predator, but they were significantly shorter for the much larger ( $328 \pm 34.4 \mu\text{m}$ ) chydorids than for the spiny morphs of *B. calyciflorus* ( $200 \pm 23 \mu\text{m}$ ). The female is cannibalistic, feeding on young conspecific females and mated males. The reproductive parameters of *A. hyalinus* were estimated from life table experiments. The average lifespan of the rotifer was  $< 4$  days and age at first reproduction 24 - 36 hours. With unlimited food (*Brachionus angularis*), *A. hyalinus* showed a total lifetime fecundity of  $19 \pm 3.98$ , with population growth rates exceeding 1.25. Males appeared regularly in the cultures, but it was not clear whether crowding or food limitation acted as a stimulus for mictic reproduction. Each male, with a lifespan of  $\sim 24$  hours, could mate more than 4 times, the duration of each copulation being  $62.4 \pm 16.14$  seconds. A single female could also be mated 4 - 5 times, even concurrently by up to three males. The mated female produced  $4.5 \pm 1.05$  resting eggs. Attempts to hatch them were unsuccessful.

## Anhydrobiosis of *Adineta* sp.: costs and benefits

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All bdelloid rotifers are able to undergo dormancy (anhydrobiosis) successfully, and most authors agree that populations appear 'rejuvenated' after dormancy. A bdelloid species was found to disregard the time spent in anhydrobiosis and to keep fecundity and longevity rates unaffected, regardless to the duration of dormancy. In this study, we have induced anhydrobiosis to another bdelloid species, *Adineta* sp., and investigated life-history traits 1) of the recovered animals, and 2) of the offspring produced after dormancy. In the first experiment a cohort was dried when 8-days old, kept dry for 7 days and rehydrated. The recovery percentage was about 75%, and the recovered rotifers had significantly higher fecundity than, and similar longevity as hydrated controls, if the dry period is disregarded. In the second experiment we studied the life cycles of the orthoclone (*D*) produced by recovered mothers aged 18-days 'anagraphically', but 11-days 'physiologically', and of the orthoclone (*E*) produced by same mothers aged 25-days 'anagraphically', but 18-days 'physiologically'. These orthoclones were coupled to control ones, established by hydrated mothers aged 11-days (*B*) and 18-days (*C*) respectively. For comparison, another orthoclone (*A*) was isolated from young (7-days old) hydrated rotifers and used as baseline. If compared to the traits of *A* orthoclone *D* did not differ in both survival and fecundity, while the remaining clones did. The offspring produced after dormancy had fecundity and longevity significantly higher than control hydrated cohorts, and gained as many as 5 eggs/female/lifetime over the respective controls. Maternal age affected the life-history traits of the orthoclones of either lines remarkably, causing to both the same loss of fitness, but on the whole, the offspring produced by rotifers experiencing anhydrobiosis had higher fitness than those whose parents were kept hydrated.

***Macrotrachela quadricornifera***  
**protagonist of a space experiment**

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*Macrotrachela quadricornifera*, a bdelloid rotifer, is the animal model of an experiment scheduled on the International Space Station (*ISS*). The experiment focuses on the role of the cytoskeleton during oogenesis and early development. Assuming that the arrangement of cytoskeletal components are perturbed by weightlessness, the morphology of embryos produced and/or developed in the absence of gravity will be studied. The experiment will last about 3 months.

The bdelloids will travel desiccated. They will then be rehydrated on the *ISS* and cultivated for several generations. Cultivation, and all other experimental procedures will be performed automatically in a tailor-made bioreactor. In the bioreactor, under a software control, the culture medium and food will be renewed daily, and at predefined times, and eggs will be transferred to another culture chamber to start the next generation. After each egg transfer, the previous population will be desiccated and stored until they are returned to earth. On return, each generation will be rehydrated, cultivated and processed for further investigations (morphology, life-history studies, development, etc.). During the experiment on *ISS* a total of 5 generations will be run. Here we present the outline of the *ISS* experiment, the expectations and state-of-the-art of ground-based research run so far on the main topics of the planned experiment, i.e. anhydrobiosis, embryogenesis and bioreactor. The first topic has been investigated by considering two aspects: 1) the morphology of desiccated rotifers at SEM and Confocal Laser Scanning Microscope, and 2) a fine desiccation protocol reliable enough to produce reproducible results. Embryogenesis has been approached at the morphological level under ground conditions: cleavage and part of gastrulation have been studied under light and confocal microscopes. The bioreactor study is the result of collaboration between the scientific researchers and an Italian factory charged with building the necessary hardware and software.



## **Induction of heat shock protein 60 (HSP60) in rotifers exposed to arsenic and heavy metals.**

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Rotifers may respond to pollutant exposure and other abiotic/biotic factors by inducing the synthesis of stress proteins. Rotifers were exposed to four concentrations of Cu (20 - 63 ppb) and to low (10 ppb) and high (5 - 150 ppb) concentrations of As, Cr, Cu, Ni, Pb, and Zn both singly and in combined exposures. Proteins were separated and identified using Western Blotting. Results show that HSP60 induction response depends on both concentration and exposure time. Induction increases proportionally with exposure time in rotifers exposed to low concentrations of CuSO<sub>4</sub> while at high concentrations immediate response is elevated followed by a dramatic decrease in expression. Induction of HSP60 was increased in rotifers exposed to low levels of As, Cr, Cu, and Ni as compared to rotifers not exposed to heavy metals. The susceptibility of rotifers to environmental changes due to pollutants can be used as indicator of water quality and/or as a model for toxicity testing.

## The rotifer communities of temporary waters in northeast Thailand

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The rotifer communities of 431 temporary waters in northeast Thailand were investigated during the rainy seasons of 1999-2002. The samples were collected qualitatively from small temporary pools, shallow roadside canals and paddy fields, using a 60- $\mu$ m mesh net. One hundred and eighteen species were identified. The majority of the species recorded are widely distributed tropical taxa, similar to those found in the permanent waters of Thailand. The most diverse genus was *Lecane* with 38 species (32.2%), followed by *Trichocerca* (15 species, 12.7%) and *Brachionus* (12 species, 10.2%). The most frequently recorded species were *Polyarthra* cf. *vulgaris* Carlin, *Lecane papuana* (Murray), *Plationus patulus* (Muller), *Lecane bulla* (Gosse), *Filinia longiseta* (Ehrenberg), *Brachionus quadridentatus* Hermann and *B. falcatus* Zacharias with 53.4, 37.6, 37.4, 32.0, 25.3, 22.3 and 21.6% of the sampled localities, respectively. Other infrequently recorded rotifers were *Brachionus durgae* Dhanapathi, *B. lyratus* Shephard, *Lepadella dactyliseta* Stenroos, *L. vandenbrandei* Gillard, *Lecane decipiens* (Murray), *L. donneri* Chengalath & Mulamoottil, *L. doryssa* Haring, *L. monostyla* (Day), *L. obtusa* (Murray), *L. ruttneri* Hauer, *L. thienemanni* (Hauer), *Testudinella parva* (Terntz), *Trichocerca chattoni* (De Beauchamp), *T. hollaerti* De Smet, *T. inermis* (Linder), *T. insulana* (Hauer), *T. longiseta* (Schrank), *T. stylata* (Gosse) and *Macrochaetus collinsi* (Gosse).

## Motion of pond dwelling bdelloid and monogonont rotifers: a morpho-functional approach

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Rotifera employ several different methods of locomotion that involve the beating of cilia on the corona, the contraction of body musculature and, sometimes, retraction of the foot. Both cilia and muscles may contribute to the movement either alternately or simultaneously. The relationship between body shape, methods of locomotion and muscle arrangement was investigated using a confocal laser scanning microscope (CLSM) to observe the musculature and a scanning electron microscope (SEM) for the external morphology. Benthic and planktonic rotifers with different methods of locomotion were collected from a pond for study. These were *Notommata glyphura*, *Dicranophorus forcipatus*, *Macrotrachela quadricornifera*, *Adineta* sp., *Hexarthra mira* and *Floscularia ringens*. The organization of the body muscles was largely uniform in the two bdelloids, *M. quadricornifera* and *Adineta* sp. However, this was not the case in the monogononts. *Notommata glyphura* and *D. forcipatus* presented a similar arrangement of musculature, particularly complex at the level of the head; the foot of *F. ringens* had a well-developed musculature consisting of six bundles; *H. mira* had strong muscles in each arm. The use of both CLSM and SEM provides a more detailed description of the anatomy and external morphology of rotifers and provides new contributions to our knowledge of the complicated anatomy of such microscopic animals.

## Factors affecting egg-ratios in planktonic rotifer populations under culture conditions

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The Edmondson's egg ratio (number of amictic eggs to the females) is an important variable, widely used to understand and predict the patterns of population growth in planktonic rotifers under field conditions. It is also useful as an indicator of the rotifer health under culture conditions. Generally, an inverse relationship exists between the egg ratio and the density of females in a population. However, a number of biotic and abiotic factors influence the egg ratio. Temperature for example, via its influence on the frequency of egg production and the hatching times of parthenogenetic eggs, causes strong changes in the egg ratio which may be unrelated to the population growth rates. Similarly, if invertebrate predators, such as *Asplanchna*, selectively feed on only the eggs but not the females, the egg ratios could be much lower than expected. We have observed this, particularly under field conditions. The easy detachment of eggs from some members of Brachionidae, especially, if there is an enhanced reproduction under high food levels, may also cause an underestimation of the egg ratio. In this review, we discuss the role of diel changes in the egg deposits, influence of frequency of food addition, problems of distinguishing between different egg types and negative effect of stresses such as toxicants and diet quality on the egg ratio of selected rotifer species.

## **Changes in rotifer communities regarding to the water-level fluctuations in the floodplain Gemenc, Danube, Hungary**

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At the floodplain of Danube at Gemenc we examined the planktonic rotifer communities in three river-arms that hydrodynamically differ (one with active flow, one closed at the beginning, and one which was revitalized). Depending on the water level in the arms, the current speed differs. Consequently, the physico-chemical parameters (temperature, conductivity, transparency, dissolved oxygen content) also differ. At high water level, during floods, the rotifer communities of the area will be uniform but at low water levels the area is carved up into different waterbodies, that look like lakes. This process begins a few days after the floods. The species composition and the abundance relations of the planktonic rotifer communities reflect these hydrological changes.

## **Diapause in rotifers**

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This review focuses on more recent findings on the diapause in Monogonont rotifers, since the major reviews by Pourriot & Snell (1983) and Gilbert (1992, 1993). It covers resting egg formation, resting egg survival, and resting egg hatching as well as possible strategies involved in these processes. Data from laboratory studies as well from natural populations exist, but especially data on resting egg hatching and emergence under in situ conditions are rare. Resting or diapausing egg production can be mictic and in some cases amictic. Mictic resting egg production is depending on factors inhibiting mictic female production which can be either environmental or endogenous, and on factors inducing mictic female production. Knowledge about factors inducing mictic female production is still limited to a few species, but effects of population density may be more widespread. Recent results also show that male mating behavior may play an important role in the successful production of resting eggs. Important factors inducing hatching are related to temperature and light conditions, also desiccation may be a factor. However, desiccation may also adversely affect the survival of diapausing stages in temporary habitats. Although not too many data are available, those existing point to distinct differences between populations and the importance of clonal variation within populations regarding the initiation as well as the termination of diapause.

**Phylogeny and classification of the Gnesiotrocha  
(Rotifera, Monogononta): a comparative morphological  
and molecular phylogenetic study.**

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We re-evaluate the classification of the Gnesiotrocha by a comparative study of morphological (including SEM of trophi structure) and molecular (18S ribosomal DNA) data. This study covers all (7) families, and 14 of 21 genera of Gnesiotrocha, with an emphasis on the most diverse family, Flosculariidae (26 samples of 22 species, all genera). The results refute monophyly of the following taxa recognized in the contemporary classification of Gnesiotrocha: Flosculariacea and Collothecacea; Flosculariidae; *Ptygura*, and *Floscularia*. Members of Conochilidae are identified as belonging to an ill-defined group of taxa together with *Sinantherina*, *Octotrocha*, *Lacinularia*, some *Floscularia* and *Ptygura*. Corona shape, until now the most frequently used feature in the diagnosis of genera is disproved as a reliable morphological feature reflecting phylogenetic relations in the group. A new classification incorporating the new insights is proposed.

Evolution of Gnesiotrocha is believed to start off from primitive sessile, pseudo-colonial, microphageous filter-feeders, leading along independent lineages towards free-living, solitary (e.g., Hexarthriidae, Testudinellidae, Trochosphaeridae), or colonial (*Lacinularia*, "Conochilidae") animals, and towards animals capable of engulfing large prey (uncinate trophi: Atrochidae, Collothecidae).

**Rotifer communities of floodplain lakes of the  
Brahmaputra basin of lower Assam (N. E. India):  
composition, distribution and ecology**

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This study deals with the seasonal investigations, undertaken during 2002-2003, on the rotifer communities of fifteen floodplain lakes of the lower Assam valley of the Brahmaputra river basin (N.E. India). These lakes are characterized by low ionic concentrations (mean conductivity: 28.2-164.8  $\mu\text{S}\cdot\text{cm}^{-1}$ ), acidic-alkaline and soft to marginally hard-waters. 164 rotifer species, belonging to 39 genera and 20 families, were documented, indicating these lakes to support the most diverse rotifer habitats so far known on the Indian subcontinent. Nine species are new records to the Indian Rotifera and ten species are new additions to N. E. India. The reported species comprise 46.4 % of the Indian Rotifera and 87.2 of the fauna of this region. The present observations registered an overall richness between 67-103 species in the sampled lakes, high mean richness ( $79.1 \pm 11.0$ ) and community similarity of 53.4-86.5%. Besides, individual lakes depicted notable seasonal variations (30-68 species) and exhibited no defined pattern of qualitative diversity. Lecanidae > Brachionidae > Colurellidae > Trichocercidae comprise significant components (64.5%), while Notommatidae > Euchlanidae = Filiniidae = Testudinellidae, together, form an important fraction (18.3%) of the recorded species. Cosmopolitan elements (59.7%) dominate the rotifer taxocoenosis. In addition, pantropical (15.2%) and cosmotropical (12.2%) taxa are well represented, and biogeographically interesting species (13.4 %) constitute a notable component; important members of the last category include seven Oriental, three Australasian and eight Palaeotropical and one Afrotropical species. The examined fauna exhibits general tropical nature characterized by the predominance of *Lecane* spp. (28.0 %). Besides, *Lepadella* (9.8 %) > *Brachionus* (7.9 %) = *Trichocerca* (7.9%) > *Keratella* (4.3%) = *Filinia* (4.3%) > *Testudinella* (3.7%) are other important contributors to species richness. Littoral or periphytic rotifers (76.2%) dominate over planktonic species (23.8%) in the examined material. The rotifers,



however, comprise the dominant qualitative and quantitative component of littoral and limnetic communities in all the floodplain lakes; they register moderate species diversity, low dominance and high evenness. The examined material also indicated several interesting acidophilic elements. The  $Q_{B/T}$  quotients ranged between 1.1-2.0, reflecting a general mesotrophic status of different floodplain lakes.

## Biodiversity of freshwater rotifers (Rotifera: Eurotatoria) from North-Eastern India, with remarks on ecology

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A survey of the rotifer fauna of North-Eastern India, a global biodiversity hot-spot, revealed 188 species (205 taxa), comprising 53.2% of the Indian Rotifera. This reflects the richest diversity of the group so far known from any region of this country and also registered highest generic (39 genera) and family richness (20 families). Cosmopolitan species (59.0%) comprised the dominant fraction while pantropical (14.4%) > tropicopolitan (9.6%) elements are well represented. Besides, biogeographically interesting species represent a significant component (17.0%). These include five Australasian, nine Oriental, ten Palaeotropical, one Holarctic, one Afrotropical and four endemic taxa. The members of the first two categories impart special affinities of N. E. Indian Rotifera with the faunas of South-East Asia and Australia; this interesting and unique feature is exclusive of the rotifer diversity of N. E. India. Lecanidae (28.7%) > Brachionidae (14.4%) > Colurellidae (13.8%) > Trichocercidae (9.0%) > Notommatidae (6.9%), together comprise 72.8% of the documented species. Four genera, namely *Lecane* (28.7%) > *Lepadella* (11.1%) > *Trichocerca* (9.0%) > *Brachionus* (7.4%) represent important components of the rotifer richness. The examined taxocoenosis exhibits broadly tropical character and paucity of temperate elements; the former aspect is attributed to the collections obtained primarily from subtropical and tropical localities of the region. Acidic - slightly acidic - circum-neutral biotopes of several parts of N. E. India are characterized by occurrence of several acidophilic elements (11.3%) and relative paucity of *Brachionus* spp. (9.6%) as compared with typically alkaline environs of the Gangetic and peninsular India. The floodplain lakes of the Brahmaputra river basin and the rice-fields are most interesting habitats for the rotifer diversity. Planktonic and littoral or periphytic taxa are fairly well recorded presently while sessiles and bdelloids are yet inadequately reported. This study registered maximum richness from the state of Assam (170 species) followed by Meghalaya (116 species) and Tripura (112 species); extensive collections are still desired from states of N. E. India.

**Adaptive radiation in arid zone rotifera:  
Lake Eyre and Carnarvon Basins, Australia**

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In the first study to include the Rotifera of arid inland Australia, >80 sites in the 1,140,000 km<sup>2</sup> Lake Eyre Basin (LEB) were sampled (35 µm nets, 4 l Haney trap) during seven field trips 2000-2003. Water bodies were mostly ephemeral, not all contained water on all trips. Nevertheless, >250 rotifer species/morphotypes were identified, with ca. 15% novelty, i.e. new records for the continent (11) or new species (26). We compare the LEB surveys with an earlier study, 1994-1995, of 56 sites in the similar-latitude Carnarvon Basin of W.A. Fewer rotifer species were recorded (73), but novelty (15%) was comparable. Although many species were shared between the two basins, regional endemism is evident. We discuss briefly the results of community ordinations. Ephemerality and salinization are seen as major determinants of rotifer community composition, and a driver of adaptive radiation in both arid/semi-arid areas, particularly in response to regular 'switching' from fresh to saline, a process which appears to have had a long evolutionary history in both regions – they are effectively biodiversity 'hotspots'.

## Morphological descriptions of selected rotifers from Lake Xochimilco (Lake Xaltocan), Central Mexico

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Rotifers are an important group of planktonic organisms in Lake Xochimilco (Central Mexico), water from which has been extensively used for human consumption, farming and aquaculture for more than five centuries. Analyses of plankton samples collected during several months during 2002 revealed wide variations in the morphology of some rotifer species. The aim of the present work is to examine the range of morphological variations exhibited by some of these rotifer species from the lake. Detailed light microscopic and SEM observations were made for common and important rotifer genera (*Brachionus*, *Keratella*, *Synchaeta* and *Trichocerca*). Data on the morphology of resting eggs of some rotifer genera is also presented.

## Removal of the mate recognition pheromone and transfer among *Brachionus* species

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When *Brachionus plicatilis* Russian strain females are exposed to 50 mM of EDTA or EGTA, several surface glycoproteins are removed. SDS-PAGE of these proteins reveals 12-14 prominent bands. Females treated with EDTA for 30 minutes died, but remained intact and were used in mating bioassay with conspecifics males. Live control females elicited a male mating response in 21% of encounters, freeze-killed control females elicited responses in 23%, but EDTA extracted females elicited a mating response in only 5% of encounters. The EDTA treatment stripped away surface glycoproteins critical to mate recognition. The EDTA extract was separated using ion exchange chromatography and the fractions were tested for their ability to restore female attractiveness. Females having their surface glycoproteins stripped away by EDTA were treated with ion exchange fraction 22. Proteins in fraction 22 bound to females, restoring 80% of their original attractiveness to males. Exposing EDTA treated females to bovine serum albumin or casein had no effect on restoring their ability to elicit the male mating response. Proteins in fraction 22 were exposed to EDTA treated females from different *Brachionus* strains and species. Female attractiveness was restored in most strains of *B. plicatilis*, but no transfer of mating attractiveness was observed to *B. rotundiformis*.

**On the rotifer fauna of Disko Island, Greenland, with notes on selected species from a stagnant freshwater lake.**

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The rotifer fauna in Moraine Lake on Disko Island was investigated. 25 taxa were found. 22 of the recorded taxa are new for Disko Island and three are new for Greenland. The new findings are presented in a species list that summarizes all records of rotifers from Disko Island, inclusive several records that not earlier have been published. Furthermore, notes are given on the morphology of some selected species, inclusive *Eothenia elongata*, *Microcodides chlaena* and *Resticula nyssa*.

## First record of *Seison* (Rotifera: Seisonidea) from the Indian Ocean

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Here we present the first record of *Seison* from the Indian Ocean, which increases the geographic distribution of seisonids greatly. Fourteen specimens belonging to the genus *Seison* were found in fixed meiofauna samples taken from seagrass beds at Gazi Bay, Kenya. The specimens generally resemble *S. nebaliae*, but they also differ in some interesting points. All the specimens are sexually mature adults and relatively small. The females have a conspicuous vulva around the cloacal opening. Furthermore, SEM studies of the trophi of the Kenyan specimens revealed a close resemblance to those of *S. nebaliae*, although a basal plate was lacking as in *S. annulatus*. The SEM studies also revealed a small, ventral oral plate, a structure never observed in seisonid trophi before. We cannot firmly conclude whether the species studied here is *S. nebaliae*. The differences in size and morphology could reflect a local variation, or it could be a new species. As the samples were treated and fixed by others, we never had the opportunity to observe live specimens, and we have no information whether the seisonids from Kenya were associated with a host.

**The musculature of *Testudinella patina*  
(Rotifera: Flosculariaceae), revealed with  
confocal laser scanning microscopy (cLSM)**

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The musculature of *Testudinella patina* was observed using phalloidin-linked fluorescent dye under confocal laser scanning microscopy. The conspicuous broad retractors appear to be made up of five separate fibres, three of which are anchored in the neck region whilst two extend into the corona. Besides the broad retractors, a total of five paired longitudinal retractors are present, all of them extending into the corona. Incomplete circular muscles are found in groups in the neck region and in the medial and posterior parts of the trunk. The foot musculature comprises eight thin ventral muscles and six thicker dorsal muscles that all extend from the base of the foot to the distal part. At the base of the foot each of the dorsal muscles anchors onto a smaller, s-shaped subterminal foot muscle. The foot musculature furthermore comprises one pair of paraterminal muscles each of which anchors basally onto a subterminal foot muscle, extends into the most proximal part of the foot and attaches onto one of the dorsal foot muscles. The visceral musculature is composed of extremely delicate fibres and is restricted to an area around and posterior to the foot opening. The presence of incomplete circular muscles supports the theory that these muscles are a basal trait for Rotifera. The morphology of the broad retractors and foot muscles is much more specialized and may be autapomorphic for *Testudinella* or alternatively for this genus and its closest relatives. The present results stress that revealing muscles by staining may produce new information from even well-investigated species and that this information may contribute to a better understanding of the functional, as well as phylogenetic, aspects of rotifer biology.



**Susceptibility of ephemeral pool *Hexarthra* sp. to predation by the fairy shrimp *Branchinecta mackini*: does predation drive local extinction?**

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An extensive area of the US desert Southwest has exposures of eroded sandstones containing rocky basins that form ephemeral pools (regionally termed tinajas) following episodes of sustained rainfall or snow. Many of these pools cluster in interconnected drainages and, if of sufficient size and duration, are dominated seasonally by eubranchiopods and/or cladocerans. These waters seldom (perhaps never) contain large rotifer populations. Other pools are "perched" above the main catchment drainages in smaller, more isolated depressions; these latter systems seldom contain the larger crustaceans but often do develop dense monocultures of an apparently lithophilic species of the rotifer *Hexarthra*. I hypothesized that the rotifers persisted in these isolated pools due to the absence of either competition with or predation by potentially sympatric crustaceans. I tested this idea with a series of laboratory and field experiments, in each case exposing a range of densities of *Hexarthra* sp. to adult fairy shrimp (*Branchinecta mackini* – the dominant anostracan in this region) in 200 ml microcosms. In all cases, individual fairy shrimp had distinct negative effects on rotifer populations, apparently due to direct predatory consumption of the smaller animals. Male *B. mackini* had slightly higher individual predation rates on *Hexarthra* sp. than did females, although this differential was reduced when expressed on the basis of predator dry weight. The results indicate that, while perhaps not the sole determinant of rotifer distribution in tinajas, fairy shrimp predation can have a strong negative influence on *Hexarthra* populations.

## **Evolution of rotifer life histories**

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Life history theory makes predictions about how organisms should adapt to the selective pressures of their environments by altering different variables in their life cycle. This topic has a long tradition in rotifer research. The most popular approach to study rotifer life histories are life table response experiments. In this paper I shall review recent studies using this approach, but also studies targeting more specific points, such as: age/size at maturity, the cost of reproduction, reproductive effort, and variation in egg/offspring size.

## The biochemistry and genetics of anhydrobiosis in bdelloid rotifers

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Some eukaryotes, including bdelloid rotifer species, are able to withstand desiccation by entering a state of suspended animation. In this ametabolic condition, known as anhydrobiosis, they can remain viable for extended periods, perhaps decades, but resume normal activities on rehydration. Anhydrobiosis is thought to require accumulation of the non-reducing disaccharides trehalose (animals, fungi) or sucrose (plants), which are hypothesised to protect proteins and membranes by acting as water replacement molecules and vitrifying agents. One test of such hypotheses would be to use trehalose or sucrose to attempt to confer desiccation tolerance on sensitive cell types, such as mammalian cells in tissue culture. However, recent attempts at engineering desiccation tolerance in sensitive organisms using trehalose or sucrose have met with limited success, indicating that disaccharides alone are not sufficient for anhydrobiosis. Using clone cultures of bdelloid rotifers *Philodina roseola* and *Adineta vaga*, we now show that disaccharides are also not necessary for anhydrobiosis. *P. roseola* and *A. vaga* exhibit excellent desiccation tolerance, but completely lack trehalose or other disaccharides in both control or dehydrating animals, as determined by gas chromatography. Indeed, trehalose synthase genes (*tps*) were not detected in these rotifer genomes, suggesting that bdelloid rotifers may not have the capacity to produce trehalose under any circumstances. This is in sharp contrast to other anhydrobiotic animals such as nematodes, tardigrades and brine shrimp cysts, where trehalose is present during desiccation. Furthermore, preliminary metabolite profiles of control and dried rotifers obtained by proton NMR indicate that no small molecule analogue of trehalose is induced during dehydration. This first observation of animal anhydrobiosis without trehalose challenges our current understanding of the phenomenon and calls for a re-evaluation of existing models.

**Grazing by *Brachionus plicatilis*:  
Prey dependent or ratio dependent?**

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The functional response of an organism describes food consumption and competitive ability, and determines the dynamic stability of predator-prey relationships. Traditionally, functional responses have been considered prey dependent, but recently it has been proposed that the ratio between prey and predator is a better predictor for the functional response. We conducted microcosm experiments with phosphorus (P) deficient growth medium, using the rotifer *Brachionus plicatilis* as predator and the algae *Tetraselmis* sp. as prey. To establish different carrying capacities, the total P concentration was varied. The cultures were run for 2-3 months with a dilution rate of 0.01 d<sup>-1</sup>.

After an initial algal bloom with a subsequent increase and collapse of the rotifer population, a quasi-steady state was established. During the algal bloom, the rotifers grew exponentially until the algal bloom was grazed down. However, the growth rates of the rotifers were significantly reduced with increasing total P concentration ( $-0.116 \pm 0.04 \text{ d}^{-1} [\mu\text{M P}]^{-1}$ ,  $p=0.024$ ). During the post-bloom period, rotifer and algal densities more or less established a steady state, and no predator-prey oscillations were detected. For both algae and rotifers, the average density during the post-bloom period increased linearly with total P concentration. Slopes were  $0.34 \pm 0.05$  and  $1.08 \pm 0.05$  ( $\pm$ SE, dimension mg C  $[\mu\text{mol P}]^{-1}$ ) for algae and rotifers, respectively. Egg-ratio data suggested that the physiology of the rotifers was comparable in all cultures during this period. The fact that both prey (algae) and predator (rotifer) densities increased with increasing total P concentration suggests that the functional response of *B. plicatilis* is ratio dependent. Ongoing work will try to verify this by inverse modeling, and possible mechanisms will be discussed.

## **Rotifers of the Poodří protected landscape area**

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Poodří protected landscape area was established in 1991, to preserve fair Odra river's floodplain with a lot of ponds and pools. Many biological monitorings have been performed here. Investigations still continue, including a monitoring of rotifer settlement. It was started in spring 2000, with a focus on planktonic and littoral rotifers. Fifty rotifer taxa were identified in 145 samples.

## ***Brachionus calyciflorus*: in the centre of chemically modified trophic interactions**

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The green alga *Scenedesmus obliquus* changes from single celled algae into multicellular colonies when exposed to grazing *Brachionus calyciflorus*, and changes back into unicells again in the absence of grazing. Moreover, this change can also be induced when exposing single-celled algae to filtrate of grazing *B. calyciflorus*. These colonies are less ingested by *B. calyciflorus*. Apart from benefits there are also costs of this defence for the algae, namely increased sedimentation rates from the epilimnion. However, reduced ingestion and assimilation rates of *S. obliquus* ultimately affect the fitness of *B. calyciflorus*. Furthermore, the grazing of *B. calyciflorus* is directly reduced when exposed to filtrate of conspecifics, which gives a second, direct, density-dependent feedback to zooplankton grazing.

Different *Scenedesmus* strains show similar defence responses when exposed to information chemicals of different herbivorous zooplankton species. Therefore we hypothesise that this induced defence is a general response against grazing, possibly induced by the same information chemical(s). One of the first and best studied examples of information-chemical induced defences in freshwater ecosystems is the defence of *Brachionus calyciflorus* against its predator *Asplanchna sieboldi*. Adding *Asplanchna* to the *Brachionus-Scenedesmus* system allows us to study inducible defences in a tritrophic system. Models have been made, parameterised for this system, with three different defence strategies (no defences, permanent defences or inducible defences). These models predict that only inducible defences (in contrast to no and permanent defences) promote system stability over a large range of algal carrying capacities, meaning that the 'paradox of enrichment' does not occur when there are inducible defences. Furthermore, only with inducible defences, the biomass of each of the trophic levels increase with enrichment, contrary to the hypothesis of exploitation ecosystems (EEH). The genera considered have several properties that makes them very suitable to test these model predictions with laboratory food chains and food webs.

## Resting eggs of rotifers in the sediment of a lowland river

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During extensive maintenance works on the River Meuse, Belgium, the water level was lowered significantly. Samples of wet sediment were obtained from the otherwise permanently flooded riverbed for a study on zooplankton 'seed banks'. Simple hatching experiments under controlled conditions resulted in the emergence of various rotifer species belonging to the genera *Brachionus*, *Euchlanis*, *Notholca*, *Polyarthra* and *Synchaeta*. *Brachionus quadridentatus* was the most commonly encountered species. Hatchlings were obtained from resting eggs isolated using density gradient centrifugation, even after 18 months preservation at 4°C under a wet atmosphere. Resting egg densities in the sediment were found to be low (ca. 2-3 g<sup>-1</sup>), with a slight increase in density downstream compared to upstream and the highest densities at the shallowest, most protected sampling sites. These eggs represent a potential for autochthonous recruitment to the system, but hatching in situ needs further investigation.

## Winter rotifer assemblages in two large Estonian lakes

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Most studies on zooplankton of the northern temperate lakes deal with the vegetation period and disregard the winter season. The aim of the present study is to analyse the qualitative and quantitative structure of winter rotifer assemblages in two large and shallow lakes, strongly eutrophic Lake Võrtsjärv (270 km<sup>2</sup>, mean depth 2.8 m) and moderately eutrophic Lake Peipsi (2,611 km<sup>2</sup>, mean depth 8.3 m). The lakes are covered with ice from December to April. A total of 33 rotifer taxa have been found in winter plankton (December – March) of these lakes. Taxonomic richness is quite invariable throughout winter. The differences in trophic states are clearly expressed in the assemblage structures. On the average, rotifers make 95% of total zooplankton abundance and 90% of biomass in L. Võrtsjärv, and 89% and 66% in L. Peipsi, respectively. Rotifer densities and biomass varied strongly between years. The values were higher in L. Võrtsjärv, densities ranging 26,368–621,599 ind.m<sup>-3</sup>, and biomass 0.018–0.374 gWW.m<sup>-3</sup>. In L. Võrtsjärv, winter maximum density and biomass occurred in December and March, respectively. In L. Peipsi, the maxima took place in February. *Polyarthra dolichoptera*, *Keratella quadrata*, *K. cochlearis* and *Synchaeta verrucosa* are dominating in the winter rotifer assemblage in L. Võrtsjärv. In addition to these, *Kellicottia longispina*, *Notholca cinetura* and *N. squamula* predominate in less eutrophic L. Peipsi.



## On the hollow curve distribution phenomenon in Phylum Rotifera

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Phylum Rotifera (*sensu stricto*) is a small phylum comprising about 1800 species distributed among two classes, five orders, 34 families, and 126 genera. At each of these levels it appears that one or a few very diverse taxa (species rich) dominate the others in the number of subtaxa they possess. Such distributions have been described in many different taxonomic groups including angiosperms, insects, birds, and fishes. Within each of these assemblages, dominance is exhibited across several taxonomic levels (*i.e.*, order, family, genus, and species). This phenomenon, called the hollow curve distribution (HCD), is clearly observed when a histogram of the frequency distribution of taxonomic subunits per unit, ordered from most to fewest units, is made. Here we document the HCD in rotifers at three taxonomic levels (*i.e.*, families per order, genera per family, and species per genus) and test these distributions against several null models (*e.g.*, even distribution, truncated Poisson distribution and an augmented version of that distribution, broken stick, and sequential resource subdivision). Our results show that rotifer taxa are dominated by one or a few very diverse taxa beyond what would be expected by these null models. Such overdominance in other taxa has been inferred to reflect real differences in evolutionary success (*i.e.*, to have undergone more speciation and/or to have had fewer extinctions than other taxa at the same taxonomical level). We explore this non-random feature of rotiferan diversity by examining a variety of life history traits in an attempt to correlate them to the overdominant taxa.

**Life on the edge: rotifers from springs and ephemeral waters in the Chihuahuan Desert, Big Bend National Park (Texas, U.S.A.)**

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Desert waters and their associated flora and fauna throughout the world are at significant risk from exploitation by development for use as agricultural and dairy lands and through increased urban sprawl, each of which exacerbates the serious problem of aquifer depletion. Here we describe an ongoing study of the Rotifera inhabiting the rare and beautiful springs, associated seeps and wetlands (cienégas), and temporary waters (huecos and tinajas) of Big Bend National Park (BBNP), a  $3.23 \times 10^5$  ha region of the northern Chihuahuan Desert. On four extensive collecting trips, we collected samples from planktonic, littoral, and benthic habitats comprising nearly 101 sites representing some 16 aquatic complexes. Seventeen families of monogonont rotifers comprising 30 genera and 69 species were identified. Of these taxa, 4, 10, and 52% of families, genera, and species, respectively, were found only in springs. In non-spring water sources an additional 6 families, 40 genera, and 32 species were identified. Although our study sites are relatively depauperate in species, they yielded a rotifer fauna that is comparable to that reported from other extreme habitats (e.g., Arctic and Antarctic systems). So far we have been unable to identify 15 species of monogononts and several bdelloids; these need further taxonomic study and may be new to science. Our efforts represent the first survey of rotifers from a diverse assortment of BBNP waters.

## No Exit—Phylogeny of the Asplanchnidae (Rotifera)

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Over the years, many rotiferologists have made significant contributions concerning the biology of Family Asplanchnidae. This knowledge includes biogeographic data, information on their predatory behavior, ability to exhibit morphological plasticity, and tendency to release kairomones that induce spine production in other rotifers. Notwithstanding this extensive knowledge, only two hypotheses regarding the phylogeny of Asplanchnidae have been proposed and neither has been adequately explored: 1) Sudzuki (1964) separated *Asplanchna* into *Asplanchna* and *Asplanchnella* based on vitellarium morphology; 2) Kutikova (1983) suggested a sister relationship with Synchaetidae based on coronal morphology. Here we report cladistical studies that employed both morphological and molecular characters to test these hypotheses. Morphological (maximum parsimony) data were taken from original descriptions as well as from our own observations and secondary sources. Molecular reconstructions (neighbor joining; maximum likelihood) were based on characters from the ITS and V4 regions. So far our analysis offers some support for Kutikova's hypothesis, but none for Sudzuki's. Additional informative characters are needed to improve the morphologically study, while our molecularly based trees currently suffer from a lack of critical taxa. (Our apologies to Jean-Paul Sartre for our title.)

**The effects of two antibiotics on the rotifer *Brachionus plicatilis* through acute toxicity, birth rate of females and males and the production of diversiform eggs.**

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The experiment studied the effects of two antibiotics, chloromycetin and nicotinic norfloxacinum, on the rotifer *Brachionus plicatilis*. The results showed that the rotifer has good tolerance to these two drugs, the 24-hr LC50 being 0.873 mg.ml<sup>-1</sup> and 0.970 mg.ml<sup>-1</sup>, respectively. The two antibiotics were found to influence, to varying degrees, the birth rate of females and males and also the production of diversiform eggs within the population. When the concentration of chloromycetin was 0.025 mg.ml<sup>-1</sup> the production of parthenogenetic eggs was greatly reduced, compared with the control population, whilst the production of male eggs was increased (P<0.01). When the concentration of nicotinic norfloxacinum was 0.50-0.75 mg.ml<sup>-1</sup> the production of male eggs increased (P<0.01), while a concentration of 1.00 mg.ml<sup>-1</sup> brought about a dramatic increase in the production of parthenogenetic eggs (P<0.01). These two antibiotics can therefore be regarded as having a direct influence in the culturing of rotifers.

## The ecology of *Cephalodella* and *Elosa* in an extremely acidic (pH 2.7) lake.

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Due to ceased lignite mining activities, abandoned mining pits filled with water and subsequent weathering of pyrite and markasite led to strong acidification of such new water bodies with pH ranging from 2.3 to 3.4. Planktonic species richness is low and declines with decreasing pH. In Mining Lake 111 (ML111, pH 2.7) two rotifer species are the only planktonic metazoa (*Cephalodella hoodi* and *Elosa worallii*). The lake is thermally stratified in summer and two strongly differing subhabitats develop: The epilimnion is warm and as potential food source the bacterivorous flagellate *Ochromonas* dominates, in the hypolimnion temperatures are lower and the osmotrophic flagellate *Chlamydomonas* dominates. A similar vertical separation was found for the rotifers, *Elosa* has its abundance maximum in the upper water layers, whereas *Cephalodella* dominates the lower ones. Growth and grazing experiments revealed that *Chlamydomonas* is a suitable food source for both rotifers whereas *Ochromonas* is not and might even reduce growth of rotifers in the presence of high *Chlamydomonas* densities. These results suggest strong food limitation of rotifers in the lake. This view is supported by a series of food supplement experiments showing a positive growth response of both rotifers to a supplement of *Chlamydomonas* to natural lake water compared to untreated lake water. To understand how the rotifers sustain a population in the pelagic, the hypothesis was tested that a considerable portion of the pelagic population was recruited or subsidized from the sediment. We found a seasonal pattern of recruitment for *Cephalodella* with maxima in spring and November, but almost no recruitment for *Elosa*.

## Reproductive strategy of *Keratella cochlearis* (Gosse) in a shallow, eutrophic subtropic Chinese lake

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Lake Donghu is a shallow, eutrophic subtropic Chinese lake that is famous in China for its long-term hydrobiological and physicochemical research. In terms of chlorophyll-a, the lake can be divided into several areas of different trophic levels. Among the three fixed sampling stations, the trophic level at Station I is the highest, and that at Station III the lowest. The volume of a single amictic egg of *Keratella cochlearis* in Lake Donghu varied between  $7.5 \times 10^3$  and  $24 \times 10^3 \mu\text{m}^3$ . The egg volume increased with increasing lorica length, but the rate of increase decreased significantly with the advance of eutrophication of the water body in which the rotifer lived. Both lorica length and egg volume showed a strong negative correlation with temperature. The degree of eutrophication of the water body had a significant effect on the relationship between lorica length and temperature, but not on the relationship between egg volume and temperature.

## Seasonal changes in the litter-dwelling rotifer population of two pine-oak forest habitats near Kyiv, Ukraine

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Investigations of litter-dwelling rotifers were carried out in two plots in the pine-oak forest near Kiev, Ukraine. The first plot (4 m<sup>2</sup>) contained pine needles, oak leaves and moss litter. Samples of the soil column, 7 cm<sup>2</sup> in area and 7 cm in depth, were collected over a period of 1.5 years (1999-2000). Each sample was divided into four layers corresponding to the degree of organic decomposition of the soil, and these layers were investigated separately. Rotifers were found concentrated in the upper 1-2 cm of the soil throughout the year, with only a few individuals penetrating into the deeper layers. Total rotifer abundance showed several maxima during the course of the year, in August/September, at the end of November (after the first snow-fall) and in the second half of spring. In contrast, abundance decreased during autumn and winter frosts, and during dry periods in summer. A seasonal succession of species, and changes in the dominant species, were recorded over the period of observation. *Rotaria sordida* was almost always dominant over all seasons of the year. *Habrotrocha flaviformis*, *Macrotrachea multispinosa* dominated during warm periods, while *Adineta steineri* had its maximum abundance in winter and early spring. A short-term increase in *Otostephanos auriculatus* abundance was observed in September; later, this species disappeared altogether. In the second plot within the same forest (10 m<sup>2</sup>), samples were collected at weekly intervals during January and February 2003. The upper 1-2 cm of litter containing pine needles and oak leaves without moss were investigated. A gradual decrease in rotifer abundance was observed, here as in the first plot, through January and February. Mean abundance during the investigating period ranged from  $154.33 \pm 43.05$  to  $65.0 \pm 13.79$  specimens per gramme of dried litter.

## The insulin-like growth factor signaling pathway involved in regulating longevity of rotifers

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Food abundance plays a critical role in regulating the population dynamics of predators. Natural environments, however, rarely allow sufficient levels of resources for organisms. Therefore, the predators have evolved to adapt to various resource levels to persist in fluctuating environments. Rotifera have an ability to adapt to various nutritional levels by changing life history parameters (e.g. reproduction and lifespan). In environments rich in food, rotifers exhibit the maximum population growth rate with active reproduction, and rapidly occupy a niche gap. In contrast, under severely limited food supply, rotifers suppress reproduction and extend reproductive period and lifespan to stably maintain the population. Reproduction and lifespan are integrated via the insulin-like growth factor (IGF) signal transduction in the nematod *Caenorhabditis elegans*. The IGF pathway involved is composed of various components well conserved among eukaryotes and has been suggested to mediate the longevity by caloric restriction. We therefore assume that the IGF signaling pathway is a candidate for regulating rotifer population dynamics by controlling reproductive activity and lifespan. We first examined the action of an inhibitor specific to PI3-kinase involved in the pathway for the rotifer *Brachionus plicatilis*. This kinase was first discovered as *age-1* to regulate the longevity of *C. elegans*. As expected, the inhibitor treatment resulted in the extension of lifespan by 30% compared to the reference group without the treatment, whereas reproductive characters were not modified. These results were consistent with those observed in *C. elegans*. Subsequently, we established a highly sensitive quantifying method to examine the expression level of responsible genes in a single rotifer using a real-time RT-PCR. This method will allow us to unravel the molecular basis for life history alteration, and to further understand the mechanisms underlying the population dynamics of rotifers.



## Factors affecting the swimming speed in the rotifer *Brachionus plicatilis*

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Swimming in rotifers is an activity demanding a high energy. Therefore it can be affected by those intrinsic and extrinsic factors having a relevant influence on the body metabolic activity. This study examines the swimming speed in amictic females of *Brachionus plicatilis* in laboratory cultures. Five different stages have been examined: recent hatched females, juveniles, adult non-ovigerous females, ovigerous females with 1 attached egg and ovigerous females with 2 attached eggs. Each animal was included in one of these stages by examining the lorica size. Two temperatures, 15 and 25 °C, and two feeding conditions, presence and absence of microalgal cells, have been tested. The swimming rates were measured by using an automated motion analysis system over video recording. Swimming speed ( $\mu\text{m s}^{-1}$ ) increased with increasing body size while in the adult females it showed a decreasing trend with the number of attached eggs. Regarding the culture medium conditions, the swimming activity was higher at 25 than at 15°C and in absence than in presence of microalgal cells. The average values under the different experimental conditions ranged between about 500 for the recent hatched females and 1300  $\mu\text{m s}^{-1}$  for the adult non-ovigerous females. The present results are discussed in relation to the metabolic rates described under similar conditions.

## Rotifer abundance and vertical distribution in a tropical monomictic lake

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Given the scarce information available on vertical distribution and abundance of rotifer communities in Mesoamerican lentic tropical systems, this work aims at describing diurnal variation patterns of rotifer density and species composition along the vertical axis of the water column in a tropical monomictic lake. Samples were taken in 3 h intervals during two diurnal cycles, over a single sampling site, and at 7 different depth levels in Laguna Escondida (State of Veracruz, Mexico). Samples were collected in 1996 and 2003 during the mixing period (winter). Using the Olmstead -Tukey diagram to analyze frequency and density data. The dominant species were *Keratella tropica* and *Trichocerca pusilla* in 1996, and *Brachionus calyciflorus* and *Ascomorpha ecaudis* in 2003. Density variations in both cycles were analysed using kernel circular density estimators (EDK). The first cycle had 13 species with mean densities over 1 ind.l<sup>-1</sup> whereas the second one yielded 6. A Raleigh test was used in quantifying those variations in density at the various depths through a diurnal period. The community was formed by species whose members did not show significant differences in their vertical distribution. *Brachionus calyciflorus* was always more abundant in depths above 2 m. Other species, like *Ascomorpha saltans*, were motile throughout the diurnal cycle. Vertical profiles of temperature, pH, and dissolved oxygen were obtained; in both occasions, these profiles revealed minor variations. Main circular descriptive statistics were obtained from density data, as well as from environmental parameters and a circular variance analysis (ANDEVA) was performed which allowed the determination of significant density differences between both sampling cycles. Finally, the use of circular statistics as a descriptive tool in this field is discussed.

## Sunken sulphur open cast and its rotifers

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The zooplankton community of sunken sulphur open cast was investigated during two years. Complex physical and chemical conditions in this water body caused the formation of untypical planktonic assemblages. Rotifer populations change in time and space. In winter *P. dolichoptera* coexists with *P. bicerca*, whereas in spring *Keratella quadrata*, and in summer *Hexarthra fennica*, *K. cochlearis* and *Synchaeta tremula* predominate. In autumn *Filinia longiseta* is the dominant species. *Keratella testudo* thrived at the border of the oxycline and below the thermocline. Thermal and chemical stratification generated five types of diurnal vertical migrations of planktonic animals, including two typical and well known types. Negative environmental conditions, especially sulfides, induced spine deformations in 0.1 % of *Keratella* populations. Only 20 rotifer species were found in this impoundment.

## Behavioural reproductive isolation in a rotifer hybrid zone

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A hybrid zone between two *Brachionus plicatilis* rotifer mitochondrial DNA (mtDNA) lineages was recently described in the Iberian Peninsula between a pond (Santed2) and a lake (Gallocanta) (Gómez et al., 2002a). The patterns of mitochondrial and nuclear genetic variation observed suggested that gene flow is mainly male-mediated from the lake to the pond. Here we test two hypotheses: a) that male-mediated gene flow occurs through assortative mating between individuals from these ponds, b) the occurrence of behavioural isolation between the two mtDNA lineages. We isolated, reared and genotyped rotifer clones from resting eggs collected in the sediments of these and two other distant ponds. We devised a quick, inexpensive RFLP method to discriminate between *B. plicatilis* and its sibling species *B. 'Manjavacas'* and between both mtDNA *B. plicatilis* lineages. Behavioural no-choice tests using new-born, virgin males and females were performed between five clones. *B. 'Manjavacas'* and *B. plicatilis* were reproductively isolated. *B. plicatilis* clones did not show evidence of reproductive isolation, regardless their mtDNA lineage, except Santed2 males, which discriminated strongly against Gallocanta females. These results could help to explain the discrepancies between