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A note on the differences found between examining whole water vs. phytoplankton net (52 µm mesh) samples to characterize abundance and community composition of tintinnid ciliates (marine microzooplankton)

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Abstract

Most tintinnid species have a shortest linear dimension < 50 µm. Hence, *a priori*, nets of mesh sizes ≥ 50 µm will likely under-sample most tintinnid species. However, studies often appear (23 since 2015) using sampling with nets of meshes sizes ≥ 50 µm, reporting both tintinnid concentrations, and community composition. How biased are results from using coarse mesh nets? We provide a comparison of whole water vs. net sampling based on fortuitous, i.e. unplanned, parallel sampling. Pairs of samples from a standard monitoring station in the Bay of Villefranche (N.W. Mediterranean Sea) taken on 44 dates from 2013 to 2018 were compared. Tintinnids were enumerated in settled material from a water column sample, an integration of 6 discrete depth samples between 5 and 70 m, prepared for analysis of phytoplankton composition and in material from a plankton net (52 µm mesh) tow from 70 - 0 m, taken the same day. Despite the large confidence limits due to low raw cell counts from whole water samples, cell concentration estimates were about an order of magnitude higher than those from plankton net samples and frequently biomass estimates as well. Community composition also differed. The most common species in whole water

samples were small (diam. ≤ 20 µm), and some common forms were absent, or nearly, from the net samples. We show that, while valuable for collecting larger and rarer species, coarse net samples do not yield robust estimations of overall concentrations, nor allow identification of the dominant tintinnid species.

Introduction

Charles Kofoid was apparently the first to state that plankton nets inevitably fail to retain a certain portion of the plankton assemblage (Kofoid 1897). A few years later Lohmann showed the large differences in apparent concentrations of a wide variety of relatively small plankters sampled with a 'fine net' compared to an apparatus using filter paper (Lohmann 1903). Thus, early on, the axiom of plankton research, "no plankton sampler or combination of plankton samplers, can provide a true estimate of abundance for all components of the plankton at any one time" (Owens et al. 2013) was established. In recognition of the fact that plankton nets do not retain most soft-bodied ciliates and dinoflagellates, for microzooplankton studies, the examination of settled material from whole water samples is typically recommended; the exception is for protists with shells or skeletons such as many rhizaria taxa or tintinnid ciliates for which net sampling may be employed (e.g., Gifford & Caron 2000).

In contrast to 'naked ciliates', tintinnid ciliates can be collected using a plankton net because they are characterized by having a relatively robust lorica or shell, more or less species-specific, into which the ciliate cell can contract. The lorica is typically tubular or vase-shaped. Lorica dimensions vary widely among species with lengths ranging from about 25 to over 500 µm. The lorica oral opening, usually equivalent to the diameter of the lorica, ranges from 7 to over 130 µm depending on the species. Within this very large range of lorica dimensions there are central tendencies. The overwhelming majority of tintinnid species have loricas between 50 and 120 µm in length and 20 to 70 µm in diameter (Dolan 2010). Alder (1999) remarked that given the small sizes of many forms, nets of mesh size of 20-30 µm were needed. Pierce & Turner (1994) found that samples gathered with a 20 µm net yielded abundance estimates as high as those based on examination of material from whole water, suggesting that a 20 µm mesh net catches most tintinnids. Oddly enough, in the literature

there are no recommendations concerning appropriate plankton net mesh sizes for collecting tintinnids other than those of Alder (1999).

In contrast, qualitative and quantitative variability in catch with plankton net mesh size have been specifically studied with regard to copepods and to a lesser extent for rotifers. Likens and Gilbert (1970) found rotifer concentrations and estimations of average size to vary with net mesh aperture. Similarly, Dolan and Gallegos (1992) found that reported maximum concentrations of rotifers in estuarine systems were a function of the net mesh used for sampling. More recently, Chick et al. (2010) found that using a 20 µm net, rather than a commonly employed 63 µm net, yielded rotifer density estimates differing by 2-3 orders of magnitude. With regard to copepods, fairly precise recommendations of mesh sizes to be used depending on the size of the target species have been established (e.g. Sameoto et al. 2000). Plankton net mesh size needs to be less than 75% of the copepod carapace width to retain about 95% of the individuals; mesh sizes ≥ carapace width yield catch rates of 25 to 0 %, with catch rates rapidly decreasing with mesh size (Nichols & Thompson 1991). The steep curvilinear effects of mesh size on catch efficiency was confirmed by Hays (1994) working on calibration of the Continuous Plankton Recorder and more recently in comparisons of different plankton net systems (Skjoldal et al. 2013).

Applying the "copepod relationship" of mesh size and organismal width, to predict catch efficiency of 95% for the 'modal' tintinnid of 45 µm diameter (Dolan 2010, fig. 2), suggests a net mesh size of about 34 µm is needed to retain the majority of tintinnid species. However, many contemporary studies report tintinnid abundances and species composition from material gathered using nets of 52 - 70 µm mesh size, or larger. Application of the "copepod relationship" suggests that using a net of 52 µm mesh will severely under-sample tintinnid species with lorica oral diameters below about 60 µm, which represents about 70% of all tintinnid species (Dolan 2010, fig. 2). While studies relying on coarse net samples are not the majority of tintinnid studies, they do appear regularly. Since 2015, 23 such studies have been published (supplementary text file 1). These studies likely provide a biased view of tintinnid communities. However, the question remains "How biased? ". In this regard it is important to recall that studies carefully documenting species compositions based on whole water or

fine net sampling (20-30 µm mesh) have commonly report numerical dominance by small species, those with lorica oral diameters less than 40 µm (e.g. Verity 1987; Sanders 1987; Modigh & Castaldo2002; Sitran et al. 2007; 2008; Feng et al. 2018; Monti-Birkenmeier et al. 2019).

Here we report on the results of a comparison of samples taken with a 52 µm mesh plankton net with whole water samples. The comparison exploits fortuitous sampling done the same day on 44 dates at the same point but for different projects. For a phytoplankton taxonomic monitoring study, whole water samples from 6 depths were combined to create a single whole water sample to be examined for recording phytoplankton abundance and composition. The 52 µm net samples were taken to monitor the abundance of the large but relatively rare polymorphic tintinnid species *Cyttarocylis ampulla* (Dolan et al. 2014) Thus, the net and whole water sampling were not designed to compare methods. However, the processing of these particular pairs of samples was standardized, again fortuitously. All the samples were processed by the first author and involved complete examination of all material in an Utermöhl settling chamber, using the same inverted microscope. Thus, sample analysis involved identical expertise, equipment, and analysis. Based on the results of our comparison, we conclude that 52 µm net samples do not yield robust estimations of overall concentrations, nor biomass, nor allow identification of the numerically dominant tintinnid species. Consequently, reports of tintinnid concentrations and community composition based on coarse plankton net samples should be regarded as highly unlikely to be accurate descriptions of the entire tintinnid assemblages.

Methods

The sampling site is 'Point B' a standard oceanographic monitoring station near the entrance of the Bay of Villefranche (43°41'10"N, 7°19'003E), in the N.W. Mediterranean Sea. As part of the French National monitoring program SOMLIT, sampling is conducted weekly and a wide variety of parameters are measured. For the whole water samples considered here, discrete depth samples from 0, 10, 20, 30, 40, and 50 m were obtained using 5 l Niskin bottles. For phytoplankton analysis, a single composite integrated water column sample of 200 ml is made

from aliquots of the discrete depth samples, fixed with acid Lugol's (2 % final concentration), and stored refrigerated until analysis. Tintinnids were enumerated in material from 50 or 100 ml settled in an Utermöhl settling chamber following examination of the chamber for phytoplankton counts. Net samples were obtained with a "phytoplankton net", of a WP-2 design in overall dimensions (Tranter 1968, p154-155), fitted with a 52 μm mesh material (57 cm dia.) towed from 70 m to the surface. Assuming 100% filtration, the 1 liter cod end of net tow material is about 15 cubic meters. We acknowledge that the volume of seawater sampled may have been less as flow meters in the interior of the net were not employed to provide reliable estimates of the volumes filtered. Generally, aliquots of 1-3 ml (depending on detrital load) of net tow material, nominally representing material from 15 - 45 l, were examined in an Utermöhl chamber. All sample were examined using an Olympus inverted microscope, model IX51 equipped with DIC optics at 200x total magnification. Olympus Cell Sense Image Analysis software was used for lorica measurements and imaging. Tintinnid identifications were based on lorica morphology following Jörgensen (1924), Kofoid & Campbell (1929, 1939) and Balech (1959). Cell concentrations were transformed into biomass concentrations using the empirical conversion factor of Verity and Langdon (1984) for tintinnids relating lorica volume to carbon content (0.05 pg C per μm^3 lorica volume). The lorica volume for each species encountered was calculated as in Dolan 2010. Morphological categories of tintinnid species were created using size-classes of lorica oral diameter (LOD). Each species was assigned the average dimensions reported in Jörgensen (1924), Kofoid & Campbell (1929, 1939) or Balech (1959), or for species not described the reference works, the measured values. Size-class diameters of lorica oral diameters were binned over 4 μm intervals beginning with the overall smallest diameter encountered (13 μm) and continuing to the largest diameter encountered (136 μm). Empty lorica, which are unreliable indicators of the presence of living cells (Dolan & Yang 2017), were not enumerated. Pairs of samples were examined from 7 dates in the autumn of 2013, 12 dates in winter and spring 2014, 12 and 11 dates spread throughout the years of 2015 and 2016, respectively, and single dates in 2017 and 2018 (see supplementary data file 1).

Results and Discussion

Summary statistics of the tintinnid concentrations found in the two sets of samples are given in Table One. Average estimates of concentrations based on whole water samples were an order of magnitude greater than estimates from the net samples. However, a great deal of variability in concentrations, ranging over 3 orders of magnitude, is evident in both sets of estimates. Consequently, standard statistical tests of raw or log-transformed fail to distinguish the two data sets. However, plotting the pairs of whole water vs. net-based estimates shows that the whole water estimates were consistently at least an order of magnitude greater than the corresponding net sample based estimate for cell concentrations (Fig. 1A) but less often for biomass concentrations (Fig. 1B).

Table One. Summary statistics of the tintinnid concentrations and overall species richness in the 44 pairs of whole water and plankton net samples. The total number of cells enumerated in the whole water samples was 248 and for the net samples was 4408.

Sample Type	**Avg cells l^{-1} ± sd**	**Conc Range**	**Conc Geometric Mean**	**Avg # spp $sample^{-1}$ ±sd**	**# spp Range**	**# spp Geometric Mean**
Whole Water	75.9±64.15	0-367	54.5	3.6±1.71	0-7	3.3
52 µm Net	4.1±6.06	0.07-19.8	1.25	11.7 ±6.51	1-19	9.5

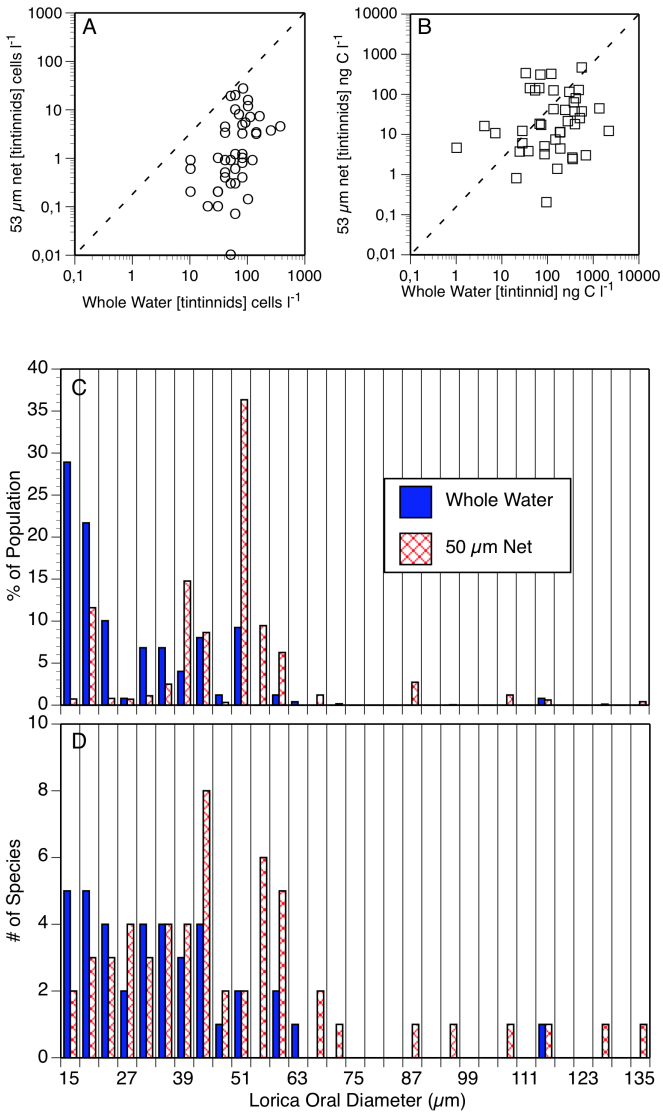

Figure 1. A and B show scatterplots of cell abundance and biomass concentration (in carbon units) estimates from whole water samples vs. material from net samples. A sample with 0 cells in the whole water sample and 0.2 cells l-1 in the net sample was omitted to allow plotting on a log-log scale. Dashed lines represent 1 to 10 relationships (order of magnitude). Note that all abundance estimates from whole water show an order of magnitude greater concentration than the net sample estimate. Biomass estimates from net samples were generally lower (33 of 43 dates) but often within an order of magnitude (23 of 43 dates) of whole water sample estimates. C shows the proportion of tintinnids found in size-classes of lorica oral diameter in whole water and net samples. All data from each set of samples was pooled (248 cells found in whole water samples, 4408 cells from net samples). Note that the majority of cells in the whole water samples were in small size classes, in contrast to the net sample population. D shows the number of species within each size-class of lorica oral diameter among the tintinnids found in whole water and in net sample material. Note the higher species

richness of the smaller size classes of the tintinnids found in whole water samples compared to those from the net material.

The tintinnid concentrations estimated from the whole water samples reported here match previously reported concentrations, from the same sampling site, in order of magnitude and variability, based on examinations of either whole water samples or samples concentrated using a 20 µm plankton concentrator. Gomez and Gorsky (2003) reported concentrations from whole water samples taken about weekly at Point B in 1998-1999 from 0 and 50 meters depth. Averaging the reported concentrations from 0 and 50 m depth (Fig. 3 in Gomez & Gorsky 2003), they found 32± 42.0 cells l^{-1}. Dolan et al. (2006) sampled Point B weekly in 2002; they reported an average concentration of 39±61.5 cells l^{-1} in integrated water column samples concentrated using a 20 µm concentrator. Thus, the estimates reported here concerning tintinnid abundances from whole water samples appear coherent with previous studies. Unfortunately, to our knowledge, there is no comparative data concerning the 52 µm net sampling. We can not exclude the possibility that net clogging may have occurred on some dates resulting in smaller volumes actually sampled compared to our assumed values. However, in our experience with the net used at the location sampled, clogging occurs relatively rarely with short-lived blooms of diatoms or salps. Likewise, although the WP 2 net design is presumably appropriate for a 52 µm mesh net, and performs similarly when fitted with larger (100-400 µm) mesh sizes (Skjoldal et al. 2013), as we did not employ a flow meter, we can not exclude the possibility that volumes sampled were less than assumed volumes.

We consistently found a larger number of species in the net samples compared to the whole water samples as suggested in the summary statistics (Table 1). The higher species diversity in the net samples reflects the relationship of sampling effort (volume sampled) and species richness known for the bay with regard to both tintinnids (Cariou et al. 1999) and certain dinoflagellate taxa (Tunin et al. 2007). As one would expect, the larger volumes sampled in the net samples yielded a larger number of species. However, the morphological characteristics of the species found in the different sets of samples differed considerably with smallest species usually numerically

dominant in the whole water samples. This is apparent in comparing the characteristics of the species pools from the two sampling methods shown in Figure 1C and 1D. In terms of the portions of the overall populations (Figure 1C), most of the tintinnids found in the whole water samples were those of small lorica oral diameter (≥20 μm). The tintinnids most commonly found in net samples were of considerably larger lorica oral diameter with the most abundant forms having oral diameters of about 50 μm. The small tintinnid forms representing dominant size-class in the whole water samples were nearly absent from the net samples. These small forms constituted 5 species in the whole water samples, only 2 of which were found in the net samples. The majority of species encountered in the whole water samples were forms with a lorica oral diameter ≤ 35 μm (20 of 38 spp) while these same size classes constituted a minority (15 out 57) of the species found in the net samples. The most common species from the whole water samples was *Salpingella faurei*, a species with lorica dimensions of 17 x 168 μm; among the net sample species it ranked far down the abundance rankings, 39 out of 57. The third most abundant species in the whole water samples, *Codonellopsis pusilla*, was absent from the net samples.

It should be noted that both of the small species mentioned above, under sampled or missing from the net material, are very common forms and nearly ubiquitous in the world ocean. For example, *S. faurei* is found in systems from Antarctica to the South Pacific and the high Arctic (Dolan et al. 2016a; 2016b; 2017). In sum, the whole water samples provided abundance estimates an order of magnitude greater than the estimates from 52 μm net material and indicated an assemblage of species dominated by forms with small lorica diameters. This finding is not surprising. As noted above, tintinnid assemblages appear to be usually dominated by small forms based on findings from a large range of coastal and open water systems investigated using whole water or fine net sampling (e.g. Verity 1987; Sanders 1987; Sitran et al. 2007; 2008; Dolan et al. 2007; Feng et al. 2018; Monti-Birkenmeier et al. 2019).

There is a lack of firm recommendations for microzooplankton sampling in both the older (see Tranter 1968, pp 150-152) and newer handbooks of zooplankton sampling (Gifford and Caron 2000) reflecting the fact that sampling needs to be adapted to the goal in mind. For example, a complete census of

species richness requires temporally intensive sampling over long periods of time while estimating average abundance can likely be derived from a few samples representative of different seasons. In terms of what sampling technique to be employed, whole water based or using some type of concentration step, varies largely due to practicality. Ideally material from large volumes (100 l) of whole water should be examined for determinations of tintinnid species richness. However, as extraneous matter in large volumes obscures visualization of individual cells, splitting and processing dozens of aliquots for each sample would be required. Thus some sort of concentration step is needed and we recommend use of 20 μm netting as a reasonable compromise between precision and accuracy, allowing processing of material from large volumes of water.

Interestingly, biomass estimates from net samples were generally lower (33 of 43 dates) but often within an order of magnitude (23 of 43 dates) of whole water sample estimates (Fig. 1B). Thus, if only "order of magnitude" tintinnid biomass estimates are needed, a 53 μm mesh net material may be adequate. Furthermore, net sampling allows detection of the larger species present in low concentrations.

Conclusions

Coarse nets (≥52 μm mesh size) can severely under sample tintinnids providing biased estimates of abundance, biomass, and community composition. While there may be instances in which a tintinnid assemblage is overwhelming dominated by large forms, those theoretically well-sampled by coarse nets, we know of no reports documenting such an assemblage. Based on the data reported here we urge that characterizations of tintinnid abundance and community composition based on coarse net sampling be viewed with considerable skepticism.

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Conflict of Interest

None declared.