

## Underestimation of rotifer abundance a much greater problem than previously appreciated

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### Abstract

Although rotifers are important components of aquatic food webs and suitable sampling methods have been described and tested in the peer-reviewed literature, they are frequently overlooked or quantified with improper methods (e.g., mesh sizes  $\geq 63 \mu\text{m}$ ) in freshwater ecology studies. As a result, we believe that the role of rotifers in aquatic food webs and ecosystem processes remains underappreciated, and this conceptual shortfall is exacerbated by the continued use of improper sampling methodology. We examined density and biomass estimates of metazoan zooplankton in the Upper Mississippi and Missouri rivers from two sampling methods. The macrozooplankton method was designed to target cladocerans, adult and juvenile copepods, by filtering 180 L water through a 63- $\mu\text{m}$  mesh. The microzooplankton method was designed to target rotifers and copepod nauplii by filtering 18 L water through a 20- $\mu\text{m}$  mesh. The macrozooplankton method underestimated the density and biomass of common rotifers by two to three orders of magnitude, a far greater amount of error than reported in previous studies. This bias in density estimates for rotifers decreased with increasing mean length of rotifers. The microzooplankton method proved to be ineffective for quantifying cladoceran species richness or cladoceran density at the species level. We urge aquatic ecologists to match their sampling methodology with the specific goals of their study. An accurate understanding of the role of rotifers in freshwater ecosystems will only be possible when the use of appropriate methodology becomes the rule rather than the exception.

Although rotifers can be the dominant metazoan zooplankton in freshwater ecosystems (Orcutt and Pace 1984; Pace et al. 1992; Pillard and Anderson 1993; Thorp et al. 1994) and are important components of aquatic food webs (Porter 1995; Jurgens et al. 1999; Miracle et al. 2007), they have been far less studied relative to crustacean zooplankton. For example, major theoretical areas of aquatic research over the last 40 years, including the size-efficiency hypothesis *sensu* (Brooks and Dodson 1965) and the trophic-cascade hypothesis *sensu* (Carpenter et al. 1985), have focused on crustacean zooplankton far more than on rotifers (Pace et al. 1998; Jurgens et al. 1999; Miracle et al. 2007).

We believe this tendency to overlook rotifers has methodological roots. Underestimation of rotifer abundance dates back to Juday (1916), who stated that the mesh of the number 20 silk bolting cloth (75  $\mu\text{m}$  mesh) was adequate for sampling the “vast majority of rotifers.” In the 1970s, several studies demonstrated that mesh sizes commonly used to sample crustacean zooplankton (e.g.,  $\geq 63 \mu\text{m}$ ) underestimate the abundance of rotifers, and recommended mesh sizes  $\leq 35 \mu\text{m}$  for quantitative sampling of rotifers (Likens and Gilbert 1970; Bottrell et al. 1976; Ejsmont-Karabin 1978). Despite these recommendations, many studies still report quantitative estimates of rotifer density or biomass based on samples filtered through mesh sizes  $> 35 \mu\text{m}$ , including mesh as large as 63 to 80  $\mu\text{m}$  (Bonecker and Lansac-Toha 1996; Ghadouani et al. 1998; Shurin 2000; Strecker et al. 2004; Geraldés and Boavida 2007). To illustrate the extent of this problem, we used Biological Abstracts BIOSIS to search for articles published from 1999 through 2007 in peer-reviewed journals with the phrase “zooplankton density” in the topic search field. We were able to access 41 articles from this search that provided quantitative estimates of zooplankton density or biomass from field surveys or experimental enclosures. Of these studies, only 23 reported quantitative estimates for rotifers, of which only 5 studies (22%) used mesh sizes  $\leq 35 \mu\text{m}$  or whole-

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water settling techniques (Table 1). Eight of the 23 studies (35%) used mesh sizes  $\geq 63 \mu\text{m}$  to report estimates of rotifer density or biomass (Table 1). We also reviewed articles from the last three International Rotifer Symposia (published in *Hydrobiologia* vols. 593, 546, and 446/447) presenting quantitative estimates of zooplankton abundance. We found 18 papers presenting quantitative estimates, of which only 50% used mesh sizes  $\leq 35 \mu\text{m}$  or whole-water settling techniques. Though not exhaustive searches, these samples of publications suggest that many studies of zooplankton in freshwater ecosystems either overlook rotifers or underestimate rotifer density and/or biomass and their contribution to the zooplankton community.

There are several reasons inappropriate sampling methods continue to be used to quantify rotifers. Studies noting underestimation of zooplankton from mesh sizes  $> 35 \mu\text{m}$  often found relatively small error rates (i.e., estimates within the same order of magnitude) or did not use sample sizes adequate to provide a reasonable estimate of effect size (Likens and Gilbert 1970; Bottrell et al. 1976; Ejsmont-Karabin 1978). Furthermore, reductions in mesh size have a negative effect on filtration rate (Tranter and Heron 1967; Likens and Gilbert 1970; Orcutt and Pace 1984). A researcher wishing to sample crustacean zooplankton and rotifers would likely have to use different methods for each group, which doubles both field and laboratory effort. Finally, the fact that rotifers are commonly ignored or have underestimated abundances reported in the literature likely has led to a perception among scientists that rotifers simply are not an important group of organisms in ecological processes for many systems.

We provide a comparison between two methods used to sample zooplankton. The first method, hereafter referred to as the microzooplankton method, used a  $20\text{-}\mu\text{m}$  mesh to sample rotifers and copepod nauplii. The second method, hereafter referred to as the macrozooplankton method, used a  $63\text{-}\mu\text{m}$  mesh to sample crustacean zooplankton (cladocerans, adult and juvenile copepods). To allow a comparison of the effec-

tiveness of each method, all zooplankton (i.e., target and non-target groups) were enumerated for both methods during this study. We report differences in density estimates for rotifers related to mesh size that are far greater than those reported in previously published studies.

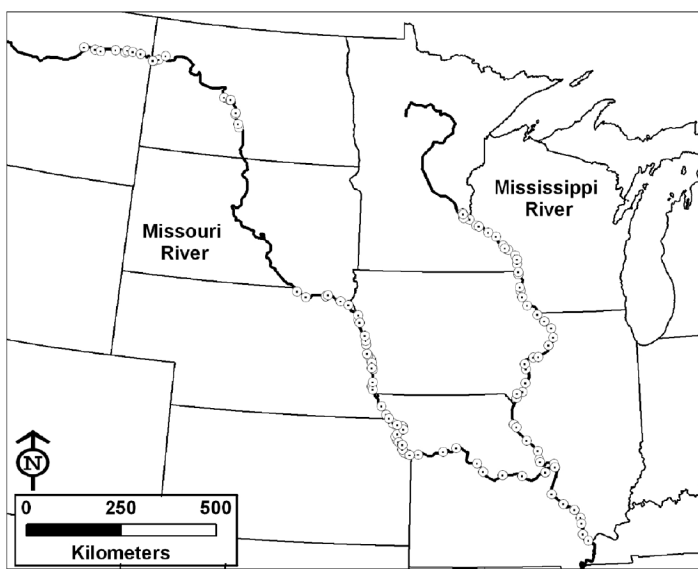
### Materials and methods

**Sampling and enumeration**—Zooplankton were collected from multiple sites in the Upper Mississippi (56 sites) and Missouri (76 sites) rivers from 15 June 2004 to 15 October 2004 (Fig. 1) for the United States Environmental Protection Agency's Great Rivers Ecosystem Environmental Monitoring and Assessment Program (Angradi 2006). Sample sites for field collections covered most of the length of each river and were determined using a stratified random design (Angradi 2006). At each site, water was obtained with a diaphragmatic pump at three locations across the river channel (one in the thalweg, and one on each side of the thalweg) and three depths (0.5 m from the surface, mid-depth, and 0.5 m from the bottom), producing depth- and spatially integrated samples at each site (Angradi 2006). For the macrozooplankton method, 180 L (20 L from each location and depth) of water was filtered through a  $63\text{-}\mu\text{m}$  mesh, whereas 18 L (2 L from each location and depth) of water was filtered through a  $20\text{-}\mu\text{m}$  mesh for the microzooplankton method. Samples were treated with  $\text{CO}_2$  from Alka Seltzer tablets as a narcotic and then preserved in 4% sucrose-buffered formalin (Haney and Hall 1973).

We counted rotifers and nauplii with a compound microscope using Sedgewick-Rafter cells and used a dissecting scope with a Ward-Whipple counting wheel or Bogorov tray to enumerate crustacean zooplankton. Sample enumeration was also attempted with settling techniques and inverted microscopes but was found to be impractical due to high concentrations of suspended solids. Rotifers were identified to genus, copepods to order, and cladocerans to species, using widely available keys (Brooks 1959; Pennak 1991; Thorp and Covich 1991). Samples were enumerated in their entirety or subsampled

**Table 1.** Mesh sizes used to provide quantitative estimates of rotifer density or biomass from published studies. Studies were selected from a Biological Abstract search of articles published from 1999 through 2007 in peer-reviewed journals with the phrase "zooplankton density" in the topic search field.

Mesh size	Number of studies	Citations
$\leq 35 \mu\text{m}$ or whole-water settling techniques	5	Chunying 1999; de Cardosa and da Motta Marques 2004; Gagneten and Ceresoli 2004; Yang et al. 2005; Trevison and Forsberg 2007
$> 35 \mu\text{m}$ to $< 63 \mu\text{m}$	7	Baranyi et al. 2002; Comerma et al. 2003; Halvorsen et al. 2004; Chen and Folt 2005; Pocięcha and Wilk-Wozniak 2006; Frutos et al. 2006; Illyova 2006
$\geq 63 \mu\text{m}$	8	Michaletz and Gale 1999; Speas 2000; Tugend and Allen 2000; Franks et al. 2001; Gonzalez et al. 2002; Ferrara et al. 2002; Bonecker and Aoyagui 2005; Geraldés and Boavida 2007
Not reported	3	Ha et al. 2003; Gophen 2003; Sharma and Bhattarai 2005
Total	23	



**Fig. 1.** Map of the North Central portion of the continental United States showing the Mississippi and Missouri rivers. Circles indicate sampling locations for the Great Rivers Environmental Monitoring and Assessment Program in 2004.

until at least 500 individuals were counted. Biomass estimates (dry weight) were obtained using published length-mass estimates (Dumont et al. 1975, Downing and Rigler 1984). We obtained a mean total length of common zooplankton from measurements of 30 individuals for each taxon under 100 $\times$  magnification using a compound microscope equipped with a digital camera and imaging software.

### Assessment

**Experimental design and analysis**—We used regression to examine the relationship between density estimates derived from the two methods. If the two methods are equally effective in sampling a particular taxon, then there should be a strong linear relationship with a slope close to one (Orcutt and Pace 1994). Differences in density estimates will result in slopes substantially different from 1 (i.e., bias) and/or weak relationships (i.e., poor precision). As a standard for comparisons, we expected accurate estimates of rotifer and nauplii density from the microzooplankton method (20- $\mu$ m mesh, 18 L water) (Likens and Gilbert 1970; Ejsmont-Karabin 1978; Orcutt and Pace 1984). Similarly, we expected that the macrozooplankton method (63- $\mu$ m mesh, 180 L water) provided accurate density estimates for crustacean zooplankton. Therefore, we used density estimates from the microzooplankton data as the independent variable in regressions for rotifers and copepod nauplii, and density estimates from the macrozooplankton data as the independent variable in regressions for crustacean zooplankton.

For rotifers, we restricted these analyses to taxa comprising at least 5% of the total catch for the microzooplankton method, which was a natural break in our data and ensured

that only adequately sampled taxa were used in analyses. All data were logarithmically transformed [ $\log_{10}(x + 1)$ ] to normalize data and improve model fit. Because the microzooplankton method sampled a small volume of water, too few cladocerans were collected to reliably characterize the species composition of this group. Hence we only report regression analyses for all cladoceran species combined and all adult copepods combined. We analyzed data from the two rivers together for these regression models after initial analysis of covariance yielded no significant differences in slope between the two rivers for any taxon tested. We also calculated mean density (# L<sup>-1</sup>) and biomass (dry weight  $\mu$ g L<sup>-1</sup>) for rotifers (across all genera), cladocerans (across all species), copepod nauplii, and adult copepods for each river to compare overall community composition from the two methods.

**Rotifer and nauplii results**—The macrozooplankton method proved unsuitable for providing accurate density or biomass estimates of rotifer genera and copepod nauplii. Five rotifer genera, *Keratella* spp., *Trichocerca* spp., *Brachionus* spp., *Synchaeta* spp., *Polyarthra* spp., and copepod nauplii, were the most abundant taxa captured (Table 2), and each accounted for greater than 5% of the total catch from the microzooplankton method. Mean density and biomass for all these taxa were greater in the microzooplankton method relative to the macrozooplankton method (Table 2). These differences were over three orders of magnitude for *Trichocerca* spp., and over two orders of magnitude for *Keratella* spp., *Synchaeta* spp., and *Polyarthra* spp. (Fig. 2). Differences in density and biomass for *Brachionus* spp. and copepod nauplii were around a factor of two or greater (Fig. 2), and the overall difference in rotifer density across all taxa was over an order of magnitude (Table 2). We found little difference (e.g., 95% confidence intervals overlap) in the estimates of rotifer generic richness between the two methods (Table 2).

Both the strength of the regressions and the slope of the relationship between the macrozooplankton and microzooplankton density estimates increased with size for rotifers and copepod nauplii, suggesting that smaller taxa readily passed through the 63  $\mu$ m mesh used in the macrozooplankton method (Fig. 3). For the smallest taxon, *Trichocerca* spp., the regression was not significant ( $R^2 = 0.000$ ;  $P \geq 0.946$ ). With increasing body size from *Keratella* spp., to *Synchaeta* spp., and *Polyarthra* spp., both the strength of the regression (as measured by  $R^2$ ) and the slope increased (Fig. 2, 3). The large rotifer *Brachionus* spp. and copepod nauplii had the greatest  $R^2$  and slope.

**Crustacean zooplankton results**—With a few exceptions, the microzooplankton method proved to be ineffective for sampling crustacean zooplankton (excluding nauplii). Cyclopoid copepods were the dominant crustacean zooplankton, with *Bosmina* cf. *longirostris* Muller, *Daphnia retrocurva* Forbes, and *Ceriodaphnia lacustris* Birge the most abundant cladoceran species (Table 2). The smaller volume

**Table 2.** Mean density and biomass for rotifers, copepod nauplii, adult copepods, and cladocerans; rotifer generic richness (number of genera L<sup>-1</sup>), and cladoceran species richness, estimated from the macrozooplankton (63 µm) and microzooplankton (20 µm) methods used to sample zooplankton from the Upper Mississippi and Missouri rivers. Also shown are mean body length estimates and the slope of the log-log regression of density between sampling methods. Numbers in parentheses show standard error of the mean.

Taxa	Mean length (µm)	Density (# L <sup>-1</sup> )		Biomass (µg L <sup>-1</sup> )		Slope log-log
		63 µm	20 µm	63 µm	20 µm	
Rotifers and nauplii:						
<i>Trichocerca</i> spp.	79.07 (3.40)	0.09 (0.03)	280.01 (33.95)	< 0.01	11.46 (1.44)	Not Significant
<i>Keratella</i> spp.	91.22 (1.06)	0.66 (0.01)	232.28 (28.76)	0.04 (0.01)	13.36 (1.71)	0.123 (0.026)
<i>Synchaeta</i> spp.	99.39 (4.87)	0.99 (0.17)	124.52 (17.35)	0.06 (0.01)	7.06 (1.02)	0.178 (0.034)
<i>Polyarthra</i> spp.	100.00 (2.77)	0.73 (0.13)	94.99 (14.60)	0.04 (0.01)	5.46 (0.85)	0.174 (0.024)
<i>Brachionus</i> spp.	179.35 (11.97)	33.46 (5.27)	88.22 (10.62)	4.10 (0.66)	11.06 (1.37)	0.777 (0.055)
All Rotifers		35.93 (5.34)	820.02 (74.92)	4.88 (0.76)	55.66 (5.38)	0.714 (0.071)
Rotifer generic richness		8.11 (0.24)	8.92 (0.21)			
Copepod nauplii	141.20 (7.97)	19.54 (3.48)	34.26 (6.52)	2.17 (0.37)	3.81 (4.29)	0.547 (0.032)
Cladocerans and Copepods:						
<i>Bosmina</i> cf. <i>longirostris</i>	386.10 (16.47)	0.76 (0.17)	0.62 (0.17)	1.04 (0.23)	0.84 (0.23)	
<i>Daphnia retrocurva</i>	1027.83 (64.61)	0.55 (0.12)	0.46 (0.15)	2.96 (0.62)	2.49 (0.80)	
<i>Ceriodaphnia lacustris</i>	527.77 (19.76)	0.51 (0.14)	0.19 (0.06)	0.96 (0.26)	0.36 (0.12)	
All Cladocerans		2.45 (0.38)	1.79 (0.37)	5.24 (0.91)	3.90 (1.05)	0.747 (0.056)
Cladoceran richness		4.17 (0.22)	0.87 (0.09)			
Cyclopoids	535.12 (28.46)	6.87 (1.15)	6.26 (1.09)	8.80 (1.47)	8.01 (1.40)	
All adult copepods		7.42 (1.18)	4.24 (0.81)	9.47 (1.59)	8.63 (1.51)	0.756 (0.041)

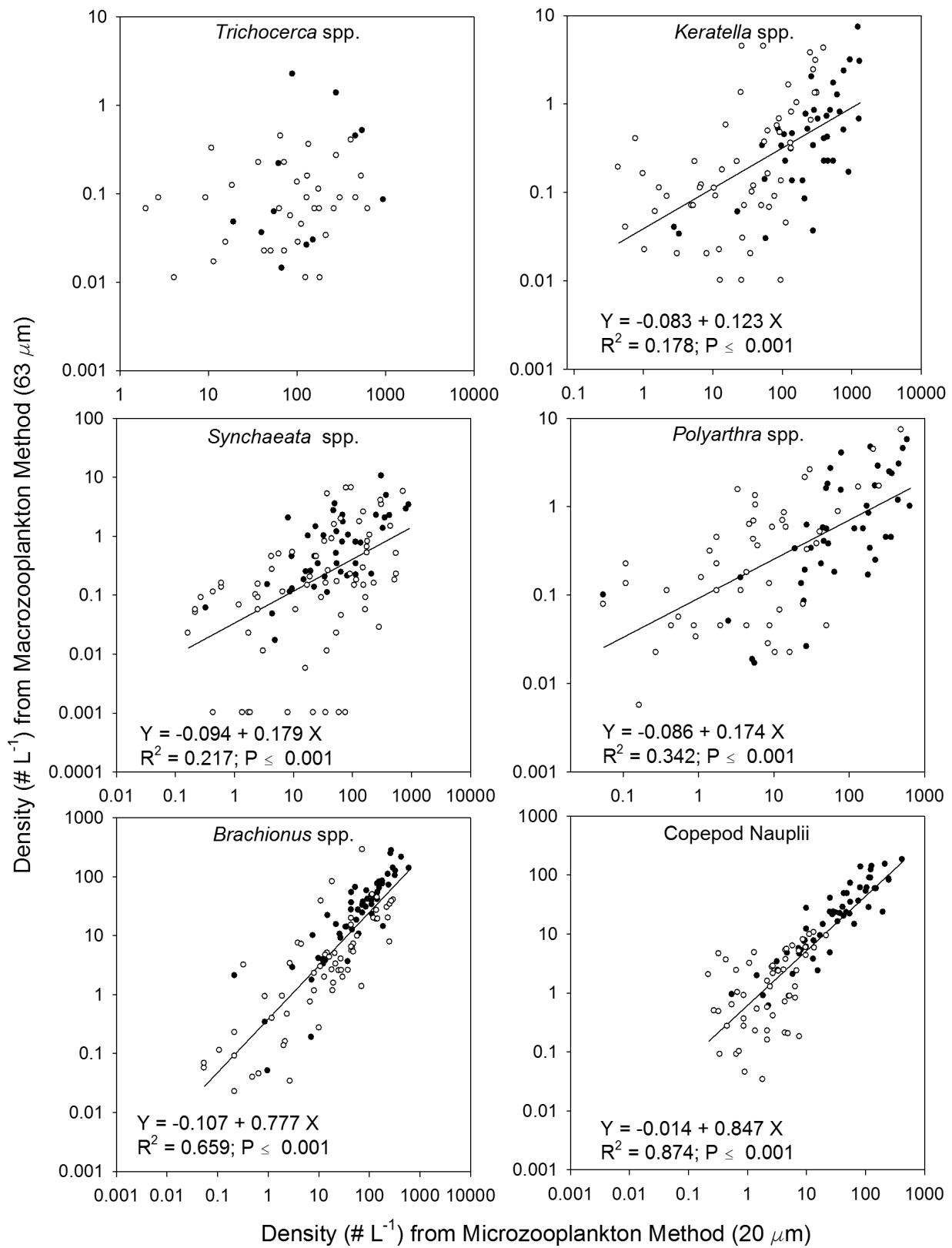
of water filtered in the microzooplankton method provided an inadequate sample of less abundant crustacean zooplankton. There were large differences in mean species richness of cladocerans between the two methods averaging over four cladoceran species per site for the macrozooplankton method and less than one species per site for the microzooplankton method (Table 2). Estimates of mean density and biomass for all adult copepods combined and all cladocerans combined were fairly similar between methods (95% confidence intervals overlapped), so the microzooplankton method appears adequate for estimating density and biomass at higher taxonomic levels (i.e., cladocerans, copepods, and rotifers; Fig. 4).

**Community structure results**—Use of the macrozooplankton method alone greatly underestimates the importance of rotifers to the zooplankton communities in both the Upper Mississippi and Missouri rivers. Data from the macrozooplankton method suggests rotifers make up less than 50% of the biomass of all zooplankton in both rivers, and less than 50% of the density of all zooplankton in the Upper Mississippi River (Fig. 5). When either the microzooplankton data are used alone, or the data from both methods are combined (microzooplankton method for rotifers and nauplii, macrozooplankton data for crustacean zooplankton), it is clear that rotifers dominate (i.e., > 65%) the zooplankton communities in terms of both density and biomass for both rivers (Fig. 5). The microzooplankton method slightly underestimated the density and biomass of adult copepods and cladocerans.

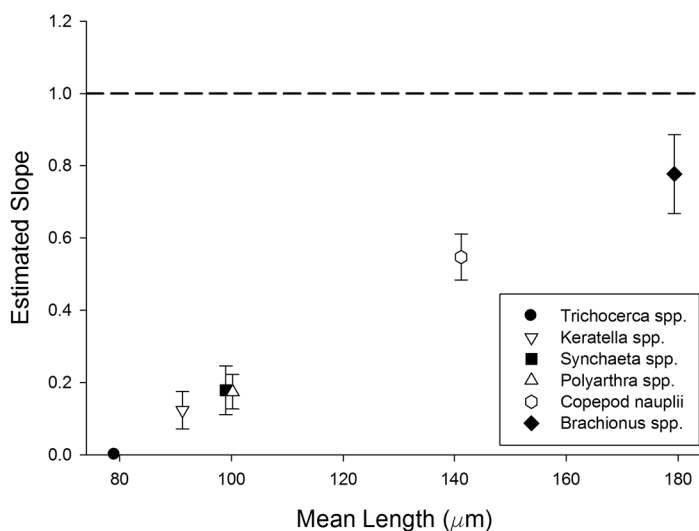
## Discussion

The still common practice of using inappropriate sampling methods to estimate rotifer abundance likely has contributed to rotifers being marginalized in aquatic ecology studies. In this study, relying on the macrozooplankton method alone would have resulted in an inaccurate description of zooplankton community structure and composition. When estimates from the macrozooplankton method were analyzed, rotifers comprised < 50% of the total density or biomass of zooplankton in the Upper Mississippi River, and less than 50% of the biomass in the Missouri River. For both rivers, the more reliable estimates obtained from the microzooplankton method demonstrate that rotifers actually dominated (i.e., comprised > 65%) the metazoan zooplankton community in terms of density and biomass. The large difference in the proportion of biomass comprised by rotifers between the two methods is understandable when the changes in density estimates for rotifers are considered. Mean density of rotifers in the Mississippi River was 52 L<sup>-1</sup> when calculated for the macrozooplankton method, but was 1134 L<sup>-1</sup> when calculated for the microzooplankton method. Similarly, density of rotifers in the Missouri River was 17 L<sup>-1</sup> when calculated for the macrozooplankton method, but was 419 L<sup>-1</sup> when calculated for the microzooplankton method.

Whereas the importance of mesh size in quantitative zooplankton sampling has been documented previously (Likens and Gilbert 1970; Bottrell et al. 1976; Ejsmont-Karabin 1978), the differences in density and biomass estimates we found were



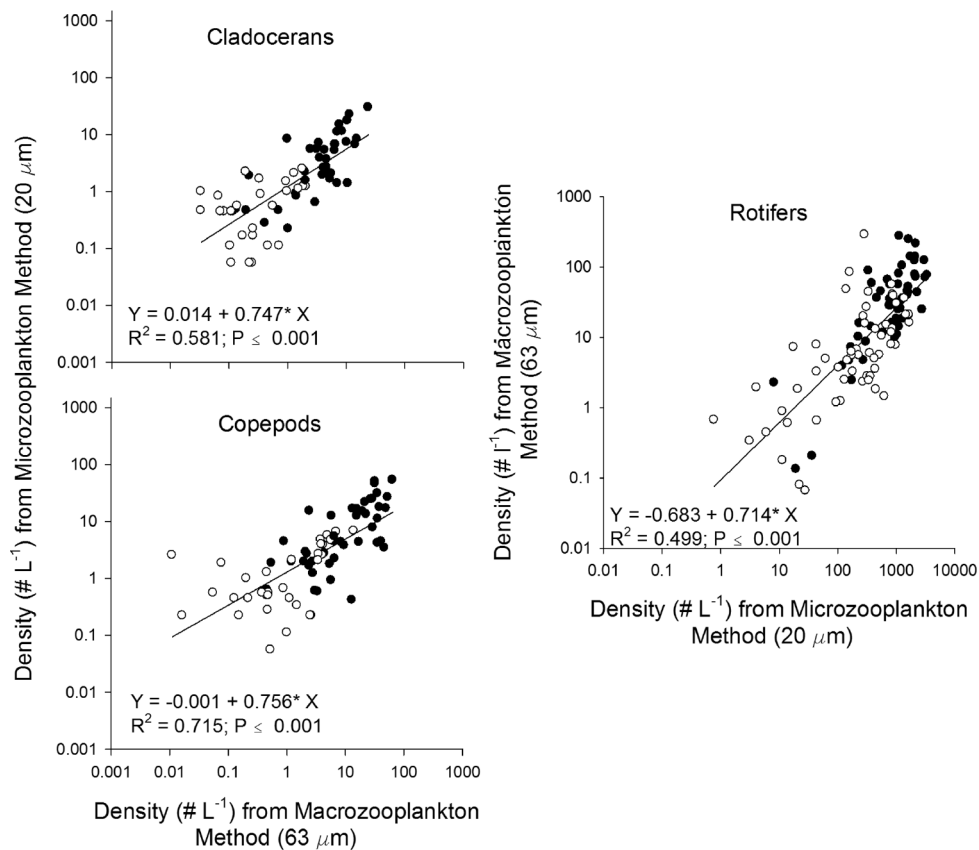
**Fig. 2.** Log-log regression plots for density (# L<sup>-1</sup>) of *Trichocerca* spp., *Keratella* spp., *Synchaeta* spp., *Polyarthra* spp., *Brachionus* spp., and copepod nauplii, estimated from the macrozooplankton (63 μm) and microzooplankton (20 μm) methods. Filled circles are Mississippi River samples, and open circles are Missouri River samples. In equations, X and Y are log<sub>10</sub>-transformed values.



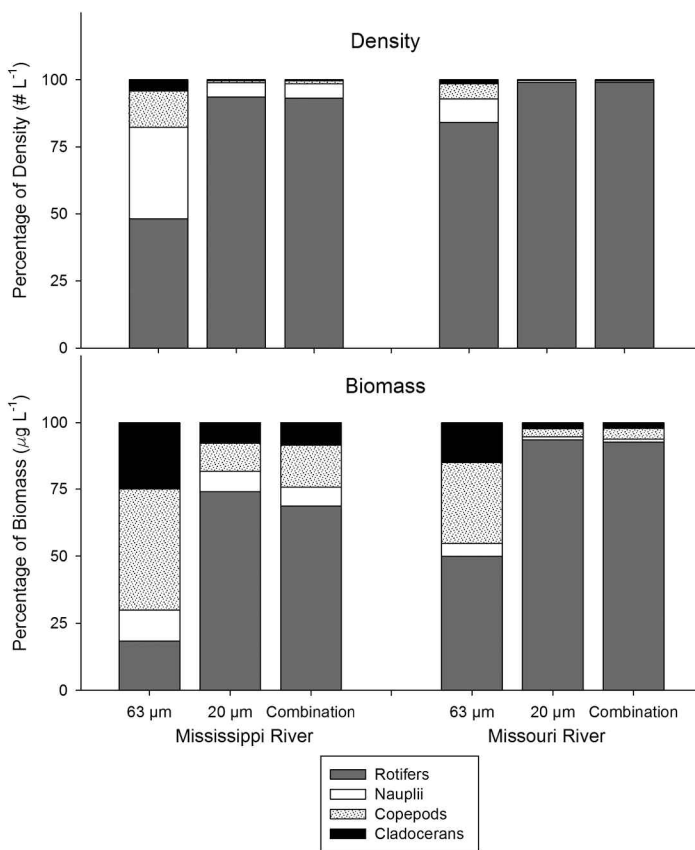
**Fig. 3.** Mean slope of the log-log regression of density (# L<sup>-1</sup>) of rotifers and copepod nauplii estimated from the macrozooplankton (63 μm) and microzooplankton (20 μm) methods plotted against mean length for each taxon. Filled circles are Mississippi River samples, and open circles are Missouri River samples. Error bars are the 95% confidence interval for mean slope. The dashed line indicates a slope = 1 where the two methods provide equivalent density estimates.

far greater in magnitude. Typical density estimates for rotifers reported by Likens and Gilbert (1970), Bottrell et al. (1976), and Ejmont-Karabin (1978) for mesh sizes ≥ 63 μm ranged from 28% to 66% of density estimates made either with fine mesh sizes (i.e., ≤ 35 μm) or from whole-water settling techniques. In our study, differences between density estimates from the two methods for copepod nauplii and *Brachionus* spp. fell within this range, but the difference between methods for all rotifers combined was greater than an order of magnitude (i.e., < 10%) and differences were greater than two orders of magnitude (i.e., < 1%) for the most common rotifer genera.

Several factors may have contributed to the substantially greater differences in rotifer density estimates between methods in this study relative to previous studies. We only identified rotifers at the genus level and it is possible that we analyzed different species with smaller body sizes than previous studies. Zooplankton in rivers and other advective environments are expected to be dominated by smaller species with short generation times (Pace et al. 1992; Thorp and Mantovani 2005). In fact, the mean lengths for common rotifer genera in the current study are shorter by up to 50 μm than those reported in previous studies (Likens and Gilbert 1970; Bottrell



**Fig. 4.** Log-log regression plots for density (# L<sup>-1</sup>) for cladocerans, adult and juvenile copepods, and rotifers estimated from the macrozooplankton (63 μm) and microzooplankton (20 μm) methods. Filled circles are Mississippi River samples, and open circles are Missouri River samples. In equations, X and Y are log<sub>10</sub>-transformed values.



**Fig. 5.** Percentage composition for mean density ( $\# L^{-1}$ ) and biomass ( $\mu g L^{-1}$ ) of rotifers, copepod nauplii, adult copepods, and cladocerans, collected from the Mississippi and Missouri rivers.

et al. 1976; Ejsmont-Karabin 1978), and both the strength of the regressions and the slope of the relationship increased with mean length in our study. Finally, methodological factors other than mesh size may have contributed to the difference between our results and previous studies. Our microzooplankton samples used a much greater volume of water (18 L) than previous studies, and our study examined data from a far greater number of sites ( $n = 132$ ) over a greater spatial scale than previous studies (Likens and Gilbert 1970; Bottrell et al. 1976; Ejsmont-Karabin 1978). These factors likely allowed for a more accurate estimate of effect size in our study.

Our results can be used to help aquatic ecologists better match methodology with study goals (Table 3). The macrozooplankton method (63- $\mu m$  mesh, 180 L water) significantly underestimated densities of common rotifers and copepod nauplii by as much as 2 to 3 orders of magnitude. Although the microzooplankton method (20- $\mu m$  mesh, 18 L water) provided accurate density estimates for all adult copepods and all cladocerans, the smaller volume of water sampled proved ineffective for estimating cladoceran species richness or cladoceran abundance at lower taxonomic levels (i.e., genus or species). Because the use of smaller mesh size (i.e.,  $\leq 35 \mu m$ ) necessitates a reduced volume of water for each sample, it is necessary to use a dual sampling approach to accurately estimate density or biomass of the entire metazoan zooplankton community at lower taxonomic levels or to calculate diversity indices. Sampling large volumes of water with large mesh sizes ( $\geq 63 \mu m$ ) is suitable for quantifying density or biomass of adult crustacean zooplankton at lower taxonomic levels and crustacean taxonomic richness. Additionally, this method also gave fairly accurate estimates of system wide taxonomic richness of rotifers, though we caution that sample size in our study was high. Because density or biomass estimates of rotifers or copepod nauplii will not be accurate when large mesh sizes are used, such estimates should not be reported and aquatic ecologists should clearly state that they are limiting their study to adult and juvenile crustacean zooplankton.

Accurate understanding of the role of rotifers in aquatic food webs and ecosystem processes will only be possible when proper sampling methodology becomes the rule rather than the exception. Rotifers are important organisms in most freshwater ecosystems and are as worthy of study and integration into theoretical constructs of ecosystem structure and function as crustacean zooplankton. Rotifers can be the dominant metazoan zooplankton in rivers and can seasonally dominate other freshwater ecosystems (Orcutt and Pace 1984; Pace et al. 1992; Pillard and Anderson 1993; Thorp et al. 1994), are important components of aquatic food webs (Porter 1995; Jurgens et al. 1999; Miracle et al. 2007), and are important prey items for fishes (Bass et al. 1997; Chick and Van Den Avyle 1999; Sampson et al. 2008). When study goals include quantifying density or biomass of the entire metazoan zooplankton community, we encourage aquatic ecologists to adopt a dual sampling approach similar to that used in this study.

**Table 3.** Study goals and associated methodological suggestions based on the results from this study. Note that filtering large volumes of water through a small mesh is impractical.

Study Goal	Suggested Method
Quantify density and biomass of the full metazoan zooplankton community at higher taxonomic levels (i.e., rotifers, cladocerans, copepods)	Small mesh and small volume of water
Taxonomic richness at the systemic scale (i.e., from multiple samples)	Large mesh and large volume of water
Quantifying density and biomass of the full metazoan zooplankton community at lower taxonomic levels (i.e., genus, species)	Dual method approach
Diversity measures or site specific estimates of taxonomic richness	Dual method approach

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