

## Comparing taxonomic and morphological biodiversity of tintinnids (planktonic ciliates) of New Caledonia

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### *Abstract*

Tintinnid ciliates are planktonic grazers of nanoplankton. They have a lorica (or shell) into which the ciliate cell can withdraw. The lorica provides information on both the identity and the ecology of the organism because characteristics of the lorica distinguish species and the diameter of the oral opening is related to the size of prey ingested. We examined the relationship between biodiversity estimates on the basis of classifying specimens as belonging to a species or a simple morphological group defined by lorica oral diameter (LOD) in a presumably species-rich area, a tropical lagoon. Two sites were sampled in the lagoon off Nouméa over an annual cycle. The tintinnid fauna was species-rich (76 species) and represented a relatively even distribution of LOD sizes compared to other tropical and temperate sites. Median LOD varied with the fraction of the chlorophyll concentration by  $>10 \mu\text{m}$ . Total chlorophyll concentration was related to tintinnid concentration and, in turn, weakly correlated with numbers of species and LOD size-classes. Numbers of species were closely related to numbers of LOD size-classes as were  $H'$  of species and  $H'$  (Shannon index) of LOD size-classes. Thus, metrics of a morphological characteristic, related to the ecology of the organisms, can be used to estimate species diversity.

With the exception of prokaryotes, organisms are distinguished from each other largely on the basis of morphology. Thus, biodiversity, generally taken to mean taxonomic diversity, should be related to morphological diversity in some manner. However, the relationship of taxonomic diversity (species, genera, family, etc.) and morphological diversity has received little attention with regard to planktonic organisms. Most studies have concerned benthic organisms and considered very large-scale, either temporal or spatial patterns. Among fossil taxa, morphological diversity, presumed to represent ecological diversity, appears to be poorly related to taxonomic diversity in terms of the appearance of high-level taxa (e.g., Foote 1997). Among extant mollusk taxa, morphological diversity also appears only weakly related to taxonomic diversity across large geographic scales (e.g., McClain et al. 2004). Tintinnid taxonomic diversity has been related to morphological diversity and phytoplankton size-

diversity across medium spatial scales (Dolan et al. 2002). However, to our knowledge, the relationship between morphological and taxonomic diversity has not been examined on medium temporal scales (weeks to months).

The general question we pose is: For a group of organisms (regardless of its definition as a taxonomic unit, a guild, a trophic level, etc.) is there a characteristic that can be directly related to taxonomic diversity in a given locality? If so, the identity of the characteristic can suggest the ecological mechanism governing taxonomic composition. For example, taxonomic diversity of a phytoplankton community may reflect physiological diversity in terms of nutrient demands or, alternatively, in terms of light-harvesting capabilities. Distinguishing between the two possibilities could allow the identification of either nutrient dynamics or the light field as a factor regulating taxonomic diversity of the phytoplankton.

In this report we summarize our investigation of morphological and taxonomic diversity in a group of planktonic herbivores, tintinnid ciliates. They are part of the microzooplankton, grazers on primarily autotrophic and heterotrophic nanoplankton. Occasionally, the feeding activity of tintinnids can dominate the consumption of phytoplankton (e.g., Karayanni et al. 2005), but they are generally a minority component of the microzooplankton compared to oligotrich ciliates or heterotrophic dinoflagellates. Nonetheless, tintinnids are much more abundant than foraminifera or radiolarians. Compared to foraminifera or radiolarians, their biogeography has received much less attention. Few generalities are known

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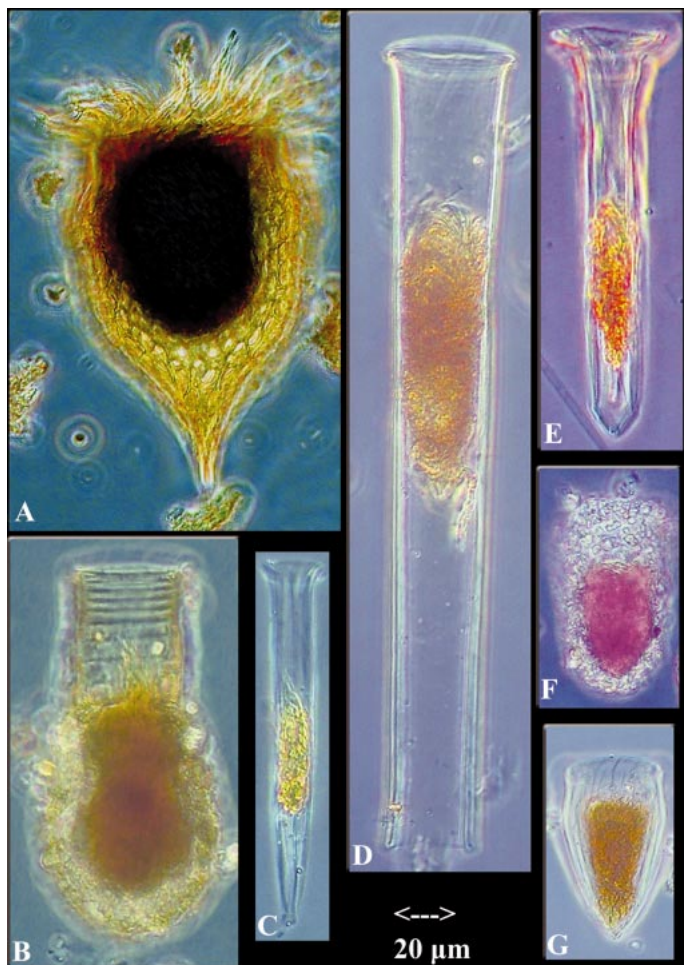


Fig. 1. Examples of tintinnid morphology. Note that although all loricas are basically tube- or vase-shaped, different species show distinct lorica architecture. Examples are species common in samples from New Caledonia: (A) *Epillocyclus brandti*, (B) *Codonellopsis morchella*, (C) *Salpingella faurei*, (D) *Eutintinnus lusus-undae*, (E) *Steenstrupiella steenstrupii*, (F) *Tintinnopsis beroidea*, (G) *Protorhabdonella curta*.

besides the fact that tintinnids supply a textbook example of the latitudinal species gradient (e.g., see fig. 3.14a in Gaston and Spicer 2003) and that high-latitude communities are distinct (e.g., Wasik 1998)

Tintinnids are ubiquitous in marine systems and a very speciose group. The standard taxonomic monographs of Kofoid and Campbell (1929, 1939) catalogued more than 700 species. Identifications were made on the basis of characteristics of the lorica or shell into which the ciliate cell can withdraw. The lorica, formed of one or more layers, is composed primarily of proteinaceous material. Although the general shape of a lorica is a tube or vase, tintinnids can show very distinct lorica morphologies (Fig. 1). Like foraminifera and radiolarians, characteristics of gross morphology define species despite the fact that some genera are known to display plasticity in lorica morphology (e.g., Williams et al. 1994).

Tintinnids, in contrast to other groups of planktonic ciliates; for example, those known as oligotrichs, are a single

order even among competing ciliate classification schemes. According to traditional ciliate taxonomy, and in agreement with the molecular data available to date (Agatha et al. 2005), they are a monophyletic group. Thus tintinnids are a coherent group ecologically as microzooplankters, morphologically as loricate ciliates, and phylogenetically as members of the order Tintinnida.

Not surprisingly, in tintinnids the diameter of the mouth-end of the lorica, the lorica oral diameter (LOD), is related to the size of the food items ingested. For example, Heinbokel (1978) found that the largest prey ingested were about half the LOD in longest dimension for a variety of tintinnid species. Studies of seasonal trends have documented shifts in overall community averages of LOD corresponding with changes in phytoplankton composition (e.g., Verity 1987). A more precise relationship was established by Dolan et al. (2002) who found that tintinnids removed prey at maximum rates that were 25% of LOD in size, based on an analysis of data from 11 species. Because lorica characteristics are related to both ecology and taxonomy, we hypothesized that morphological diversity should correspond with taxonomic diversity in this group over seasonal time scales.

Here we report on the relationship between morphological diversity, in terms of LOD diversity, and species diversity of tintinnids in a tropical lagoon system. We found that taxonomic diversity and diversity of LOD were tightly related, suggesting that temporal changes in prey-size diversity regulated temporal changes in tintinnid species diversity. We found then that a morphology-based biodiversity measure, based on a character of ecological importance, can provide evidence of the ecological mechanism regulating biodiversity.

## Materials and methods

**Study site and sampling**—Sampling was conducted in the southwest lagoon of New Caledonia. The coral reef lagoon has an average depth of about 20 m and the water column is well-mixed. Southeast trade winds dominate hydrodynamics, giving average water residence times of about 10 d; chlorophyll *a* (Chl *a*) concentrations average about  $0.5 \mu\text{g L}^{-1}$  (Bujan et al. 2000). Samples were obtained from two stations in the lagoon, N12 (14-m depth) near the city of Nouméa and M33 (24-m depth) near the middle of the lagoon (Fig. 2) at 7- to 28-d intervals from April 2002 to March 2003. Niskin bottles were used to sample five depths in the water column at each station (3, 7.2, 11.5, 15.8, and 20 m for M33; 3, 4.7, 6.5, 8.2, and 10 m for N12). Samples were kept in Niskin bottles until pooled to produce a water column composite sample at the laboratory within 1.5 h after sample collection. The water column composite sample was subsampled for chlorophyll analysis and tintinnid ciliate determinations.

**Sample processing**—A 2-liter sample of the composite water column sample was fixed with Lugol's (2% final concentration), sedimented in a graduated cylinder, and the concentrated sample was examined in a sedimentation chamber using an inverted microscope at  $\times 200$ . Tintinnid species identifications were made at  $\times 400$  and made largely on the

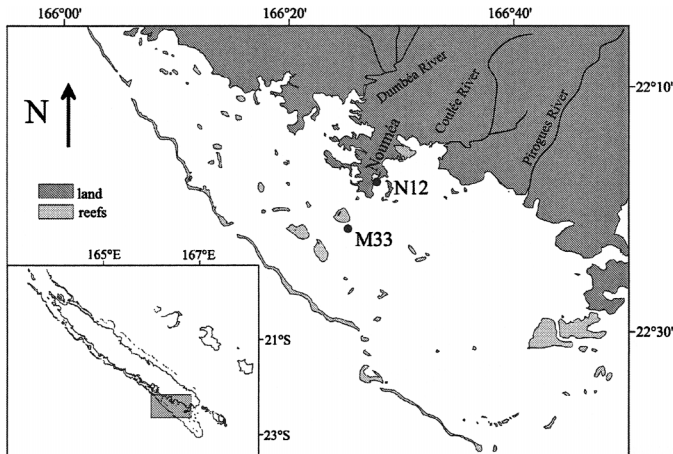


Fig. 2. Map of station locations in New Caledonia.

basis of descriptions by Kofoed and Campbell (1929, 1939), Campbell (1942), and Marshall (1969). Other works consulted for species descriptions included Campbell (1942), Hada (1938), Jorgensen (1924), and Rampi (1950).

A 'lumping' approach to identification was employed: any intermediate forms or slight variants were pooled with the morphologically closest and most numerous species. For example, as intermediate forms were observed, all varieties of *Dadayiella ganymedes* (*D. trachelium*, *D. bulbosa*, and *D. acuta*) were pooled; a single type was established for forms ranging from *Tintinnopsis parva* to *T. rapa*, hence the designation in Table 1 of *T. rapa-parva*. Forms intermediate between *Climacocylis scalaria* and *C. scalaroides* were observed so, and *C. scalaroides* forms were designated *C. scalaria*. All empty lorica were ignored.

Taxonomic diversity was estimated for each sample as the Shannon index (ln-based) and species richness. Morphological diversity was estimated by placing species into size-classes of LOD. Each species was assigned the average dimensions reported by Kofoed and Campbell (1929, 1939), Campbell (1942), and Marshall (1969). Size-class diameters were binned over 4- $\mu$ m intervals beginning with the overall smallest diameter (12  $\mu$ m) and continuing to the largest diameter encountered in a given sample. For each sample, the median LOD was identified and morphological diversity was estimated as the number of size-classes occupied and a Shannon index of morphological diversity was calculated using numbers and proportional importance of different size-classes (ln-based).

The overall diversity encountered was compared with that found in seasonal studies of other systems, which employed a method comparable to the method we used: examining large volumes (liters) of water or using fine-mesh nets (20  $\mu$ m). These studies provided lists of species found over annual cycles. The systems compared included temperate coastal waters of Japan (Kamiyama and Tsujino 1996), the northwest Mediterranean (Modigh and Casaldo 2002), the northwestern Atlantic—Buzzards Bay (Pierce and Turner 1994), Narragansett Bay (Verity 1987) and the Damariscotta Estuary (Sanders 1987), the northeastern Atlantic—the Nervion Estuary, and the southwestern Atlantic—the Bahia

Table 1. Tintinnid species encountered in samples from New Caledonia.\*

<i>Acanthostomella</i> c	<i>Epilocyliis</i> c	<i>Rhabdonella</i> w
<i>A. conicoides</i>	<i>E. acuta</i>	<i>R. amour</i>
<i>A. obtusa</i>	<i>E. acuminata</i>	<i>R. elegans</i>
<i>Amphorella</i> c	<i>E. brandti</i>	<i>R. poculum</i>
<i>A. quadrilineata</i>	<i>E. undella</i>	<i>Salpingella</i> c
<i>Amphorellopsis</i> c	<i>Eutintinnus</i> c	<i>S. acuminata</i>
<i>A. tetragona</i>	<i>E. apertus</i>	<i>S. curta</i>
<i>Canthariella</i> w	<i>E. elegans</i>	<i>S. decurtata</i>
<i>C. pyramidata</i>	<i>E. elongatus</i>	<i>S. faurei</i>
<i>Climacocylis</i> w	<i>E. fraknoii</i>	<i>Steenstrupiella</i> c
<i>C. scalaria</i>	<i>E. tubulosus</i>	<i>S. steenstrupii</i>
<i>Codonellopsis</i> c	<i>E. lusus undae</i>	<i>S. gracilus</i>
<i>C. inornata</i>	<i>E. macilentus</i>	<i>Stenosemella</i> n
<i>C. lagenula</i>	<i>E. rectus</i>	<i>S. monacense</i>
<i>C. lusitanica</i>	<i>E. sp A</i>	<i>S. nivalis</i>
<i>C. morchella</i>	<i>Favella</i> n	<i>Tintinnidium</i> n
<i>C. orthocerca</i>	<i>F. azorica</i>	<i>T. sp A</i>
<i>Coxiella</i> c	<i>F. composita</i>	<i>Tintinnopsis</i> n
<i>C. ampla</i>	<i>F. ehrenbergii</i>	<i>T. acuminata</i>
<i>C. calyptra</i>	<i>Helicostomella</i> n	<i>T. beroidea</i>
<i>C. laciosa</i>	<i>H. fusiformis</i>	<i>T. brandti</i>
<i>C. pelagica</i>	<i>H. edentata</i>	<i>T. campanula</i>
<i>C. pseudoannulata</i>	<i>Metacylis</i> n	<i>T. compressa</i>
<i>Craterella</i> c	<i>M. mediterranea</i>	<i>T. everta</i>
<i>C. tortulata</i>	<i>M. mereschkowskii</i>	<i>T. platensis</i>
<i>Dadayiella</i> w	<i>M. jorgensenii</i>	<i>T. radix</i>
<i>D. ganymedes</i>	<i>M. sp A</i>	<i>T. "rapa-parva"</i>
<i>Dictyocysta</i> c	<i>M. sp B</i>	<i>T. tocantinensis</i>
<i>D. duplex</i>	<i>Odontophorella</i> w	<i>T. turbo</i>
<i>D. lepida</i>	<i>O. sp. A</i>	<i>T. urnula</i>
<i>D. mitra</i>	<i>Protorhabdonella</i> c	<i>Undella</i> c
<i>D. occidentalis</i>	<i>P. curta</i>	<i>U. cleveii</i>
<i>D. pacifica</i>	<i>Proplectella</i> w	<i>U. sp. A</i>
	<i>P. perpusilla</i>	<i>Xystonella</i> w
		<i>X. longicaudata</i>

\* Genera noted as c = cosmopolitan, w = warm, or n = neritic, following the classification of Pierce and Turner 1993.

Blanca Estuary (Barria de Cao 1992). Data from two tropical systems were included: the coastal Caribbean waters of Jamaica (Gilron et al. 1991) and tropical estuarine and mangrove waters on the southeastern coast of India (Godhantaraman 2002). Systems were compared in terms of numbers of species and numbers of lorica size-classes. Each reported species was assigned the average dimensions reported by Kofoed and Campbell (1929, 1939), Campbell (1942), and Marshall (1969) unless data were provided in the report (e.g., Verity 1987). Consequently, unidentified species were ignored and such species (generally about 10% of the species numbers) may have occupied unique LOD size-classes in the assembly. For each system LOD frequency distribution was plotted.

Chlorophyll concentrations were estimated in three fractions for each sample: whole water, water filtered through a 10- $\mu$ m Nucleopore filter, and water filtered through a 2- $\mu$ m Nucleopore filter. For each fraction, replicate samples of 300–500 mL were filtered using Whatman GF/F filter. Material retained on the filters was extracted using methanol, and Chl *a* concentrations were determined fluorometrically (Holm-Hansen et al. 1965). The size-fractionated Chl *a* con-

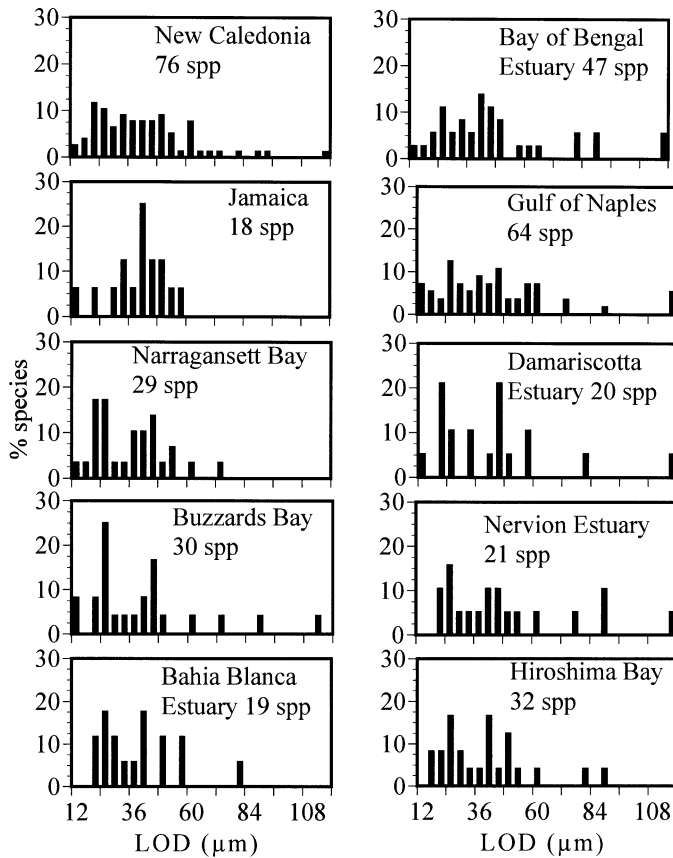


Fig. 3. Frequency distribution of LOD found in the pool of tintinnid species from the lagoon of New Caledonia compared with species pools reported in seasonal studies of tropical and temperate systems. Data for the coastal waters of the Bay of Bengal from Godhantaraman (2004), Jamaica from Gilron et al. (1991), Gulf of Naples from Modigh and Casaldo (2002), Narragansett Bay from Verity (1987), Damariscotta Estuary from Sanders (1987), Buzzards Bay from Pierce and Turner (1994), Nervion Estuary from Urrutxurtu (2004), Bahia Blanca Estuary from Barria de Cao (1992), and Hiroshima Bay from Kamiyama and Tsunjinjo (1996).

centrations ( $<2 \mu\text{m}$ ,  $2\text{--}10 \mu\text{m}$ , and  $>10 \mu\text{m}$ ) were transformed into percentages of total chlorophyll and then used to estimate an index of the size-diversity of chlorophyll (Shannon index, ln-based). Statistical relationships between diversity and concentration estimates were examined using nonparametric Spearman rank correlation.

## Results

*General characteristics of New Caledonia tintinnids*—A total of 76 species in 27 genera were found (Table 1). A complete listing of the abundance by date of each species, along with the lorica dimensions used for each species, is available on request from the corresponding author. The taxonomic richness found represents about 13% of the species and 50% of the genera in a 'global' tintinnid data set composed of the species cataloged in the monographs of Kofoid and Campbell (1929, 1939), Campbell (1942), and Marshall (1969). Following the distributional classification of genera

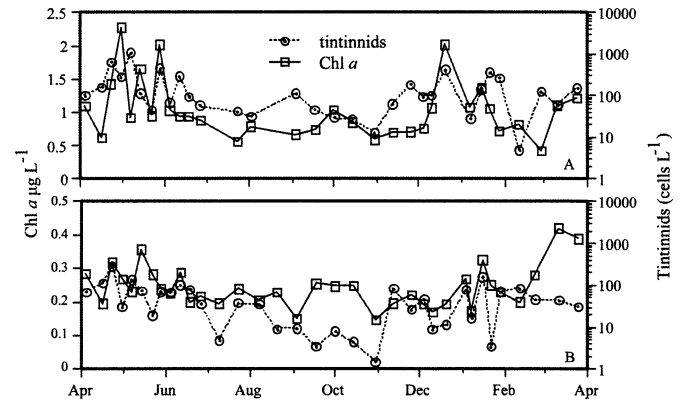


Fig. 4. Temporal changes in the concentrations of Chl *a* and tintinnids between April 2002 and March 2003. (A) Data from the nearshore Sta. N12 and (B) data from the midlagoon Sta. M33. Statistical relationships are given in Table 2.

proposed by Pierce and Turner (1993), most of the New Caledonia species (43) belong to cosmopolitan genera with the remainder species of either neritic (25) or warm-water genera (9). Thus, the tintinnid fauna of New Caledonia is not dominated taxonomically by tropical forms.

Compared to other systems, the tropical tintinnid fauna of New Caledonia appears rich in both numbers of species and size-classes of LOD (Fig. 3). Comparing communities found over an annual cycle, tropical systems do not necessarily show higher species or morphological diversity. Whereas the tintinnid community found in coastal waters of India appears to be more diverse than those of the temperate communities of the Atlantic and Pacific (47 vs. 20–32 species), Jamaican waters harbored relatively few species (18), representing only 9 size-classes of LOD. Thus, the diversity found New Caledonia waters can not be attributed to latitude alone. In contrast to the disparity found among tropical systems, temperate coastal communities appear remarkably consistent. Excluding the northwestern Mediterranean, which is arguably not a typical temperate site, the systems from the northern Pacific to three coasts of the Atlantic ranged narrowly in numbers of species (19–30) and numbers of LOD size-classes (9–13).

*Temporal trends*—At Sta. N12, chlorophyll averaged about  $1 \mu\text{g L}^{-1}$ , and tintinnids averaged  $250 \text{ cell L}^{-1}$ . The midlagoon station showed lower average concentrations of both chlorophyll ( $0.25 \mu\text{g L}^{-1}$ ) and tintinnids ( $50 \text{ cells L}^{-1}$ ). Tintinnid and total chlorophyll concentrations covaried loosely at both the nearshore Sta. N12 and the midlagoon Sta. M33 (Fig. 4). Chlorophyll and tintinnid concentrations were highest during the austral autumn and lowest in the austral spring period when water temperatures are lowest ( $21\text{--}22^\circ\text{C}$ ). Pooling data from the two stations, chlorophyll and tintinnid concentrations were significantly correlated (Table 2).

A morphological characteristic of the tintinnid community can be related to a chlorophyll size parameter: the percentage greater than  $10 \mu\text{m}$ . Shifts in the proportion of chlorophyll greater than  $10 \mu\text{m}$ , which did not appear to follow any seasonal rhythm, were generally followed by shifts in the

Table 2. Spearman rank correlation coefficients (nonparametric) of pooled data (n = 64).\*

	Chl a	Chl a >10 μm	Chl size H'	Tin conc.	Median LOD	No. of tin spp.	Tin spp. H'	No. of tin LODs	tin LOD H'
Chl a		0.785***	0.352**	0.543***	0.364**	0.410***	0.214	0.400***	0.103
% Chl a >10 μm			0.432***	0.396***	0.334**	0.314*	0.129	0.270*	0.044
Chl size H'				0.193	0.111	-0.046	-0.195	0.035	-0.172
Tin concentration					0.079	0.814***	0.448***	0.746***	0.273*
Median LOD						0.350	0.118	0.212	0.175
No. of tin spp.							0.762***	0.833***	0.834***
tin spp H'								0.608***	0.570***
No. of tin LODs									0.596***
tin LOD H'									

\* Tin = tintinnid ids. \* p < 0.05, \*\* p < 0.005, \*\*\* p < 0.0005.

median size of tintinnid LOD (Fig. 5). The percentage of chlorophyll greater than 10 μm was, on average, much higher in the nearshore station (about 40%) compared with that of the midlagoon station (about 20%). These differences corresponded to differences in median LOD values between the two stations, as the nearshore community averaged about 40 μm, and the midlagoon community averaged around 30 μm. Pooling data from both stations, the percentage of chlorophyll >10 μm was significantly correlated with the median LOD of the tintinnid community (Table 2).

Temporal changes in metrics of tintinnid diversity closely tracked one another. The numbers of species oscillated with the numbers of LOD size-classes found (Fig. 6). Both were highest during the austral autumn and lowest in the austral spring when both chlorophyll and tintinnid concentrations were minimal. An especially close association was found between H' values of species and LOD diversity (Fig. 7). Not surprisingly then, diversity metrics were strongly correlated with each other and to a lesser degree with tintinnid concentration (Table 2).

Scatter plots showed that taxonomic and morphological diversity measures were not related to each other in the same fashion, corresponding to the differences observed in tem-

poral patterns. For the two closest relationships, numbers of species and LOD size classes displayed a curvilinear relationship, while H' of species and H' of LOD size-classes showed a near linear relationship (Fig. 8).

Discussion

Tintinnid abundance in the lagoon was similar to that of other systems, given the concentration of Chl a. The average concentrations of 10<sup>1</sup>-10<sup>2</sup> tintinnid cells L<sup>-1</sup> corresponded with Chl a concentrations ranging from 0.2 to 2 μg L<sup>-1</sup>, which is similar to reports for the southwestern Atlantic, (Thompson and Alder 2005), the Mediterranean (Dolan 2000), and Indian Ocean (Modigh et al. 2003). Although the abundance of tintinnids appeared predictable given chlorophyll concentration, the community composition was not.

Taxonomically, the assemblage did not appear to be a predictable assemblage simply from the location of New Caledonia. Species of tropical or warm-water genera were not dominant, but rather, species of cosmopolitan or neritic genera were. *Tintinnopsis* and *Eutintinnus* species dominated samples from the nearshore and midlagoon stations, respectively (Table 1). Species of these genera are common in both temperate and tropical coastal systems, ranging from both

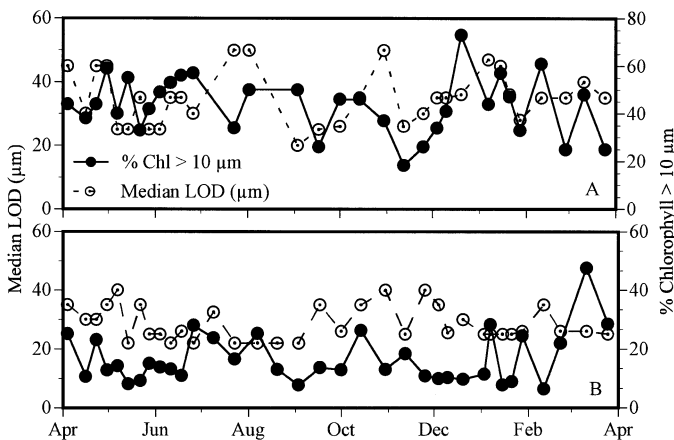


Fig. 5. Temporal changes in the relative importance of the larger fraction of chlorophyll greater than 10 μm and the median LOD of the tintinnid community. (A) Data from the nearshore Sta. N12 and (B) data from the midlagoon Sta. M33. Statistical relationships are given in Table 2.

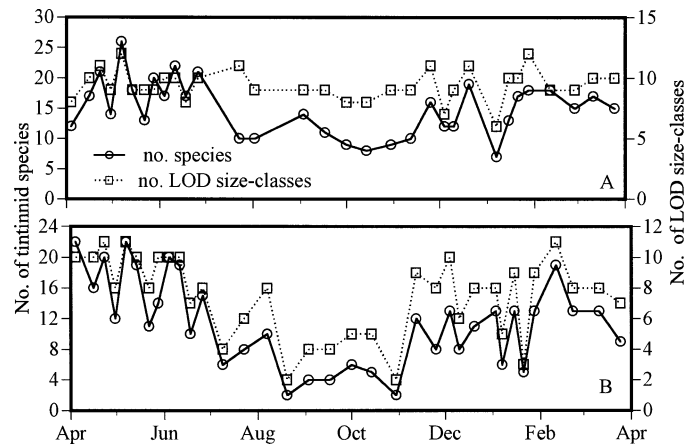


Fig. 6. Temporal changes in the numbers of species and numbers of LOD grouped in 4-μm size-classes. (A) Data from the nearshore Sta. N12 and (B) data from the midlagoon Sta. M33. Statistical relationships are given in Table 2.

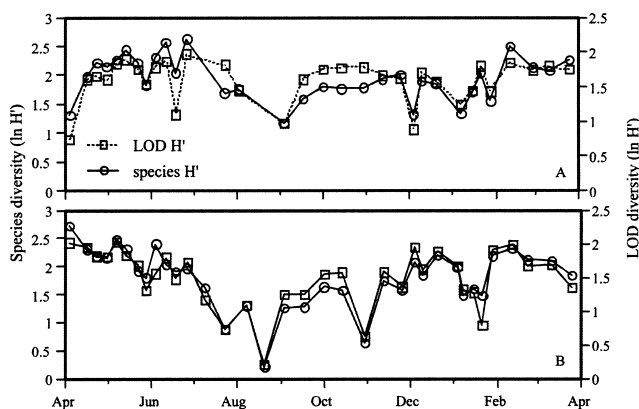


Fig. 7. Temporal changes in species diversity and diversity of LOD grouped in 4- $\mu\text{m}$  size-classes; diversity is estimated as the Shannon index,  $H'$ . (A) Data from the nearshore Sta. N12 and (B) data from the midlagoon Sta. M33. Statistical relationships are given in Table 2.

sides of the North Atlantic (Sanders 1987; Verity 1987; Ur-rutxurtu 2004), to Jamaica (Gilron et al. 1991), India (Godhantaraman 2002), and the northwestern Mediterranean Sea (Cariou et al. 1999; Modigh and Castalado 2002). An explanation might be that the relatively shallow nature of a lagoon environment favors species of these genera, which are known to form benthic cysts.

In taxonomic terms there did not appear to be a well-defined tintinnid assemblage; that is, a tintinnid 'fingerprint' (i.e., Modigh et al. 2003) for the lagoon of New Caledonia. However, comparing the frequency distributions of LOD among various systems (Fig. 3), the New Caledonia community appears distinct in that most of the LOD size-classes between 10 and 100  $\mu\text{m}$  were occupied.

The temperate communities of tintinnids, ranging across three coasts of the Atlantic and the North Pacific, were quite similar to each other in both the low number (10–13) and wide dispersion (20–120  $\mu\text{m}$ ) of LOD size-classes. Most of the temperate communities were dominated by two LOD size-classes; one around 20  $\mu\text{m}$  and another around 40  $\mu\text{m}$ . The two peaks in species-richness are likely related to seasonal changes in the size-spectrum of the phytoplankton community. Many temperate systems are characterized by the occurrence of two temporally and compositionally distinct peaks of phytoplankton concentration.

Field studies in distinct systems, both temperate and tropical, have shown that the tintinnid community characteristic of average LOD changes seasonally with the size spectrum of prey (Middlebrook et al. 1987; Verity 1987; Gilron et al. 1991). Laboratory studies of tintinnids established that LOD determines the maximum size of food particle ingested (Heinbokel 1978), and for ciliates in general with oral cilia, the minimum size of prey is likely related to the distance between the membranelles of the oral cilia (Fenchel 1987). Reviewing data on 11 tintinnid species, Dolan et al. (2002) found that LOD was about 4 times the size of food items fed on most efficiently, in terms of clearance rate. Given these relationships, it is not surprising that in the lagoon of Nouméa, the most common LOD size-class was 20–24  $\mu\text{m}$ , as most of the chlorophyll stock was <10  $\mu\text{m}$  in size. Nor

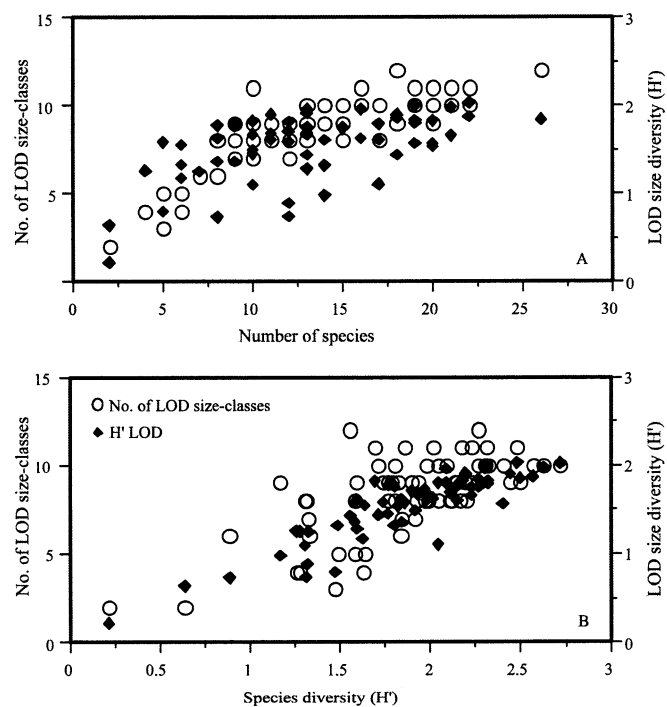


Fig. 8. Scatterplots of the relationships between taxonomic and morphological metrics of the biodiversity of New Caledonia tintinnids. (A) Species richness versus numbers of LOD size-classes and  $H'$  of LOD size-class diversity. (B) Relationships between  $H'$  of species diversity and LOD size-class  $H'$  and the number of LOD size-classes. Note the curvilinear relationship of species and LOD size-class richness compared to the near linear relationship of species and LOD  $H'$ .

is it surprising that a measure of community morphology, the community median LOD, varied with the proportion of chlorophyll >10  $\mu\text{m}$  in size (Fig. 5). Verity (1987) previously noted a similar relationship for Narragansett Bay tintinnids. Such observations support the idea put forward by Admiraal and Venekamp (1986) that community LOD is controlled by the size spectrum of phytoplankton prey items.

The surprising finding in our data was not that species diversity was related to LOD diversity, but rather to the strength of the relationship. The number of species found was reflected in the number of LOD size-classes present in a curvilinear manner (Fig. 8). When species abundance was low (<10 species), nearly each species represented a unique size-class; at higher species abundance, a size-class was occupied by up to five species. Biodiversity measured as  $H'$  values, indicating both the number of different species and their relative abundance, were nearly equivalent to LOD  $H'$  values (Figs. 7 and 8). This reflects the fact that a given LOD size-class, when composed of several species, was often dominated by a single species.

The close match of diversity metrics based on LOD or species suggests that in tintinnids, LOD data can be employed as proxy measure of diversity. We employed 4- $\mu\text{m}$  size-classes as the finest distinction possible and reasonable. LOD appears to be about four times the size of prey most efficiently filtered (Dolan et al. 2002), thus 4- $\mu\text{m}$  classes would differ in "preferred prey size" by 1  $\mu\text{m}$ ; intuitively,

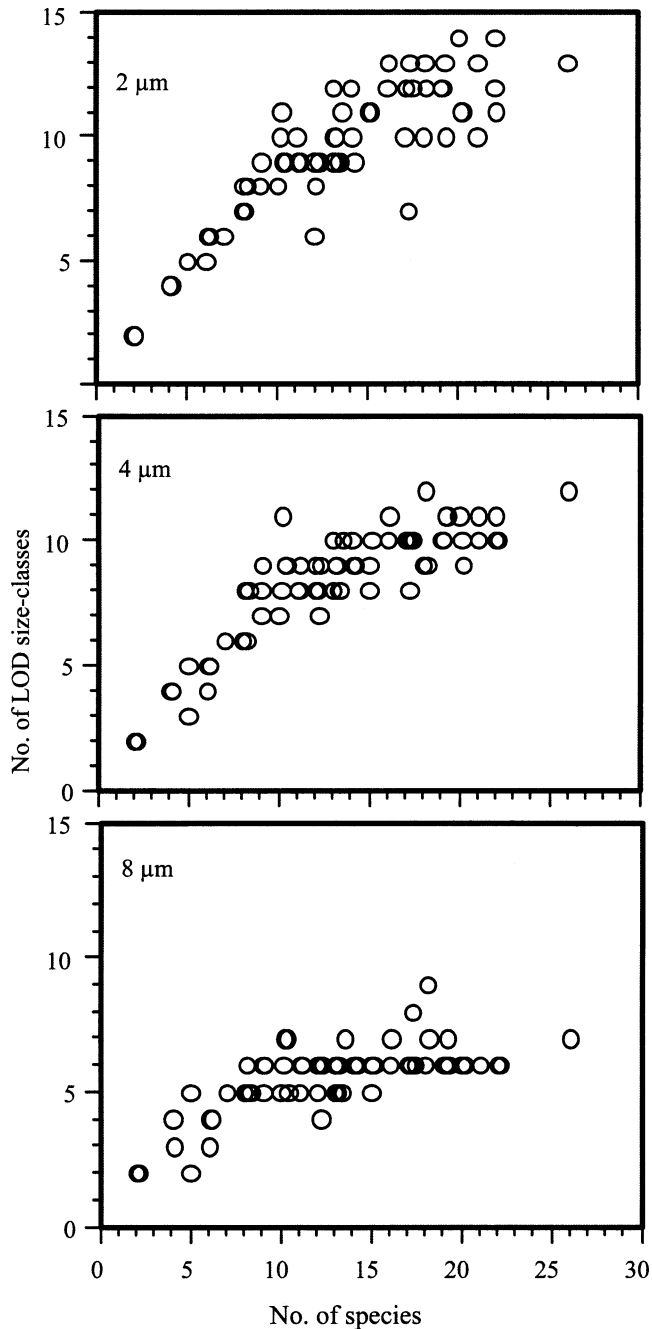


Fig. 9. Scatterplots of species richness versus richness of LODs using groupings of 2-, 4-, and 8- $\mu\text{m}$  size-classes. See Discussion for details.

an absolute lower limit of size distinction. Second, specimens were examined at  $\times 200$  or  $\times 400$ ; therefore, LOD estimates made in assigning species were then  $\pm 2 \mu\text{m}$  magnitude.

In a previous study, the relationship of morphological diversity and taxonomic diversity was examined using standard deviations (SDs) of average community lorica dimensions (Dolan 2000). This approach showed that SD was an indicator of taxonomic diversity, especially SD of lorica lengths, but was weakly correlated comparing communities

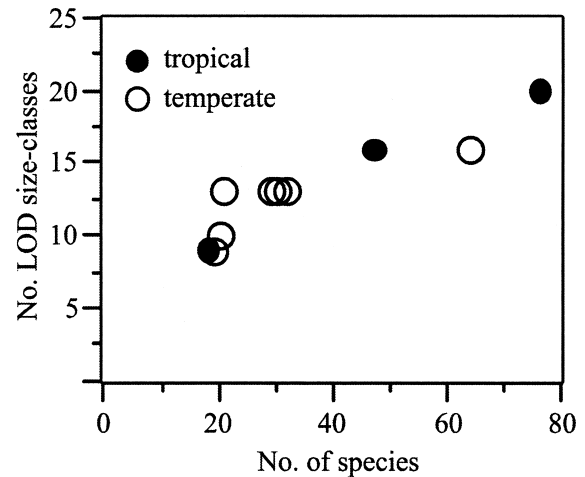


Fig. 10. Scatterplots of LOD size-class richness and species richness of the tintinnid community encountered throughout annual cycles across different systems. Tropical systems are coastal waters of New Caledonia, Bay of Bengal, and Jamaica. Temperate systems are Buzzards Bay, Hiroshima Bay, Narragansett Bay, Damariscotta River Estuary, Nervion River Estuary, Bahia Blanca Estuary, and the Gulf of Naples. Data sources given in the legend to Fig. 3.

across a large spatial scale (Dolan 2000). In a subsequent study, diversity of LOD size-classes was employed and shown to be closely related to both taxonomic diversity and the size diversity of phytoplankton across a large spatial scale (Dolan et al. 2002). In contrast to the data presented here, LOD size-classes were variable, set as equal to the number of species encountered, to maximize the possibility of distinguishing species via LOD (Dolan et al. 2002). The method we used here does not require knowledge of the number of species present; each sample is analyzed in a similar manner.

Automated or semiautomated systems of plankton analysis capable of producing data on tintinnid LODs potentially can be employed to estimate diversity. Sophisticated systems capable of resolving species within a tintinnid genus have been described (Culverhouse et al. 1994; Williams et al. 1994). However, our analysis suggests that relatively crude data on LOD sizes may suffice. Clearly, the image capture and analysis systems must be capable of resolving differences of about  $2 \mu\text{m}$  and distinguishing empty from occupied lorica. It should be noted that results are dependant on the resolution of size-classes. As an example, Fig. 9 shows the relationship between numbers of species and LOD size-classes using different magnitudes of size-classes. Grouping in 8- $\mu\text{m}$  intervals underestimates species abundance compared with 4- $\mu\text{m}$  grouping. Using a finer grouping of 2- $\mu\text{m}$  intervals yields estimates closer to our estimates of actual species richness. However, if the data employed were individual LOD measurements (rather than assigned LOD values), the 2- $\mu\text{m}$  grouping may yield more LOD groups than species, because individual variability within a species exists.

The relationship between total species richness in various systems and LOD size-class richness in those systems is shown in Fig. 10. In this crude cross-system comparison,

morphological diversity is also closely related to taxonomic diversity. The abundances of LOD size-classes found appears to be one-half to one-quarter of the number species described as occurring in the system. This suggests then, that most species are not the sole occupants of a given size-class. Over an annual cycle, two to four species can be found for each LOD size class.

In our study, each species was assigned a name on the basis of lorica morphology, including a reliance on lorica dimensions, and each species was assigned an average LOD on the basis of a description of the species. Thus, no allowance was made for plasticity in a given species. This is a possible source of error. Species exhibiting multiple morphologies in a sample would inflate diversity estimates. Species exhibiting distinct morphologies in distinct samples are less likely to have influenced diversity metrics. We have no data to permit an assessment of either possibility. However, species of genera known to show considerable plasticity of lorica morphology such as *Favella* (Laval-Peuto 1983), *Cymatocylis* (Wasik 1998), and *Ptychocylis* (Davis 1981) were rare or absent in our samples. Species of the genus best known to show seasonal shifts in lorica dimensions, *Tintinnopsis* (Gold and Morales 1976), varied in length but little in LOD in our samples, with the exception of *T. campanula*, a minor component of the tintinnid community. LOD appears to be a conservative characteristic (Laval-Peuto and Brownlee 1986).

Morphological diversity studied here is a relatively simple analysis, focusing on a single character, LOD, compared to investigations of multicellular organisms. Morphological diversity in fossil or living taxa has been defined as an estimate derived from a consideration of a multivariate *morphospace* (e.g., Roy et al. 2004). In protistan and metazoan taxa, compared to gastropods, for example, perhaps both the strength and weakness of investigating morphological diversity is the relative simplicity of their morphologies. Thus, the limited number of characters available increases the opportunity to find and focus on a character or characters related to their ecological niche. However, it should be recalled that characters should not be restricted to morphology, but rather, extended to include physiology.

Tintinnids in the tropical Pacific lagoon of New Caledonia are a species-rich assemblage; 76 species were distinguished. The community showed temporal changes in diversity and morphology (median LOD), which were only weakly related to changes in trophic resources measured as Chl *a*. However, metrics of morphological diversity based on LOD were correlated with metrics of taxonomic diversity. Because LOD is related to the size of prey exploited in tintinnids, correlation of taxonomic and LOD diversity supports the hypothesis that the taxonomic diversity of tintinnids in the lagoon reflects a trophic diversity. Although the relationship of primary producer diversity generating consumer diversity is intuitively appealing, this has rarely been shown (Turner and Hawkins 2004). Furthermore, metrics of LOD diversity in tintinnids appear to be a good proxy measure of taxonomic diversity both within and among systems.

## References

ADMIRAAL, W., AND L. A. H. VENEKAMP. 1986. Significance of tintinnid grazing during blooms of *Phaeocystis pouchetii* (Hap-

- topyceae) in Dutch coastal waters. *Neth. J. Sea Res.* **20**: 61–66.
- AGATHA, S., M. C. STRÜDER-KYPKE, A. BERAN, AND D.H. LYNN. 2005. *Pelagostrobilidium neptuni* (Montagnes and Taylor, 1994) and *Strombidium biarmatum* nov. spec. (Ciliophora, Oligotrichea): Phylogenetic position inferred from morphology, ontogenesis, and gene sequence data. *Eur. J. Protistol.* **41**: 65–83.
- BARRIA DE CAO, M. S. 1992. Abundance and species composition of *Tintinnina* (Ciliophora) in Bahia Blanca Estuary, Argentina. *Est. Coast. Shelf Sci.* **34**: 295–303.
- BUJAN, S., C. GRENZ, R. FICHEZ, AND P. DOUILLET. 2000. Evolution saisonnière du cycle biogéochimique dans le lagon sud-ouest de Nouvelle-Calédonie. Application d'un modèle compartimental. *C. R. Acad. Sci. Paris, Sci. Vie* **323**: 225–233.
- CAMPBELL, A. S. 1942. The Oceanic Tintinnoida of the plankton gathered during the last cruise of the Carnegie. Carnegie Institution of Washington, Publication 537.
- CARIOU, J. B., J. R. DOLAN, AND S. DALLOT. 1999. A preliminary study of tintinnid diversity in the NW Mediterranean Sea. *J. Plank. Res.* **21**: 1065–1075.
- CULVERHOUSE, P. F., R. ELLIS, R. G. SIMPSON, R. WILLIAMS, R. W. PIERCE, AND J. T. TURNER. 1994. Automatic categorisation of five species of *Cymatocylis* (Protozoa, Tintinnida) by artificial neural network. *Mar. Ecol. Prog. Ser.* **107**: 273–280.
- DAVIS, C. C. 1981. Variations of lorica shape in the genus *Ptychocylis* (Protozoa: Tintinnia) in relation to species identification. *J. Plank. Res.* **3**: 433–443.
- DOLAN, J. R. 2000. Tintinnid ciliate diversity in the Mediterranean Sea: Longitudinal patterns related to water column structure in late spring-early summer. *Aquat. Microb. Ecol.* **22**: 20–30.
- , H. CLAUSTRE, F. CARLOTTI, S. PLOUNEVEZ, AND T. MOUTIN. 2002. Microzooplankton diversity: Relationships of tintinnid ciliates with resources, competitors and predators from the Atlantic coast of Morocco to the eastern Mediterranean. *Deep Sea Res. I* **49**: 1217–1232.
- FENCHEL, T. 1987. *Ecology of Protozoa: The Biology of Free-living Phagotrophic Protists*. Springer-Verlag.
- FOOTE, M. 1997. The evolution of morphological diversity. *Ann. Rev. Ecol. Syst.* **28**: 129–152.
- GASTON, K. J., AND J. I. SPICER. 2003. *Biodiversity an Introduction*, 2nd ed. Blackwell.
- GILRON, G. L., D. H. LYNN, AND J. C. ROFF. 1991. The annual cycle of biomass and production of tintinnine ciliates in a tropical neretic region near Kingston, Jamaica. *Marine Microbial Food Webs* **5**: 95–113.
- GODHANTARAMAN, N. 2002. Seasonal variations in species composition, abundance, biomass and estimated production rates of tintinnids at tropical estuarine and mangrove waters, Parangipettai, southeast coast of India. *J. Mar. Sci.* **36**: 161–171.
- GOLD, K., AND E. A. MORALES. 1976. Studies on the sizes, shapes, and the development of the lorica of agglutinated tintinnida. *Biol. Bull.* **150**: 377–392.
- HADA, Y. 1938. Studies on the Tintinnoida from the Western Tropical Pacific. *J. Fac. Sci. Hokkaido Imper. Univ. Ser. IV Zool.* **6**: 87–190.
- HEINBOKEL, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. II. Grazing rates of field populations. *Mar. Biol.* **47**: 191–197.
- HOLM-HANSEN, O. C. F. LORENZEN, R. W. HOMES, AND J. D. H. STRICKLAND. 1965. Fluorometric determinations of chlorophyll. *J. Cons. Perm. Int. Explor. Mer.* **30**: 3–15.
- JÖRGENSEN, E. 1924. Mediterranean Tintinnidae. Report of the Danish Oceanographical Expeditions 1908–1910 to the Mediterranean and adjacent seas. Vol. II. Biology, No. 8, J.3 (Thor expedition). Copenhagen.



- KAMIYAMA, T., AND M. TSUJINO. 1996. Seasonal variation in the species composition of tintinnid ciliates in Hiroshima Bay, the Seto Inland Sea of Japan. *J. Plank. Res.* **18**: 2313–2337.
- KARAYANNI H., U. CHRISTAKI, F. VAN WAMBEKE, M. DENIS, AND T. MOUTIN. 2005. Influence of ciliated protozoa and heterotrophic nanoflagellates on the fate of primary production in the northeast Atlantic Ocean. *J. Geophys. Res. Oceans* **110**: No. C07S1.
- KOFOID, C. A., AND A. S. CAMPBELL. 1929. A conspectus of the marine and fresh-water ciliata belonging to the suborder tintinnoinea, with descriptions of new species principally from the Agassiz expedition to the eastern tropical Pacific 1904–1905. *Univ. Calif. Publ. Zool.* **34**: 1–403.
- , AND ———. 1939. The tintinnoinea of the eastern tropical Pacific. *Bull. Mus. Compar. Zool. Harvard Coll.* **84**: 1–473.
- LAVAL-PEUTO, M. 1983. Sexual reproduction in *Favella ehrenbergii* (Ciliophora, Tintinnia): Taxonomic implications. *Protistologica* **19**: 503–512.
- , AND D. C. BROWNLEE. 1986. Identification and systematics of the tintinnia (Ciliophora): Evaluation and suggestions for improvement. *Ann. Inst. Océanogr. Paris* **62**: 69–84.
- MARSHALL, S. M. 1969. Protozoa, order Tintinnia, p. 117–124. *In* Conseil International pour l'Exploration de la Mer, Fiches d'Identification de Zooplancton, fiches.
- MCCLAINE, C. R., N. A. JOHNSON, AND M. A. REX. 2004. Morphological disparity as a biodiversity metric in lower bathyal and abyssal gastropod assemblages. *Evolution* **58**: 338–348.
- MIDDLEBROOK, K., C. W. EMERSON, J. C. ROFF, AND D. H. LYNN. 1987. Distribution and abundance of tintinnids in the Quoddy region of the Bay of Fundy. *Can. J. Zool.* **65**: 594–601.
- MODIGH, M., AND S. CASTALADO. 2002. Variability and persistence in tintinnid assemblages at a Mediterranean coastal site. *Aquat. Microb. Ecol.* **28**: 299–311.
- , S. CASTALADO, M. SAGGIOMO, AND I. SANTARPIA. 2003. Distribution of tintinnid species. *Hydrobiologia* **503**: 251–262.
- PIERCE, R. W., AND J. T. TURNER. 1993. Global biogeography of marine tintinnids. *Mar. Ecol. Prog. Ser.* **94**: 11–26.
- , AND ———. 1994. Plankton studies in Buzzards Bay, Massachusetts, USA. IV. Tintinnids, 1987–1988. *Mar. Ecol. Prog. Ser.* **112**: 235–240.
- RAMPLI, L. 1950. Tintinnoidi delle acque di Monaco raccolti dall'Eider nell'anno 1913. *Bull. Inst. Océanogr. n.* **965**: 1–7.
- ROY, K., D. JABLONSKI, AND J. W. VALENTINE. 2004. Beyond species richness: Biogeographic patterns and biodiversity dynamics using other metrics of diversity, p. 151–170. *In* M. V. Lomolino and L.R. Heaney [eds.], *Frontiers of Biogeography: New Directions in the Geography of Nature*. Sinauer Assoc.
- SANDERS, R. W. 1987. Tintinnids and other microzooplankton—seasonal distributions and relationships to resources and hydrography in a Maine estuary. *J. Plank. Res.* **9**: 65–77.
- THOMPSON, G. A., AND V. A. ALDER. 2005. Patterns in tintinnid species composition and abundance in relation to hydrological conditions of the Southwestern Atlantic during austral spring. *Aquat. Microb. Ecol.* **40**: 85–101.
- TURNER, J. R. G., AND B. A. HAWKINS. 2004. The global diversity gradient, p. 170–190. *In* M. V. Lomolino and L.R. Heaney [eds.] *Frontiers of Biogeography: New Directions in the Geography of Nature*. Sinauer Associates.
- URRUTXURTU, I. 2004. Seasonal succession of tintinnids in the Nervion River Estuary, Basque Country, Spain. *J. Plank. Res.* **26**: 307–314.
- VERITY, P. G. 1987. Abundance, community composition, size distribution, and production rates of tintinnids in Narragansett Bay, Rhode Island. *Est. Coast. Shelf Sci.* **24**: 671–690.
- WASIK, A. 1998. Antarctic tintinnids: Their ecology, morphology, ultrastructure and polymorphism. *Acta Protozool.* **37**: 5–15.
- WILLIAMS, R., H. MCCALL, R. W. PIERCE, AND J. T. TURNER. 1994. Speciation of the tintinnid genus *Cymatocylis* by morphometric analysis of the loricae. *Mar. Ecol. Prog. Ser.* **107**: 263–272.

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