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Quantifying rotifer species richness in temperate lakes

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SUMMARY

1. Biodiversity assessments of lakes depend on the ability to identify the complement of species present, although the degree of sampling required is often uncertain. We utilise long-term data to predict rotifer species richness in three habitats in three Polish lakes using rarefaction sampling methods.
2. Richness in littoral and psammon habitats did not saturate, even with up to 130 samples. Highest richness was observed in psammon habitat (119 species) in Lake Mikolajskie, followed by littoral habitat in Lakes Łuknajno (114 species) and Kuc (110 species). Littoral habitats in Lakes Łuknajno (56%) and Kuc (51%) had the most species not shared with other habitats in the same lake.
3. Species richness (Chao2) estimates ranged between 44 for pelagic and 135 for psammon habitat in Lake Mikolajskie, to 100 for psammon and 137 for littoral habitat in Lake Kuc, and 65 for pelagic and 162 for littoral habitat in Lake Łuknajno. Whole lake estimates were 167, 205 and 171 species, respectively, for these lakes, higher than the 150 to 160 species predicted by Dumont and Segers (Hydrobiologia, 1996, **341**, 125).
4. Using standardised sampling, richness was significantly higher in littoral than either pelagic or psammon habitats. Contrasts of standardised rarefaction curves revealed that richness in Lakes Kuc and Mikolajskie was described as well by littoral-only or psammon-only samples, respectively, as by those randomly drawn from across all habitats in the lake.
5. Species richness estimates for Lake Mikolajskie were highest in summer, followed by autumn and spring. Interannual estimates differed by up to 427%, nearly an order of magnitude greater than maximal seasonal variation of 70%.
6. Results indicate that much higher sampling intensity is required to establish species richness than is presently carried out in most lakes. Because many species can be detected only with very intensive sampling, conservation programmes must consider sampling intensity when designing studies.

Keywords: littoral, planktonic, rarefaction, rotifers, species diversity

Introduction

Biodiversity patterns in lakes integrate species' dispersal capabilities, environmental tolerances and biological interactions among residents. Community composition can be and often is further influenced by human perturbations. In order to infer natural or human-mediated changes in community composition, the entire species complement (i.e. species richness) must be sampled and identified repeatedly. Because sampling an entire ecosystem is typically prohibitive, ecologists often resort to subsampling techniques and the subsequent application of statistical methods to infer total species richness. Investigators may use any of three classes of techniques to infer species richness (see Hortal, Borges & Gaspar, 2006). First, they may utilise non-parametric estimators of species

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abundance or species occurrence patterns in samples. These estimators have been developed to correct for systematic bias in underestimating total species richness, such as the sum total of species observed pooled across samples (e.g. Walther & Moore, 2005). Several methods have been developed to estimate species richness from samples (e.g. Smith & van Belle, 1984) or modified from mark-recapture situations where the distribution of species among samples is equivalent to that of individuals sampled at different points in time (Chao, 1987). Chao, Jackknife and bootstrap estimators are based on the observed frequency of rare species in the community and add the number of species that occur in only one or two samples to the observed total species richness in the case of the jackknife estimator, or add the proportion of samples that have a particular species present for bootstrap estimation to correct the bias (Smith & van Belle, 1984; Chao, 1987).

Second, investigators may develop parametric models of relative species abundance distributions, following which the complement of remaining, unidentified species can be extrapolated. Commonly used distributions associated with this approach are the lognormal and log-series in which the complement of species is extrapolated through integration of each distribution.

Third, they may fit asymptotic equations to describe the relationship between cumulative species richness and sampling effort. Total species richness is then extrapolated based upon an infinite sampling size. Commonly used functions include modified logarithmic, modified power, negative exponential and Weilbull (see Walther & Moore, 2005 with references therein). The Michaelis–Menten function, derived to model enzyme kinetics, has received considerable attention as it is a relatively simple function with only two fitted parameters and has often been used in describing asymptotic curves of species richness (e.g. Colwell & Coddington, 1994; Keating & Quinn, 1998).

Unlike estimators of total species richness that are based on the entire pool of samples, species accumulation or rarefaction curves describe the relationship between the number of species accumulated and sampling effort. This relationship then allows for comparisons of species richness accumulation among different sites or communities, standardised for differences in sample sizes (e.g. Colwell & Coddington, 1994; Colwell, Mao & Chang, 2004). Two types of species richness accumulation curves may be developed. First, a species-accumulation curve describes the relationship between cumulative number of species and the number of individuals censused in a single sample. Second, a sample-accumulation curve describes cumulative species richness when samples are sequentially and randomly added from replicated sets of samples. The former relationship can be described as an individual-based rarefaction curve, the latter as a sample-based rarefaction curve (Colwell, Mao & Chang, 2004). One benefit of sample-based rarefaction curves is that they incorporate between-sample heterogeneity, unlike their individual-based counterparts (Gotelli & Colwell, 2001; Ugland, Gray & Ellingsen, 2003).

Rarefaction sampling has an extensive history in terrestrial ecology (for recent examples see Colwell *et al.*, 2004; King & Porter, 2005; Wunderle, Henriques & Willig, 2006). Aquatic ecologists have used rarefaction sampling for studies on marine ecosystems (Ugland *et al.*, 2003; Vallet & Dauvin, 2004; George, 2005), rivers (McCabe & Gotelli, 2003; Koel, 2004) and lakes (Benson & Magnuson, 1992; Allen *et al.*, 1999; Willis & Magnuson, 2000; Wolfe, 2003; Bouchard, Gajewski & Hamilton, 2004). Despite the usefulness of this technique, its application to zooplankton assemblages has been infrequent (but see Dumont & Segers, 1996; Arnott, Magnuson & Yan, 1998).

Dumont & Segers (1996) analysed cladoceran and rotifer species richness patterns in North America, South America, Europe and Africa using hyperbolic regression and Chao's non-parametric (see Methods) estimator. Rotifer species richness was better characterised than that of cladocerans (Dumont & Segers, 1996). These analyses suggested that temperate lakes should contain between 150 and 160 rotifer species, whereas tropical lakes should contain in excess of 210 species. The authors noted that assessment of species richness in a Brazilian lake was, however, encumbered by seasonal succession of species. Similarly, Arnott *et al.* (1998) noted that species richness of cladocerans in Canadian Shield lakes increased with the number of seasonal, interannual and spatial samples collected, and that single samples recovered only 33% of the total estimated species complement.

In this study, we utilise rarefaction sampling to develop incidence-based species accumulation curves for rotifer species in three well-studied Polish lakes. We test the number of samples needed to characterise rotifer species richness in different habitats within these lakes, whether different habitats and different lakes support similar levels of richness and whether sampling multiple habitats within one lake yields higher species richness than sampling only one habitat with the same sampling intensity.

Methods

Study sites

For this study, we selected three lakes from the Polish lake district for which biological composition has been well studied owing to cultural eutrophication concerns (e.g. Papinska, 1981; Karabin, Ejsmont-Karabin & Kornatowska, 1997; Pieczynska, Kolodziejczyk & Rybak, 1998; Ejsmont-Karabin, 2003, 2005). Study systems include mesotrophic Lake Kuc (53°49′N, 21°24′E), eutrophic Lake Mikolajskie (53°47′N, 21°36′E) and meso-eutrophic Lake Łuknajno (53°49′N, 21°38′E), all situated in the Great Masurian lakes district of north-eastern Poland. The lakes have respective areas of 99, 498 and 680 ha, respectively, and maximum depths of 28, 25.9 and 3 m (Brodzińska *et al.*, 1999).

Samples were collected from littoral, pelagic and psammon habitats in Lake Kuc between 1996 and 2005, from littoral and pelagic habitats in Lake Łuknajno from 1985 to 2002, and from littoral, psammon and pelagic habitats of Lake Mikolajskie from 1985 to 2005. Pelagic zooplankton was collected with a 5-L sampler at random locations on the lakes. Samples were condensed by filtration through a 30-μm mesh net, fixed with Lugol's solution and later with formalin in a laboratory.

Littoral samples were collected from randomly located open water sites with a 1-L Patalas sampler and concentrated with a 30-μm mesh size plankton net. Sessile rotifers were collected together with their plant substratum by submerging a 1-L glass in a weedy station and arranging a few aquatic plants (or their fragments) loosely in it. Small fragments of leaves were examined under the microscope and sessile rotifers were identified and enumerated. The remaining macrophyte fragments were fixed with 2% formalin and filtered on a plankton net. Rotifers collected on the net were transferred into bottles and enumerated. These samples consisted of free-living rotifer species associated with vegetation.

Psammon samples (2 cm thick) were cut out with a sharp-edged cylinder (area = 28 cm^2) from random locations among three beaches. The samples were transferred to glass containers and rinsed six times with tap water. After sedimentation of sand grains (10 s), the sample was filtered through a plankton net. All rotifers were identified and enumerated in five subsamples, each equal to 5% of the sample. The first subsample was analysed alive and the remaining subsamples were fixed with 4% formalin. All samples were identified and enumerated by one individual, J. E-K., although cryptic speciation could influence richness estimates. Taxonomic keys used were Kutikova (1970); Koste (1978); Shiel & Koste (1993); Nogrady, Pourriot & Segers (1995); Segers (1995); Shiel (1995); De Smet (1996), and De Smet & Pourriot (1997).

Model development and analysis

To estimate total species richness in a lake, as well as habitat-specific richness in each lake, we developed sample-based rarefaction curves (as per Gotelli & Colwell, 2001) using the software EstimateS, v. 7.5 (Colwell, 2004). While there was a significant correlation ($r = 0.22$, $P < 0.001$, $n = 498$) between the number of species identified in samples and the number of individuals counted, there was no significant difference in the number of animals counted between habitats (ANOVA, $F = 1.09$, d.f. = 2,495, $P = 0.34$). Mean numbers of animals counted were 2043 (461 SE) for littoral habitat, 2790 (241 SE) for pelagic habitat and 2175 (435 SE) for psammon. Thus, to the extent that counts varied, they were highest for pelagic and lowest for littoral, opposite the diversity patterns reported below (see Results).

For total lake species richness, samples from all habitats and dates for a single lake were randomised without replacement, selected (from $n = 1$ to m, where m is equal to the total number of samples) and the cumulative number of different species tabulated. This procedure was repeated for each of 100 bootstrap iterations. The software provided estimates of rarefied species richness ($\hat{S}_{MaoTao}$), which is the expected species accumulation curve based on the data in lieu of a resampled total observed species (S_{obs}) or sample-based rarefaction (Colwell *et al.*, 2004).

We also calculated two estimates of total species richness – the bias-corrected Chao2 and Jackknife2 –

that have been determined to be among the most reliable predictors for incidence data of this type (Hortal *et al.*, 2006). Both of these estimators have been used to reduce the bias in underestimating species richness and have been shown to be accurate to true species richness relative to other non-parametric estimators, especially when based on small sample sizes and when the sampling scale is on the order of individuals or traps (Colwell & Coddington, 1994; Hortal *et al.*, 2006). Walther & Moore (2005) reviewed estimator performance for a variety of taxa.

The bias-corrected Chao2 and Jackknife2 estimators of total species richness are respectively formulated as:

$$S_{\text{chao2}} = S_{\text{obs}} + \left[\frac{m-1}{m}\right]\left[\frac{Q_1(Q_1-1)}{2(Q_2+1)}\right]$$

and

$$S_{\text{jack2}} = S_{\text{obs}} + \left[\frac{Q_1(2m-3)}{m} - \frac{Q_2(m-2)^2}{m(m-1)}\right]$$

where m is the total number of samples, Q_1 is the frequency of unique species and Q_2 is the frequency of duplicates (Smith & van Belle, 1984; Colwell & Coddington, 1994).

The same procedure was repeated to determine species richness for each habitat within each lake, pooling samples across years. To determine whether species richness varied across habitats or across lakes, we conducted an two-factor ANOVA without replication, on estimated species richness values for a rarefied (i.e. standardised) samples size of $n = 27$. This sample size was selected because it was the smallest sample size other than littoral (six) in Lake Mikolajskie, which was excluded from analysis. We adjusted the resulting sums of squares because the design was unbalanced. Tukey's test was used to contrast mean species richness when significant differences were found.

To determine whether species richness varied across seasons in one lake, we calculated the Chao2 estimator and Jackknife2 values for Lake Mikolajskie by pooling data from across the 9 years for which data were available. We also assessed interannual patterns in species richness in this lake by pooling data across seasons. We considered this lake most suitable for these calculations as total sample size (243) greatly exceeded those for our other two study lakes (141, 114).

To determine how best to deploy sampling effort, we contrasted estimated cumulative species richness from multiples of duplicate (Lakes Łuknajno and Mikolajskie) or triplicate (Lake Kuc) samples taken randomly from all habitats versus those taken from a single habitat. For example, if four sets of samples were taken randomly from each of three habitats and combined (all habitats), 12 samples were taken from individual habitats. Littoral habitat from Lake Mikolajskie was excluded from this analysis because of low sample size. The maximum number of samples used in this rarefaction analysis was constrained to the minimum sample size from each of the habitats (either $n = \text{m}/2$ or $n = \text{m}/3$ depending on whether data was available for two or three habitats per lake). This procedure was repeated for 100 bootstrap iterations for each lake. Lines were fit to cumulative species richness using a Michaelis–Menten function.

Although we present the rarefaction curves, we did not extrapolate total species richness using Jackknife2 or Chao2 estimators or analyse patterns statistically owing both to low statistical power and to a lack of independence among samples. That is, estimators are not suitable to the analysis of pooled duplicate or triplicate samples and samples in this rarefaction analysis taken from each habitat were also present in the treatment of all habitats combined.

Results

Observed rotifer species richness ranged between 129 species in 114 samples from Lake Łuknajno, to 146 species in 243 samples from Lake Mikolajskie and 166 species in 141 samples from Lake Kuc (Table 1; Appendix). Observed richness was highest in littoral habitat in lakes Kuc (110 species) and Łuknajno (114 species) and in psammon in Lake Mikolajskie (119 species). Species complementarity across habitats within a lake varied in a similar manner. For example, 51% and 56% of the species encountered in littoral habitat in lakes Kuc and Łuknajno, respectively, were not shared with other habitats within the same system (Table 1). Forty-five percent of rotifer species encountered in Lake Mikolajskie were exclusively found in psammon habitat, the highest ratio in that lake.

Rarefaction (resampling) analysis was used to build species richness accumulation curves for rotifers in each lake and in each habitat in each lake (Fig. 1).

Table 1 Number of species observed, number of samples analysed, number of individuals per sample and the proportion of unique species in each habitat for three Polish lakes

Lake	Number of species sampled in lake	Habitats sampled	Number of samples	Number of individuals per sample	Number of species present per habitat	Proportion of unique species compared with other habitats
Kuc	166	Littoral	37	1247	110	0.51
		Pelagic	27	6987	62	0.38
		Psammon	77	4412	65	0.46
Łuknajno	129	Littoral	29	3232	114	0.56
		Pelagic	85	3656	65	0.23
Mikolajskie	146	Littoral	6	1207	63	0.19
		Pelagic	107	1042	43	0.28
		Psammon	130	850	119	0.45

Chao2's species incidence function and the Jackknife2 richness estimator were used to estimate total lake species richness, as well as habitat-specific species richness (Table 2; Fig. 1). Total estimated species number for Lakes Kuc, Łuknajno and Mikolajskie were 206, 171 and 167, respectively, using the Chao2 estimate, and 225, 187 and 183 with the Jackknife2 estimator (Table 2).

In Lake Kuc, the rarefaction curve for littoral habitat rose more steeply and attained a higher value than psammon habitat, which, in turn, exceeded the value for pelagic habitat (Fig. 1). In Lake Łuknajno, the rarefaction curve for littoral habitat rose more steeply than that of pelagic habitat and showed little sign of achieving an asymptote; by contrast, pelagic richness was much better characterised and was asymptotic with all samples considered (Fig. 1). In Lake Mikolajskie, littoral habitat was poorly characterised owing to the dearth of samples (six), but appeared to be highly speciose and perhaps richer than either of the other habitats. Asymptotic patterns were observed for the well-characterised psammon and pelagic habitats, the former estimated to be more than three times richer than the latter (Fig. 1).

A two-way ANOVA without replication was used to assess species accumulation curves for the three habitats in the three lakes, rarefied to only the first 27 samples. Standardised species richness differed significantly across habitats ($F = 26.3$, d.f. = 2,2, $P = 0.04$) but not among lakes ($F = 4.5$, d.f. = 2,2, $P = 0.18$). Species richness in littoral habitat (106 species) was significantly greater than that in either pelagic (51 species) or psammon (61 species) habitats, which did not differ from each other (Tukey HSD, $q = 9.95$, d.f. = 2, $P < 0.05$).

Estimated species richness for Lake Mikolajskie in spring was lower than that of autumn, which, in turn was slightly lower than that of summer (Table 3). When rarefied to a sample size of $n = 33$, estimated richness was not significantly different as measured by overlapping confidence intervals for $\hat{S}_{MaoTao}$. Variability among the Chao2 estimates of seasonal richness was more than twice that of the $\hat{S}_{MaoTao}$ estimates (CV = 25% and 10%, respectively).

Species richness differences among years for Lake Mikolajskie were much more pronounced than those among seasons. For example, the highest annual Chao2 and Jackknife2 estimators were 362% and 427% greater, respectively, than the lowest annual values, while these differences between seasons were only 70% and 59%, respectively (Tables 3 and 4). Rarefied species richness for 1988 appeared much lower than all other years. Based on overlapping confidence intervals for $\hat{S}_{MaoTao}$, rarefied species richness was similar in a group comprising 1998, 2001 and 2004, and within another group of the remaining years (Table 4). Similar to the pattern observed for seasonal variability, interannual variability in the Chao2 estimate was twice that of the $\hat{S}_{MaoTao}$ estimate (CV = 46% and 23%, respectively).

A second set of rarefaction analyses were conducted to determine whether estimated species richness differed with comparable levels of sampling effort expended on a single habitat or across habitats within a lake. In Lake Kuc, cumulative species richness for littoral-only samples paralleled that for whole lake samples and far exceeded values for either psammon or pelagic habitats (Fig. 2). Littoral-only samples from Lake Łuknajno also yielded higher estimated species richness than that of pelagic habitat and from

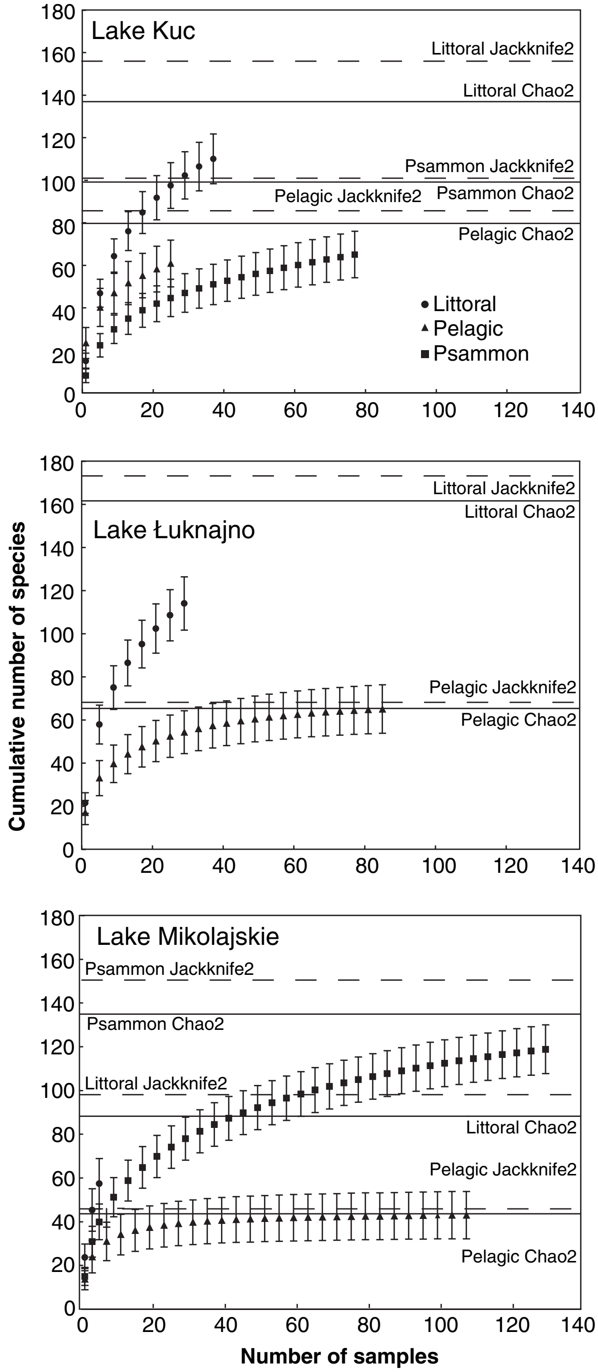

Fig. 1 Sample-based rarefaction curves of rotifer species richness, $\hat{S}_{MaoTao}$ (with 95% CI) for each habitat. Total species richness in each habitat is indicated by the Chao2 (solid line) and Jackknife2 (dashed line) estimators. Only every 4th point is shown for the curves.

combined littoral and pelagic habitats (owing to the high redundancy of species in the pelagic habitat; Table 1). Psammon-only samples yielded species richness values comparable to combined psammon and pelagic habitats in Lake Mikolajskie (Fig. 2).

Table 2 Sample sizes, rarefied species richness at $n = 114$ samples ($\hat{S}_{MaoTao}$) and Chao2 and Jackknife2 estimates of whole lake species richness with samples pooled across dates and habitats

Lake	Sample size	$\hat{S}_{MaoTao,n=114}$ (95% CI)	Chao2 mean (95% CI)	Jackknife2
Kuc	141	159 (148–169)	206 (184–251)	225
Łuknajno	114	129 (118–140)	171 (148–224)	187
Mikolajskie	243	125 (116–134)	167 (154–196)	183

Table 3 Seasonal differences in sample sizes, rarefied species richness at $n = 33$ ($\hat{S}_{MaoTao}$) and Chao2 and Jackkife2 estimates of whole lake species richness for Lake Mikolajskie with samples pooled across habitats. Littoral habitat is excluded from the analysis because of small sample size (six).

Season	Sample size	$\hat{S}_{MaoTao,n=33}$ (95% CI)	Chao2 mean (95% CI)	Jackknife2
Spring	33	66 (54–78)	82 (72–111)	93
Summer	88	80 (69–91)	132 (118–166)	148
Autumn	116	77 (67–88)	130 (115–171)	142

Table 4 Interannual differences in sample sizes, rarefied species richness at $n = 6$ ($\hat{S}_{MaoTao}$) and Chao2 and Jackkife2 estimates of whole lake species richness for Lake Mikolajskie with samples pooled across habitats. Littoral habitat is excluded from the analysis because of small sample size.

Year	Sample size	$\hat{S}_{MaoTao,n=6}$ (95% CI)	Chao2 mean (95% CI)	Jackknife2
1988	6	26 (17–35)	26 (26–28)	26
1997	32	42 (33–51)	65 (63–73)	67
1998	13	28 (19–37)	36 (34–52)	41
1999	21	41 (32–50)	72 (64–98)	81
2000	22	42 (33–51)	79 (68–111)	86
2001	14	31 (22–40)	39 (37–55)	43
2002	62	43 (35–52)	90 (85–108)	100
2004	12	27 (19–35)	56 (40–116)	55
2005	36	48 (40–56)	120 (109–143)	137

Discussion

Species richness of Polish lake rotifer communities differed widely among habitats in this study. In Lakes Kuc and Łuknajno, species richness was greatest in littoral habitats, while in Lake Mikolajskie psammon habitat yielded highest richness values (Fig. 1). It is possible that littoral habitat was also richest in Lake

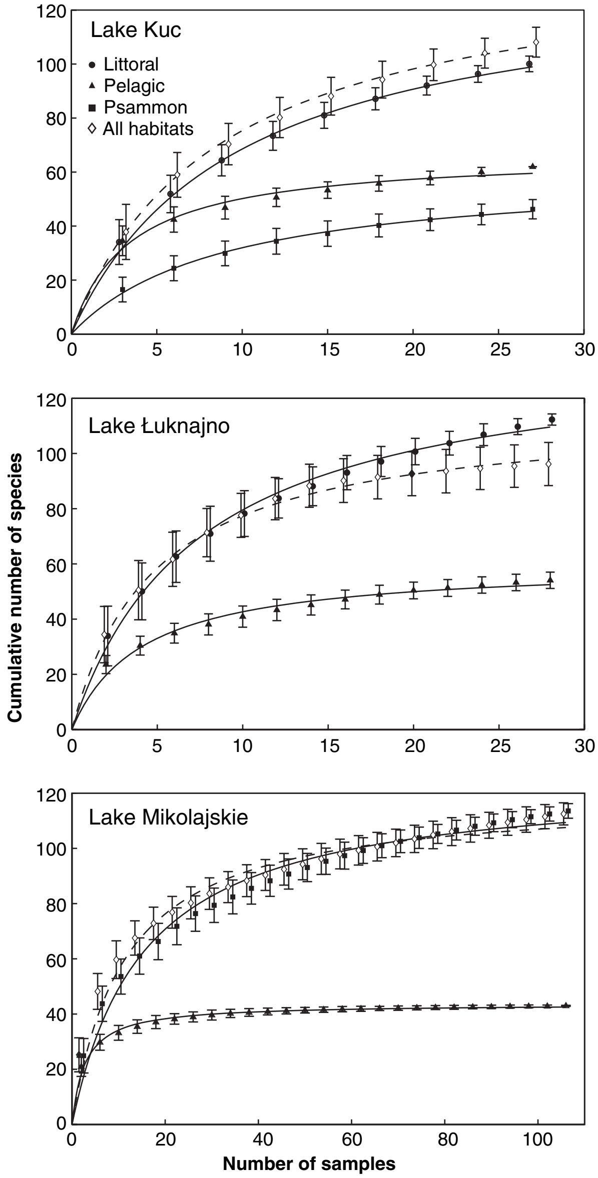

Fig. 2 Sample-based rarefaction curves of mean (± 1 SD) rotifer species richness from bootstrapped resampling for separate and pooled habitats. The rarefaction curves were fitted with the Michaelis–Menten function. The dashed line indicates the fitted curve for the species list of all habitats combined. Only every 2nd point is shown for the curves.

Mikolajskie, although low sample size precluded a formal examination of this possibility. In all three lakes, pelagic habitat – ironically the habitat most reported upon in limnological studies – yielded the lowest species richness values. Investigators interested in establishing the total complement of rotifer species in lakes ought to include sampling of littoral areas. In Lake Kuc, for example, comparable levels of sampling in littoral areas or whole lake regions yielded similar species richness values (Fig. 2).

No clear pattern existed with respect to trophic state of a lake and its species richness, as meso-eutrophic Lake Łuknajno had higher diversity than the lakes on either side of the productivity scale. However, Lake Łuknajno is the largest of the three lakes surveyed and previous work has established a positive correlation between area and species richness (Hoffman & Dodson, 2005; Karatayev, Burlakova & Dodson, 2005).

Estimated (Chao2) species richness values for each of the three lakes in our study were slightly to moderately higher than the 150 to 160 species predicted by Dumont & Segers (1996). Total species richness estimated using the Jackknife2 procedure was substantially higher than Dumont & Segers (1996) projections as well as our Chao2 estimations (Table 2). Because our samples spanned across seasons, years and habitats and involved in between 114 and 243 samples per lake and >2000 individuals counted per sample, we are confident that the estimated richness values adequately reflects natural variation in these lakes. By contrast, richness estimates for Lakes Broa and Glubokoe were developed using 12 plankton samples from each lake (Dumont & Segers, 1996); the authors estimated that a minimum of 28 samples would be required from each of these lakes to record total species richness. In addition, our analyses explicitly considered habitat differences. Consequently, the under-reporting that influenced the results for Lake Broa (Dumont & Segers, 1996) should not apply here. Other rarefaction studies illustrate that inclusion of additional samples (i.e. habitats) into simulations results in higher species richness estimates (e.g. Olszewski, 2004). For example, King & Porter (2005) determined that a combination of three capture methods yielded higher cumulative ant species richness estimates than any one of the three methods individually. Likewise, estimated bird species richness in Amazonian lowland forest was greater when two habitat types were combined than when either was considered alone (Wunderle *et al.*, 2006).

Larger interannual than seasonal variation of species richness in Lake Mikolajskie is contrary to results found in Arnott *et al.* (1998). The coefficient of variation for this lake was nearly twice that of seasonal variation (46% and 25%), unlike the pattern observed for crustacean zooplankton richness for eight Ontario lakes (13% and 16%, respectively, Arnott *et al.*, 1998).

Differences between the two studies is largely an artefact resulting from differences in sampling effort, thus a meaningful comparison of the Chao estimators is not recommended unless sampling effort is standardised across studies. Variability in sample sizes for interannual richness estimates for Lake Mikolajskie was 71% (Table 4) whereas Arnott *et al.* (1998) used a fixed sampling effort of 6 monthly samples. Richness estimators and their variability are heavily influenced by sample size, as estimated species richness increases as a function of increasing sample size.

Both measures of richness used in this study – Chao2's incidence-based estimate and the second-order Jackknife2 estimator (see Hortal *et al.*, 2006) – yielded results that were quite similar (mean difference 7.6%) and never dissimilar enough to switch the rank order of species richness between habitats. This finding was not unexpected, as Hortal *et al.* (2006) recommended both of these measures for samples of the type studied here.

Results obtained from this study provide some guidance for sampling strategies, although no clear rules. As sampling intensity increases, so too does the prospect of recording rare species. However, even intensive sampling strategies may provide relatively incomplete faunal lists, depending on the habitat and lake sampled. For example, in 107 samples we recovered between 93% and 98% of estimated rotifer species in pelagic habitat from Lake Mikolajskie and between 93% and 97% of pelagic species in Lake Łuknajno, but only 72% to 78% of taxa in Lake Kuc were recovered in 27 samples (see Fig. 2). Littoral habitats in Lakes Kuc and Łuknajno were sampled with 70–80% and 67–72% completeness with respective sample sizes of 37 and 29. Even the most intensively sampled habitat in this study, psammon in Lake Mikolajskie (130 samples), recovered only 79–89% of estimated species richness. If the lakes analysed here are representative of temperate lake ecosystems, then many studies may significantly under-report species richness because many use sample sizes much smaller than those described here.

Under-reporting of species richness can obscure changes in native biodiversity patterns if such assessments are conducted as part of a conservation strategy. For example, if under-reporting occurred during initial assessments of a lake, then many rare species would never be recognised as inhabitants of the system. If, on the other hand, under-reporting occurred during subsequent investigations, erroneous conclusions could be drawn that rare species had been extirpated. These errors could have profound management implications concerning whether a lake receives protected status based on its assemblage of rare species, or, on the success of programmes intended to protect rare species. This study – which utilised samples collected over a 17-year span and analysed by a single taxonomist – illustrates that most rare species would not be detected with the level of sampling utilised in most studies. While these rare species may contribute little to ecosystem function, they are the cornerstone of conservation programmes and detection of their presence requires intensive sampling.

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Appendix Species list for each habitat within Lakes Kuc, Łuknajno and Mikolajskie. Abbreviations for habitat types are littoral (Lit), pelagic (Pel) and psammon (Psa).

	L. Kuc			L. Łuknajno		L. Mikolajskie		
Species	Lit	Pel	Psa	Lit	Pel	Lit	Pel	Psa
Anuraeopsis fissa (Gosse)				x	x	x	x	x
Ascomorpha ecaudis Perty		x		x	x		x	
Ascomorpha ovalis (Bergendal)	x	x	x		x		x	x
Ascomorpha saltans Bartsch	x	x		x	x		x	x
Aspelta circinator (Gosse)								x
Asplanchna brightwelli Gosse				x	x		x	
Asplanchna herricki De Guerne				x				
Asplanchna priodonta Gosse		x		x	x		x	x
Brachionus angularis Gosse	x	x		x	x		x	x
Brachionus calyciflorus Pallas		x		x	x		x	
Brachionus quadridentatus Hermann				x				x
Bryceella tenella (Bryce)								x
Cephalodella apocolea Myers	x			x		x		
Cephalodella auriculata (Müller)	x	x	x	x	x	x		x
Cephalodella catellina (Müller)	x		x	x	x	x		x
Cephalodella compacta Wiszniewski			x					x
Cephalodella eva (Gosse)	x	x		x		x		x
Cephalodella exigua (Gosse)	x			x		x		x
Cephalodella forficula (Ehrenberg)	x			x				x
Cephalodella gibba (Ehrenberg)	x		x	x	x	x		x
Cephalodella gibboides Wulfert	x		x					
Cephalodella gigantea Remane								x
Cephalodella gracilis (Ehrenberg)	x		x	x				
Cephalodella hoodi (Gosse)						x		x
Cephalodella hyalina Myers				x				
Cephalodella intuta Myers	x							
Cephalodella limosa Wulfert	x							
Cephalodella megalocephala (Glasscott)						x		
Cephalodella megalotrocha Wiszniewski	x							x
Cephalodella misgurnus Wulfert			x					
Cephalodella nana Myers				x				
Cephalodella reimanni Donner	x					x		x
Cephalodella sterea (Gosse)	x	x		x		x		x
Cephalodella tachyphora Myers								x
Cephalodella tantilloides Hauer				x				
Cephalodella tenuior (Gosse)	x		x	x		x		x
Cephalodella tenuiseta (Burn)			x	x				x
Cephalodella ventripes (Dixon-Nuttall)	x	x		x		x		x
Collotheca algicola (Hudson)				x				

Appendix (*Continued*)

	L. Kuc			L. Łuknajno		L. Mikolajskie		
Species	Lit	Pel	Psa	Lit	Pel	Lit	Pel	Psa
Collotheca ambigua (Hudson)				x				
Collotheca campanulata (Dobie)	x			x				
Collotheca libera (Zacharias)		x					x	x
Collotheca mutabilis (Hudson)	x	x		x	x		x	x
Collotheca ornata (Ehrenberg)	x							x
Collotheca pelagica (Rousselet)		x		x	x		x	x
Collotheca sp.		x						
Collotheca trilobata (Collins)				x				
Collotheca wiszniewskii Varga			x					x
Colurella adriatica Ehrenberg	x	x	x	x	x	x		x
Colurella colurus (Ehrenberg)			x	x	x	x		x
Colurella geophila Donner								x
Colurella hindenburgi Steinecke			x	x				x
Colurella obtusa (Gosse)	x	x	x	x	x	x		x
Colurella sulcata (Stenroos)	x			x				
Colurella uncinata (Müller)	x	x	x	x	x	x		x
Conochiloides natans (Seligo)		x			x		x	
Conochilus hippocrepis (Schrank)	x	x				x	x	
Conochilus unicornis Rousselet	x	x		x	x	x	x	x
Dicranophorus capucinus Harr. et Myers			x					x
Dicranophorus forcipatus (Müller)				x				x
Dicranophorus grandis (Ehrenberg)	x			x				x
Dicranophorus hercules Wiszniewski			x	x				x
Dicranophorus leptodon Wiszniewski			x					
Dicranophorus longidactylum Fadeev								x
Dicranophorus luetkeni (Bergendal)			x					x
Dicranophorus robustus (Harr. et Myers)								x
Dicranophorus rostratus (Dix.-Nut. et F.)				x				x
Dicranophorus secretus Donner								x
Encentrum acrodon Wulfert				x				
Encentrum diglandula (Zavadovsky)								x
Encentrum marinum (Dujardin)			x	x				x
Encentrum sutor Wiszniewski						x		x
Encentrum uncinatum (Milne)			x					
Erignatha clastopis (Gosse)	x					x		x
Euchlanis contorta (Wulfert)								x
Euchlanis dapidula Parise				x				x
Euchlanis deflexa Gosse	x	x		x	x	x		
Euchlanis dilatata Ehrenberg	x	x		x	x	x	x	x
Euchlanis incisa Carlin	x							x
Euchlanis lyra Hudson						x		x
Euchlanis meneta Myers				x				
Euchlanis oropha Gosse								x
Euchlanis triquetra Ehrenberg	x							
Filinia longiseta (Ehrenberg)							x	x
Filinia terminalis (Plate)		x		x	x		x	x
Floscularia janus (Hudson)	x			x				
Gastropus stylifer Imhof	x	x		x	x	x	x	x
Harringia eupoda (Gosse)	x							
Hexarthra mira (Hudson)				x				
Kellicottia longispina (Kellicott)	x	x		x	x	x	x	x
Keratella cochlearis (Gosse)	x	x		x	x	x	x	x
Keratella hiemalis Carlin		x			x		x	
Keratella quadrata (Müller)		x		x	x		x	x

Appendix (*Continued*)

Species	L. Kuc Lit	L. Kuc Pel	L. Kuc Psa	L. Łuknajno Lit	L. Łuknajno Pel	L. Mikolajskie Lit	L. Mikolajskie Pel	L. Mikolajskie Psa
Keratella testudo (Ehrenberg)					x			
Keratella valga (Ehrenberg)					x			
Kostea wockei (Koste)								x
Lacinularia flosculosa (Müller)	x							
Lecane aculeata (Jakubski)			x					
Lecane arcuata (Bryce)			x					x
Lecane arcula Harring	x	x		x				
Lecane bifurca (Bryce)	x							x
Lecane bulla (Gosse)	x	x	x	x	x			x
Lecane clara (Bryce)								x
Lecane closterocerca (Schmarda)	x	x	x	x	x	x		x
Lecane curvicornis (Murray)	x							
Lecane depressa (Bryce)	x							
Lecane flexilis (Gosse)	x	x	x	x	x	x		x
Lecane furcata (Murray)	x		x	x		x		x
Lecane gwileti (Tarnogradski)			x		x			
Lecane hamata (Stokes)	x	x	x	x		x		x
Lecane inermis (Bryce)				x		x		
Lecane intrasinuata (Olofsson)	x		x			x		
Lecane latissima Yamamoto			x					
Lecane levistyla (Olofsson)			x					x
Lecane ludwigii (Eckstein)	x	x						x
Lecane luna (Müller)	x	x	x	x	x	x		x
Lecane lunaris (Ehrenberg)	x		x	x	x	x		x
Lecane nana (Murray)	x							x
Lecane opias (Harring et Myers)			x					
Lecane paxiana Hauer	x							
Lecane perpusilla (Hauer)	x		x					
Lecane psammophila (Wiszniewski)			x					x
Lecane pyriformis (Daday)	x			x				x
Lecane quadridentata (Ehrenberg)	x							
Lecane scutata (Harring et Myers)	x		x					x
Lecane stenroosi (Meissner)			x					x
Lecane stichaea (Harring)								x
Lecane subtilis (Harring et Myers)			x				x	
Lecane tenuiseta (Harring)	x	x	x	x				
Lepadella acuminata (Ehrenberg)	x		x	x		x		x
Lepadella ehrenbergi (Perty)	x	x						
Lepadella elliptica (Wulfert)	x		x					
Lepadella heterodactyla Faddev		x						
Lepadella ovalis (Müller)		x	x	x				
Lepadella patella (Müller)	x	x	x	x	x	x		x
Lepadella quadricarinata (Stenroos)	x		x			x		x
Lepadella rhomboides (Gosse)				x				
Lepadella triptera Ehrenberg	x			x		x		x
Lindia euchromatica Edmondson	x			x		x		
Lindia torulosa Dujardin	x		x					x
Lindia truncata (Jennings)	x		x	x		x		
Lophocharis oxysternoon (Gosse)				x				
Lophocharis salpina (Ehrenberg)	x	x		x	x			x
Monommata dentata Wulfert	x							
Monommata longiseta (Müller)	x			x	x	x		x
Myersinella tetraglena (Wiszniewski)			x					
Mytilina crassipes (Lucks)	x							

Appendix (*Continued*)

	L. Kuc			L. Łuknajno		L. Mikolajskie		
Species	Lit	Pel	Psa	Lit	Pel	Lit	Pel	Psa
Mytilina mucronata (Müller)					x			
Mytilina ventralis (Ehrenberg)	x			x		x		
Notholca acuminata (Ehrenberg)					x			x
Notholca foliacea (Ehrenberg)		x	x		x	x		x
Notholca labis Gosse		x			x		x	x
Notholca squamula (Müller)		x					x	x
Notommata aurita (Müller)	x		x					x
Notommata cyrtopus Gosse	x		x	x		x		x
Notommata doneta Harring et Myers	x							
Notommata glyphura Wulfert			x					
Notommata omentata Wulfert	x							
Notommata silpha (Gosse)	x							
Notommata tripus Ehrenberg	x							
Paradicranophorus aculeatus (Neis.-Sh.)								x
Plationus patulus (Müller)				x	x			
Platyias quadricornis (Ehrenberg)	x							
Pleurotrocha petromyzon Ehrenberg	x	x						
Pleurotrocha robusta (Glasscott)	x							
Ploesoma hudsoni (Imhof)		x	x	x	x			
Polyarthra dolichoptera Idelson		x		x	x		x	x
Polyarthra euryptera Wierzejski					x		x	
Polyarthra luminosa Kutikova		x						
Polyarthra major Burckhardt	x	x		x	x	x	x	x
Polyarthra remata Skorikov		x		x	x	x	x	x
Polyarthra vulgaris Carlin	x	x		x	x	x	x	x
Pompholyx sulcata Hudson		x		x	x	x	x	x
Proales decipiens (Ehrenberg)				x				
Proales fallaciosa Wulfert	x							
Proales gigantea (Glascott)				x				
Proales globulifera (Hauer)	x							
Proales minima (Montet)			x			x		x
Proales wesenbergi Wulfert			x					
Proalides tentaculatus De Beauchamp							x	
Ptygura beauchampi Edmondson	x			x				
Ptygura longicornis (Davis)				x				
Ptygura melicerta Ehrenberg	x			x		x		
Ptygura pilula (Cubitt)	x							
Ptygura velata (Gosse)	x							
Scaridium longicaudum (Müller)	x			x		x		
Sinantherina socialis (L.)				x				
Squatinella bifurca (Bolton)	x			x				
Squatinella rostrum (Schmarda)						x		
Stephanoceros fimbriatus (Goldfuss)	x							
Synchaeta kitina Rousselet	x	x		x	x	x	x	x
Synchaeta lakowitziana Lucks		x		x	x		x	x
Synchaeta oblonga Ehrenberg				x	x	x		x
Synchaeta pectinata Ehrenberg		x		x	x		x	x
Synchaeta stylata Wierzejski				x				
Synchaeta tremula (Müller)		x		x	x		x	
Taphrocampa annulosa Gosse	x							
Testudinella carlini Bartos	x							
Testudinella parva (Ternetz)	x			x				
Testudinella patina (Hermann)	x			x	x			
Testudinella truncata (Gosse)	x					x		x

Appendix (*Continued*)

	L. Kuc			L. Łuknajno		L. Mikolajskie		
Species	Lit	Pel	Psa	Lit	Pel	Lit	Pel	Psa
Trichocerca bidens (Lucks)	x			x				
Trichocerca brachyura (Gosse)					x			
Trichocerca capucina (Wierz. et Zach.)		x		x	x		x	x
Trichocerca cylindrica (Imhof)					x		x	
Trichocerca dixon-nuttallii (Jennings)				x			x	
Trichocerca elongata (Gosse)	x							
Trichocerca iernis (Gosse)				x				
Trichocerca insignis (Herrick)						x		
Trichocerca intermedia (Stenroos)	x		x	x				x
Trichocerca jenningsi Voigt		x						
Trichocerca musculus (Hauer)	x							
Trichocerca myersi (Hauer)			x					x
Trichocerca porcellus (Gosse)	x			x	x	x		x
Trichocerca pusilla (Lauterborn)				x	x	x	x	x
Trichocerca rattus (Müller)	x					x		x
Trichocerca relicta Donner				x				
Trichocerca rousseleti (Voigt)	x	x			x	x	x	
Trichocerca similis (Wierzejski)		x		x	x		x	x
Trichocerca stylata (Gosse)							x	
Trichocerca sulcata (Jennings)				x				
Trichocerca taurocephala (Hauer)			x	x				x
Trichocerca tenuior (Gosse)	x	x	x					x
Trichocerca tigris (Müller)				x	x			x
Trichocerca uncinata (Voigt)						x		x
Trichocerca vernalis (Hauer)				x				
Trichocerca weberi (Jennings)			x		x			
Trichotria pocillum (Müller)	x	x	x	x		x		x
Trichotria tetractis (Ehrenberg)								x
Trichotria truncata (Whitelegge)	x							
Wierzejskiella sabulosa (Wiszniewski)			x					x
Wierzejskiella velox (Wiszniewski)								x