PANAMJAS
Pan-American Journal of Aquatic Sciences

Body size in relation to rotifer abundance underestimation using a net of large mesh size in a tropical reservoir

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Abstract: Rotifer abundances of an eutrophic tropical reservoir, sampled during one year, were underestimated by a 68µm mesh size net as compared to a net of 20µm, resulting in differences in community structure characterization.

Key words: body width, zooplankton, Morisita-Horn.

Tamanho corporal em relação a subestimação de abundância de rotíferos utilizando uma rede de malha grande numa represa tropical. Resumo: Abundancias de rotíferos numa represa eutrófica tropical, amostrada durante o curso de um ano, foram subestimadas usando uma rede de malha de 68µm quando comparado com uma malha de 20µm, resultando em diferenças na caracterização da estrutura da comunidade.
Palavras-chave: largura corporal, zooplâncton, Morisita-Horn.

Although Orcutt & Pace (1984) and James (1991) recommended the analysis of whole, unfiltered samples, quantitative sampling of planktonic rotifers is usually carried out using nets or sieves to concentrate the samples in the field (May & Wallace 2019). Chick *et al.* (2010) and Thomas *et al.* (2017) reviewed the use of different mesh sizes for sampling rotifers. Corroborating the older literature (Likens & Gilbert 1970, Bottrell *et al.* 1976, Ejsmont-Karabin 1978), they presented convincing data that use of plankton nets of 63-64µm mesh size can severely underestimate rotifer abundance as compared to a mesh size of 20µm. Most recently, Rocha *et al*. (2021) found large degrees of underestimation of richness and abundance of rotifers in tropical rivers when using a 65µm mesh net as compared to one of 20µm. Here, I present further data indicating such underestimation, when comparing meshes of 20 and 68µm, in a tropical eutrophic reservoir. Samples were taken over the course of a year, to increase the probability of finding different taxa in abundance. In addition, this data set is to be combined with similar data sets for the years 1999-2001 and 2005-2008, to analyze zooplankton seasonality in this reservoir.

The reservoir is a small (ca 13 hectares), shallow (ca 2m mean depth) urban water body in Campo Grande, Mato Grosso do Sul (20.503º S 54.617º W) in Brazil. Two replicate samples, of 4L in volume each, per mesh size were taken, at approximate monthly intervals during the course of a year (November 2010 to January 2012), at the same point at the outlet of the reservoir, at mid-depth using a bottle sampler, and concentrated by passing through nylon monofilament plankton nets with mesh sizes of 20 and 68µm. The concentrated sample were fixed to a final concentration of 4% formaldehyde. Total water samples were taken on each sampling date to aid in taxonomic identification. In the laboratory, each fixed sample was concentrated to approximately 100mL, and five 1mL sub-samples taken with a Stempel pipette and counted in a Sedgewick-Rafter chamber (Bottrell *et al.* 1976, McCauley 1984, Wetzel & Likens 1991), at a magnification of x100, using a compound light microscope. Abundances (expressed as numbers per L) for each sample were calculated by multiplying the mean number of organisms of the five sub-samples by the volume of the concentrated sample and dividing by 4 (Wetzel & Likens 1991). Mean abundances and standard deviations were calculated

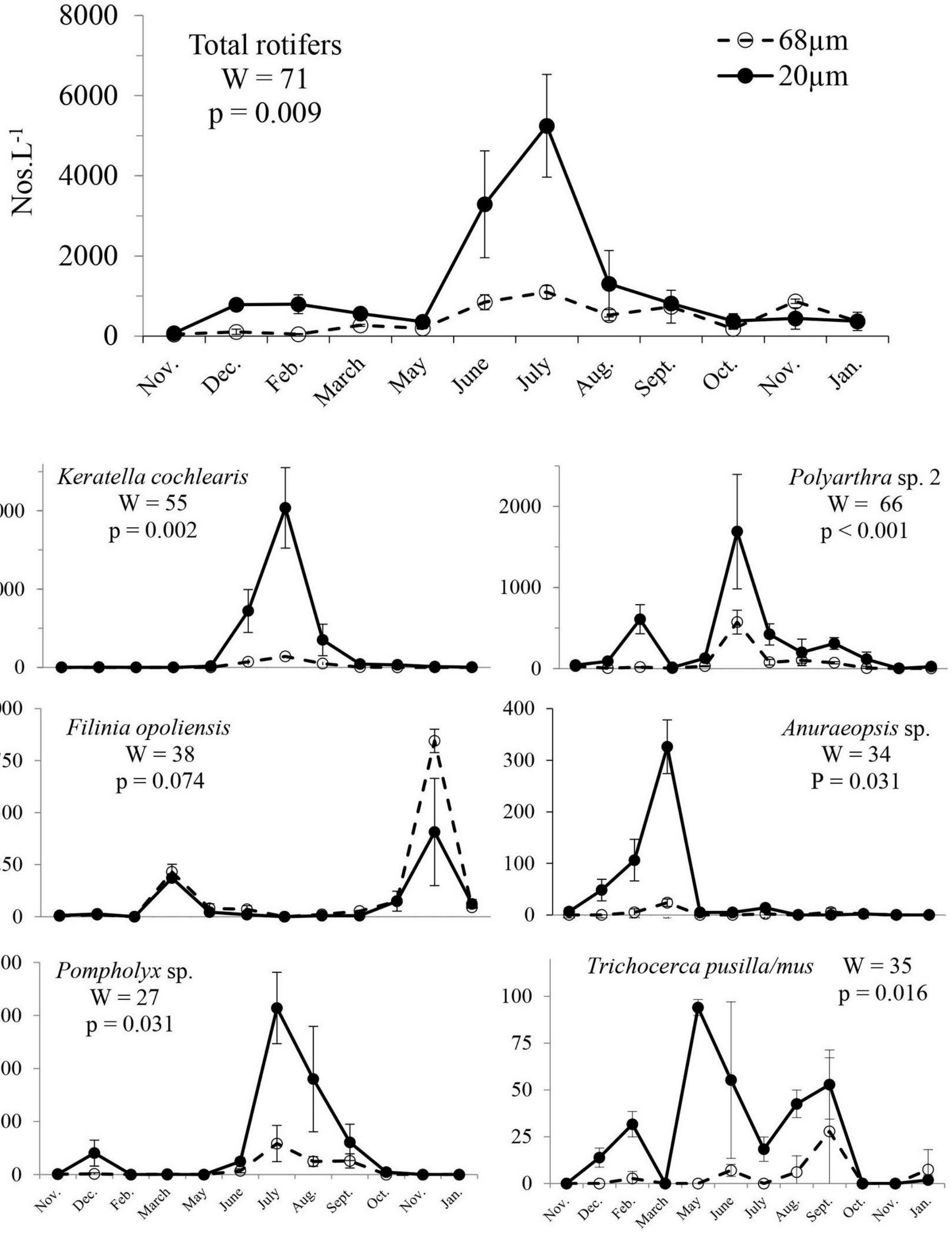

Figure 1. Temporal variation from November 2010 to January 2012 in mean abundance (numbers per liter abbreviated as Nos.L^{-1}on the y-axis) of the total rotifers and the six more abundant taxa encountered in the reservoir, for the two mesh sizes. Standard deviations of the samples and the Wilcoxon signed rank test W and p values for mesh size are shown.

for each mesh size on each sampling date. Differences in abundance between samples of each mesh size were examined by the Wilcoxon signed rank test, and taxon richness and Shannon-Wiener Diversity Index values (to the natural log base) were calculated (Hammer *et al.* 2001). The Morisita-Horn Index of Community Similarity, comparing the two mesh sizes, based on taxa absolute abundances, was calculated empirically for each sampling date (Chao *et al.* 2015). During counting, for each taxon, measurements were made of total lengths (body, and spines when present) and widths of the first one or more individuals encountered in randomly selected sub-samples from (generally) 20µm mesh filtered samples, during the course of the year; an ocular micrometer was used at magnifications of x100 and (almost exclusively) x200. A fixed number of organisms to be measured was not established; more individuals of more abundant taxa were measured, while low numbers of rare taxa were measured, because insufficient numbers of the latter were found. The illoricate species *Synchaeta pectinata* was measured from live samples and relaxed (uncontracted) individuals in the fixed samples.

In the temporal analysis, abundances in the 20µm mesh samples were significantly greater for total rotifers, and five of the six more abundant taxa, with the exception of *Filinia opoliensis* (Fig. 1). The *Trichocerca* taxon in the figure showed characteristics intermediate between the species *pusilla* and *mus*, and was thus termed *Trichocerca pusilla/mus*. Because of the low abundances encountered, the remaining taxa found in the present study (see below) were not analyzed. Taxon richness was generally greater in the 20µm mesh samples, while diversity showed no particular pattern, lower richness tending to be offset by greater evenness in the 68µm samples (Fig. 2). On nine of the twelve sampling dates, values of the Morisita-Horn Index of Similarity were considerably less than 1 (complete similarity) (Fig. 3), indicating that characterization of community structure was strongly influenced by sampling with different mesh sizes.

In Table I are presented the values of total length (body and spines) and width of the rotifer taxa encountered in the reservoir during the sampling period. The same data are presented in Figure 4 with the exception of *Trichocerca* sp., *Lecane* spp., *Brachionus havanaensis* and *B. caudatus*. They were removed because relatively few measurements were made for the latter taxa (at least 21 individuals were measured for each taxon shown in Figure 4), and to increase graphical readability. Also for the latter purpose, the standard deviations are not shown in the figure. Five taxa not listed in the table were encountered in low numbers, in the fixed and live samples, namely *Lepadella* sp. (on four dates in 20µm mesh samples and on one date in a 68µm mesh sample), *Colurella* sp. (on two dates in 20µm mesh samples), *Filinia* cf. *brachiata* (on one date in a 20µm mesh sample), *Platyias quadricornis* (on one date in a 68µm mesh sample),

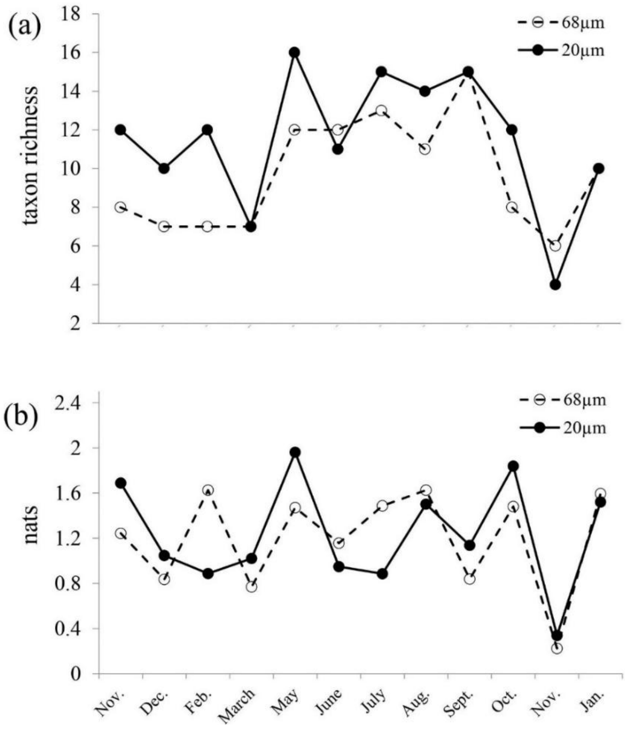

Figure 2. Temporal changes in rotifer taxon richness (a) and Shannon-Weiner diversity (in nats) (b), for the two mesh sizes.

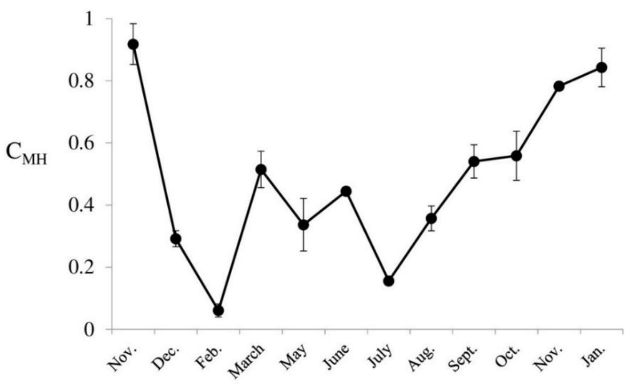

Figure 3. Temporal changes in the Morisita-Horn Index of Similarity (C_{MH}), based on absolute abundances of rotifers, for the two mesh sizes. 95% Confidence Intervals (based on 200 bootstrap replications) are shown.

and *Asplanchna* sp. (on one date in a 68µm mesh sample).

Most of the taxa encountered (especially those with high abundances (Fig. 1)) were of small body size, comparable with the sizes of the taxa encountered by Duncan (1983), Chick *et al.* (2010) and Rocha *et al.* (2021) and considered by these authors to be especially small. Eutrophic environments, as in the present study, tend to be dominated by small-bodied species (Ejsmont-Karabin 2012), although large species can also be favoured in such environments (Stemberger &

Table I. Mean total lengths (body and spines) and widths (µm), with standard deviations, and numbers of individual lengths measured (n), of the rotifer taxa encountered in the reservoir, generally measured from the 20 µm samples. Taxa are listed in order of increasing length. The taxa shown in Figure 1 are indicated in bold. In the final column are listed the abbreviations identifying the taxa in Figure 4.

Taxon	Length	Width	n	Abbrev.
Polyarthra sp. 1	67 (±8)	52 (±6)	40	*Poly1*
Trichocerca pusilla/mus	68 (±11)	40 (±6)	87	*Tp/m*
***Anuraeopsis* sp.**	73 (±7)	44 (±4)	56	*Anur*
Lecane spp.	86 (±31)	66 (±24)	20	
***Pompholyx* sp.**	87 (±6)	76 (±5)	65	*Pomph*
Brachionus angularis	98 (±6)	82 (±5)	34	*Bang*
***Polyarthra* sp. 2**	105 (±13)	70 (±9)	327	*Poly2*
Hexarthra sp.	128 (±20)	92 (±14)	38	*Hex*
Trichocerca sp.	134 (±25)	44 (±8)	8	
Keratella cochlearis	138 (±12)	51 (±4)	42	*Kcoch*
Brachionus havanaensis	142 (±17)	57 (±20)	3	
Filinia opoliensis	159 (±13)	66 (±9)	74	*Fopol*
Keratella tropica	169 (±36)	66 (±14)	67	*Ktrop*
Brachionus mirus	173 (±24)	81 (±11)	60	*Bmirus*
Brachionus caudatus	229 (±36)	123 (±19)	10	
Synchaeta pectinata	253 (±60)	170 (±40)	22	*Spect*
Kellicottia bostoniensis	279 (±35)	44 (±6)	21	*Kbost*
Brachionus dorcas	314 (±37)	206 (±24)	29	*Bdorc*
Brachionus falcatus	319 (±51)	119 (±19)	51	*Bfalc*

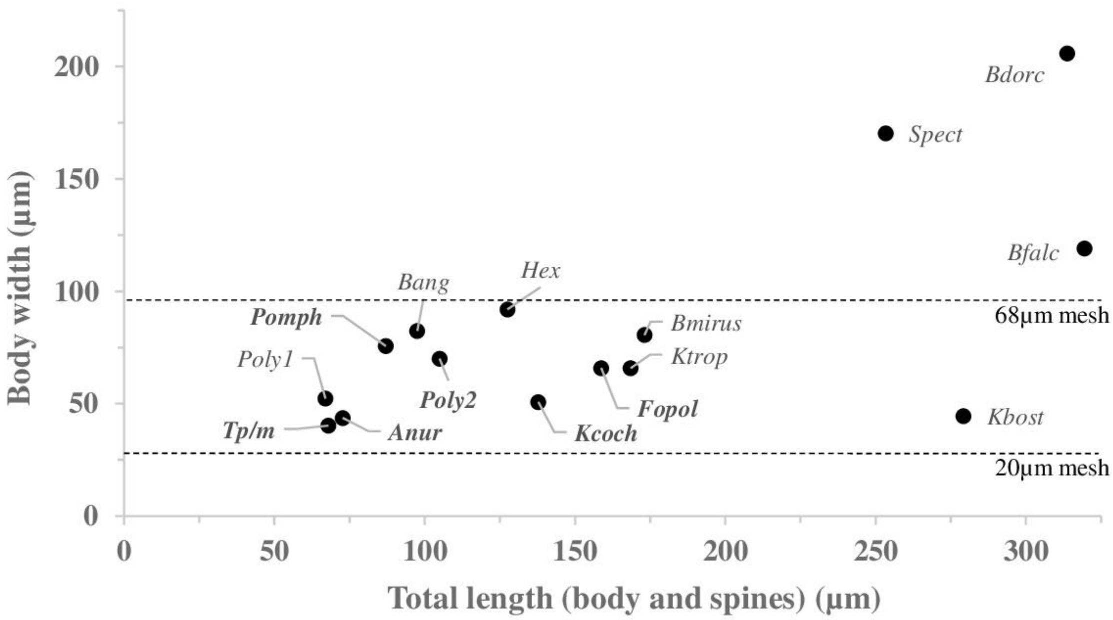

Figure 4. Relationship between mean total lengths (body and spines) and widths (µm) of the rotifer taxa encountered in the reservoir. The dashed lines indicate the calculated diagonal distances between knots for the two mesh sizes examined. The abbreviations for the taxa are shown in Table I. The taxa shown in Figure 1 are indicated in bold.

Gilbert 1985); three large taxa (*Brachionus dorcas*, *B. falcatus* and *Synchaeta pectinata*) were recorded here (Fig. 4). Small-bodied taxa also seem to be favoured in rivers and in tropical environments (Duncan 1983, Chick *et al.* 2010, Rocha *et al.* 2021). All five underestimated taxa (Figures 1 and 4, Table I) had lengths ≥68µm; thus, mesh size of 68µm does not signify that organisms of greater than 68µm in length are retained, as organisms can pass through the mesh in a longitudinal orientation (Pinto Coelho 2004). Body width would be more important than length, in relation to the diagonal distance between knots of the net meshes (calculated as 96µm for the 68µm mesh and 28µm for the 20µm mesh) (Ejsmont-

Karabin 1978), in determining retention efficiency for dorso-ventrally flattened taxa (Rocha *et al.* 2021). For example, in the present study, *Keratella cochlearis* was underestimated, probably due to its narrow width, despite having a length greater than 96µm. For more cylindrical taxa, such as *Filinia* and *Trichocerca*, body width in relation to the lateral distance between knots (namely 20 and 68µm) might be most important. *Filinia*, with a diameter close to 68µm, was not underestimated by the latter mesh size. Of the nineteen taxa examined, only four had body widths (considerably) greater than 96µm (*Brachionus caudatus*, *B. dorcas*, *B. falcatus* and *Synchaeta pectinata*), and thus not expected to be underestimated by the 68µm mesh net. All taxa had body widths greater than 28µm and thus expected to be retained efficiently by the 20µm mesh net.

Besides the importance of body size, other aspects of taxon morphology could be important. The setae of *Filinia* and arms or protuberances (as in *Hexarthra*) might increase retention efficiency. Additionally, distortion/contraction of body shape, especially of illoricate taxa, during filtration could decrease retention efficiency, while organisms can adhere to the netting or be destroyed during filtration and thus not backwashed into the sample (Likens & Gilbert 1970, Ruttner-Kolisko 1977, Ejsmont-Karabin 1978, James 1991).

While the efficiency of zooplankton sampling is affected by the number and spatial distribution of samples, type of sampling equipment and methods of preservation and enumeration (Bottrell *et al.* 1976, Ruttner-Kolisko 1977, de Bernardi 1984, McCauley 1984, Nie & Vijverberg 1985, James 1991, Wetzel & Likens 1991, Pinto Coelho 2004, Mack *et al.* 2012, Suthers *et al.* 2019, Appel *et al.* 2020), the role of net mesh size must be considered of baseline importance. Figure 4 can be considered as a general indication of sampling efficiency based on body size, dependent on mesh size, and could perhaps be extrapolated to evaluate the efficiency of other mesh sizes not analyzed here (Rodrıguez *et al.* 2013). I emphasize that knowledge of the body size of the taxa under study is of importance not only with regard to analysing ecosystem structure and function (Gianuca *et al.* 2016., Hébert *et al.* 2017, Kwong & Pakhomov 2021), but also when choosing sampling methodology. Thus, for example, one could recommend total unfiltered samples for small rotifers (Orcutt & Pace 1984, James 1991), 20µm mesh for most rotifers and copepod nauplii (Chick *et al.* 2010, Thomas *et al.* 2017, Rocha *et al.* 2021, this study) and ≥55µm for other crustaceans (James 1991, Chick *et al.* 2010, Thomas *et al.* 2017). Waters with elevated concentrations of suspended solids, as in eutrophic systems, might incentivize the use of mesh sizes larger than 20µm, because of clogging (Ejsmont-Karabin 1978, Shiel *et al.* 1982, Evans & Sell 1985, Mack *et al.* 2012). However, while this might permit the filtering of larger volumes of water, and thus sufficient numbers of organisms to obtain low sample coefficients of variation, I show here that the correct characterization of the community structure, skewed toward larger organisms, will be compromised.

Ethical statement

The present investigation did not involve the use of regulated animals and did not require approval by an ethical Committee

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Received: August 2022
Accepted: December 2022
Published: April 2023