Vol. 28: 299–311, 2002 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published July 25

Variability and persistence in tintinnid assemblages at a Mediterranean coastal site

M. Modigh*, S. Castaldo

Stazione Zoologica 'A. Dohrn', Villa Comunale, 80121 Naples, Italy

ABSTRACT: Seasonal variations in tintinnid abundance and species composition were studied weekly for 4 yr at a coastal site in the Gulf of Naples (Tyrrhenian Sea). Of the 57 identified tintinnid species, only 7 accounted for 81% of tintinnid abundance. Recurrent seasonal patterns were observed for the most common species. The characteristics of the tintinnid community over this 4 yr study are similar to those reported for tintinnid assemblages at the same study site in 1984-85 in terms of total abundance, species composition and timing of the dominant species. According to reports in the literature the persistence in time of the dominant tintinnid species appears to be a general feature, a sort of 'fingerprint' of each area. In contrast, in the Gulf of Naples only *Tintinnopsis beroidea*, reported as dominant in 1934, continues to be dominant in this area today; the other dominant species of the 1930s were rarely found during this study. Changes and persistence of tintinnid species in the Gulf of Naples are discussed in relation to major changes in phytoplankton populations in the period 1984 to 2000. Chlorophyll concentrations integrated over the whole water column are now only 50% of the former values and only 34% of former surface values. We also compare our findings to reports from other coastal sites to evaluate the distribution of key tintinnid species, in particular for the western Mediterranean Sea.

KEY WORDS: Tintinnids · Diversity · Mediterranean

INTRODUCTION

The importance of protozoan and small metazoan grazers in transferring microbial production to higher trophic levels has been well assessed (e.g. Sherr et al. 1986, Capriulo et al. 1991, Pierce & Turner 1992, Paffenhöfer 1998). Tintinnids, or loricate ciliates, are the most thoroughly investigated component of microzooplankton communities and have been reported to ingest up to 27% of annual primary production in coastal waters (Capriulo & Carpenter 1983, Verity 1987).

However, tintinnids comprise only a minor part of the ciliate assemblage in most areas, ranging from 5 to 10% in the Mediterranean Sea (Margalef 1963, Dolan 2000). Information on the by far more abundant naked choreotrichs is mainly limited to bulk properties (abundance, biomass) while data on species composition of naked ciliate communities are scarce. Several species of pelagic ciliates have not yet been described (Laval-Peuto & Rassoulzadegan 1988, Foissner 1999), and a consensus for the diversity of naked ciliate communities is still lacking (Finlay & Fenchel 1999, Foissner 1999). On the other hand, there is an abundant literature on tintinnid species distributions (see Pierce & Turner 1993 for a review), and tintinnids have been defined as ideal organisms to track changes in the microbial compartment (Thompson et al. 1999, Dolan 2000, Pitta et al. 2001).

However, before we can assess whether changes have occurred in the microbial populations, basic knowledge on their diversity is required. The tintinnid lorica provides a convenient basis for identification, but taxonomic determination exclusively based upon lorica morphology is not devoid of problems. Lorica

*E-mail: modigh@alpha.szn.it

polymorphism is frequent and re-examinations of several tintinnid species have suggested that many species should be reduced to synonyms (e.g. Bakker & Phaff 1976, Laval-Peuto 1981, 1982, Laval Peuto & Brownlee 1986). Laval-Peuto & Brownlee (1986) have pointed out the necessity of using other criteria (e.g. cytological, ecological, biogeographical) along with the characteristics of the lorica for tintinnid taxonomy. To date, such studies are limited to a small number of species and, as lorica morphology remains the only general basis for tintinnid taxonomy, it was used also in the present study.

To track changes in tintinnid assemblages over time, observations over several years need to be analysed for any single site. Seasonal and interannual variability at the local scale must be recorded to assess whether an area is characterised by a well-defined association of tintinnid species.

The present paper provides data on seasonal variations in tintinnid abundance and species diversity over 4 yr, 1997 to 2001, at a coastal station in the Gulf of Naples (Mediterranean Sea). The study site, Stn MC, is positioned in an area with alternatively coastal and offshore characteristics and has been the site of continuous sampling for hydrographic and biological parameters since 1984 (Scotto di Carlo et al. 1985, Mazzocchi & Ribera d'Alcalà 1995, Modigh et al. 1996, Ribera d'Alcalà et al. 2002).

Analyses of weekly microzooplankton samples over 3 yr in the Gulf of Naples have shown the numerical dominance of naked choreotrichs and noticeable recurrences in the seasonal succession among genera of mixotrophic ciliates (Modigh 2001). Total ciliate abundance spanned over 3 orders of magnitude, but the relative contribution of different size classes remained constant over time. However, a more detailed description of the dynamics of the ciliate community was not possible at the time since the taxonomy of the naked ciliates was not determined. There is no previous information, therefore, on species composition for these protists in the Gulf of Naples.

During the long-term study carried out at Stn MC, a notable decrease in phytoplankton biomass was recorded for the period 1984 to 2001. At present, chlorophyll concentrations are 50%, integrated over the 0 to 60 m layer, and only 34% of the surface values of the data recorded in 1984. The decrease in chlorophyll values has been accompanied by an increment in the number of nanoplanktonic solitary diatoms and phytoflagellates (Ribera d'Alcalà et al. 2002). Data on tintinnid species and abundance are available for Stn MC for the first year of the long-term study (Scotto di Carlo et al. 1985, Petrillo 1988). A change in tintinnid assemblages may have been expected in response to the observed changes in phytoplankton populations, in particular in relation to the increment in nanoplanktonic cells, since these are the preferential food for tintinnids (Verity 1987, Pitta et al. 2001). Higher tintinnid abundance and an increase in the number of tintinnid species have been reported as presumably related to prey abundance (Verity 1987, Pierce & Turner 1994). Furthermore, Verity (1987) hypothesised that succession among tintinnid species was related to phytoplankton prey size, with smaller tintinnids reported when prey organisms were prevailingly small.

Here we describe the tintinnid assemblages at Stn MC over 4 yr and define the dominant tintinnid species in the Gulf of Naples. We then compare our data with those of Scotto di Carlo et al. (1985) and of Issel (1934). Moreover, to test for similarities in the occurrence of the most abundant and frequently observed tintinnid species, we compare these data to those reported for other western Mediterranean sites.

MATERIALS AND METHODS

Microzooplankton samples were collected weekly at Stn MC, located 2 nautical miles (3.7 km) offshore in the Gulf of Naples on the 80 m isobath. The present study focused on tintinnid assemblages in the weekly microzooplankton samples collected from January 1997 to May 2001, with an interruption between January and May 2000. A conductivity, temperature, depth (CTD) Niskin rosette sampler was employed to obtain samples for chlorophyll and microzooplankton analyses. Samples for chlorophyll measurements were collected at 7 depths in the 0 to 60 m layer, filtered onto Whatman GF/F filters, extracted in 90% acetone and read spectrofluorometrically (Neveux & Panouse 1987). In addition, chlorophyll size fractions (>10, 2 to 10 and <2 µm) were measured for 1 yr (January 1998 to March 1999) by means of differential filtration through Nuclepore membranes of appropriate mesh size. Microzooplankton samples were drawn from the surface Niskin bottle and preserved in 2% final concentration of borate formalin. From June 2000 till June 2001, an additional sample was drawn from the same surface Niskin bottle and preserved in acidic Lugol's iodine at 1% final solution. A total of 190 formalin preserved samples and 47 Lugol's samples were analysed. Sample preservation and preparation for chlorophyll and microzooplankton analyses are described in detail in Modigh (2001).

Aliquots of the microzooplankton samples were settled in sedimentation chambers and examined with an inverted Zeiss microscope at 320× magnification. For the last year of this study, 1 formalin and 1 Lugol's preserved sample were counted for each sampling. In general, a subsample of 100 ml was scanned; on aver-

age 273 ± 178 cells were counted (Lugol's). Linear dimensions of the lorica and of the cell were measured with an ocular micrometer, and the volumes were calculated referring to simple geometrical shapes. Tintinnid biomass in terms of carbon was calculated from cell volume using the conversion factors of 0.14 pg C μm^{-3} for formalin and 0.19 pg C μm^{-3} for Lugol's preserved ciliates (Putt & Stoecker 1989). Heavily agglutinated tintinnid lorica were only rarely observed, and for those ciliates having an agglutinated non-transparent lorica the equation established by Verity & Langdon (1984) was employed: $C = V \times 0.053 + 444.5$, where C is the carbon biomass (pg C) and V is the lorica volume (μm^3).

Tintinnid species were identified on the basis of lorica morphology following the descriptions of Kofoid & Campbell (1929, 1939) and Balech (1959); Jörgensen (1924) was consulted for the comparison with historical data sets. For each sample, the total number of species and the Shannon diversity index ($\log_2$ based) were calculated. Pearson's coefficient of correlation was calculated to test the relationship between the different parameters measured and tintinnid numbers, Shannon diversity index and number of species. Parametric 1-way ANOVA was employed for the comparison of annual means for the different parameters considered. Such analysis requires homogeneity of variances between the sets of data, which was generally not the case. Thus, the data were transformed to $\log_{10}(x + 1)$ for normalization.

To evaluate sampling error, a jack-knife estimation for the cumulative species as a function of the number of samples examined was performed. This method predicts how many species would have been recorded had the sampling been more intensive and produces an estimated collector's curve (Magurran 1988). A cluster analysis (Bray-Curtis coefficient of faunal similarity) was performed to discern diversity of tintinnid assemblages on a seasonal scale. To normalise the data, a transformation of the abundance values was applied (species abundance/total tintinnid abundance for each sample). Cluster analysis and jack-knife calculations were made using the 'Biodiversity Professional' program (beta release; McAleece N, Lambshead PJD, Paterson GLJ, Gage JD 1997; through the Scottish Association for Marine Science, UK, web site [available at: http://www.sams.ac.uk/dml/projects/benthic/bdpro/]).

RESULTS

Surface temperature followed a sinusoidal pattern with the annual minimum at the end of March (14.2 ± 0.8°C) and maximum in August (27.8 ± 0.7°C). Surface salinity varied between 36.52 and 38.32 psu (average 37.66 ± 0.33). During winter, homothermic conditions were recorded along the water column. Seasonal stratification occurred from April to the end of October, mainly due to warming of the surface layer. Chlorophyll concentrations varied between 0.14 and 7.45 µg chlorophyll *a* (chl *a*) l^{-1} at the surface. In winter, the distribution was fairly homogeneous throughout the water column, while during the period of stratification highest chl *a* concentrations occurred in the first few metres of the water column.

Tintinnid abundance ranged from 0 to 30.5×10^3 cells l^{-1}. Average tintinnid abundance over the whole period of study was $1.1 \pm 3.1 \times 10^3$ cells l^{-1} (Fig. 1A). Interannual differences in mean tintinnid numbers, as well as differences between the replicate samples analysed in 2000-01, were not significant (ANOVA). Tintinnids (in Lugol's samples) represented 7.8 ± 8.3 and 7.9 ± 8.0 % of ciliate numbers and biomass, respectively; the highest contribution occurred in spring, when up to 33 % of ciliate abundance was due to tintinnids. Lowest tintinnid numbers occurred in winter, and this group was absent in 3 samples collected in January in different years.

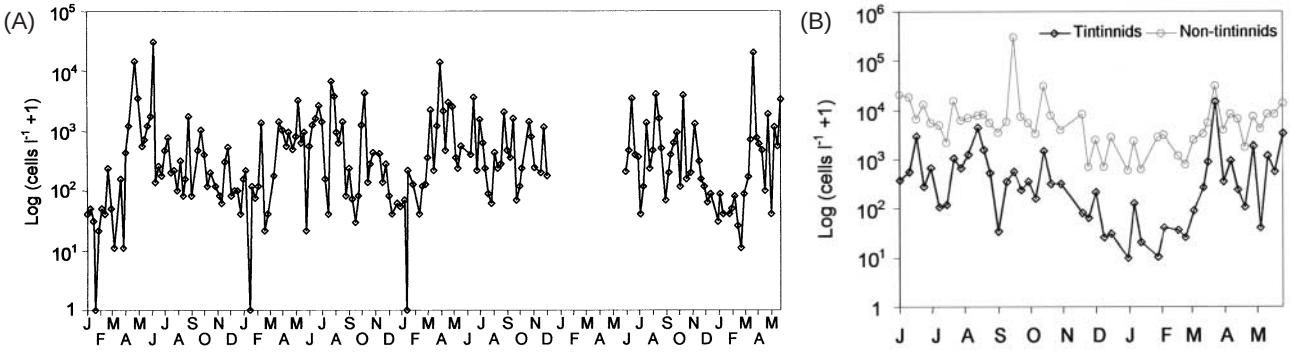

Fig. 1. (A) Variation in tintinnid abundance at Stn MC, 1997 to 2001. (B) Tintinnid and non-tintinnid ciliate abundance in Lugol's fixed samples at Stn MC, June 2000 to May 2001

Table 1. Pearson's correlation coefficients between temperature, salinity and chlorophyll *a* (chl *a*) concentrations (µg chl *a* l^{-1}) and abundance (cells l^{-1}), biomass (µg C l^{-1}) and number of tintinnid species. For temperature, salinity and total chlorophyll, N = 190; for chlorophyll size fractions, N = 58. $^{*}p < 0.05$, $^{**}p < 0.01$

	Tintinnids		
	Abundance	Biomass	No. of species
Salinity	−0.24**	−0.35**	−0.10
Temperature	0.04**	0.13**	−0.04
Total chl *a*	0.29**	0.38**	0.11
Chl *a* <2 µm	0.76**	0.80**	0.39**
Chl *a* 2 to 10 µm	0.47**	0.44**	0.14
Chl *a* >10 µm	0.20**	0.20**	0.05

The majority of the ciliates were naked choreotrichs: 74 ± 25% of abundance in the Lugol's samples (1 yr) and 68 ± 19% in the formalin samples (4 yr) due to poorer preservation of naked ciliates in formalin. Total ciliate abundance in the Lugol's preserved samples varied between 590 and 298 500 cells l^{-1} (average 14 ± 43 × 10^3 cells l^{-1}) and biomass ranged from 1.0 to 134 µg C l^{-1} (average 15.3 ± 25.5 µg C l^{-1}).

Highest numbers of naked ciliates occurred during periods of water column stratification. In most cases, high tintinnid numbers coincided with high naked ciliate concentrations, but the opposite was not always true. During the period of water column stratification, naked ciliate concentrations remained at times high for several weeks, but tintinnids were abundant on only 1 sampling occasion (Fig. 1B).

Tintinnid abundances were positively correlated with chl *a* concentrations and negatively correlated with salinity, while no correlation with temperature was found. The relationship between tintinnid abundance and biomass and the different chl *a* size fractions revealed decreasing correlations with increasing size of the chl *a* fractions (Table 1).

Taxonomy was determined to the species level most of the time; 26 tintinnid genera and 57 species were identified (Table 2) for the entire study; 3.7% of individuals were determined to genus level and 2.1% were counted as undetermined tintinnids. When first encountered, the undetermined tintinnids were given a code that was then maintained throughout the entire sampling period; no sample contained more than one undetermined tintinnid species.

Seven tintinnid species accounted for 81% of total tintinnid numbers: *Tintinnopsis minuta*, *T. beroidea*, *Metacylis annulifera*, *Eutintinnus tubulosus*, *Helicostomella subulata*, *Salpingella curta* and *S. decurtata* (in rank order). Lorica dimensions of these common species are reported in Table 3. Polymorphism is very pronounced in the genus *Tintinnopsis*, and the distinction between *T. beroidea* and *T. minuta* (as well as other species of this genus) is uncertain (Margalef & Duran 1953, Bakker & Phaff 1976). We based our assignment for these 2 species on the size and proportion of lorica length and lorica oral diameter. In addition, the lorica of the small *Tintinnopsis* spp. were not heavily agglutinated, and the cell was in general clearly visible.

Fluctuations in tintinnid abundances were very sharp, and peaks in abundance could be ascribed to a small number of species. In most cases, exceptionally high tintinnid concentrations were encountered on only 1 sampling occasion, and 1 species contributed >80% to total tintinnid numbers. Variations in abundance for some of the most frequently observed and abundant species are shown in Fig. 2. The most abundant species showed a similar pattern of occurrence for each of the 4 yr; differ-

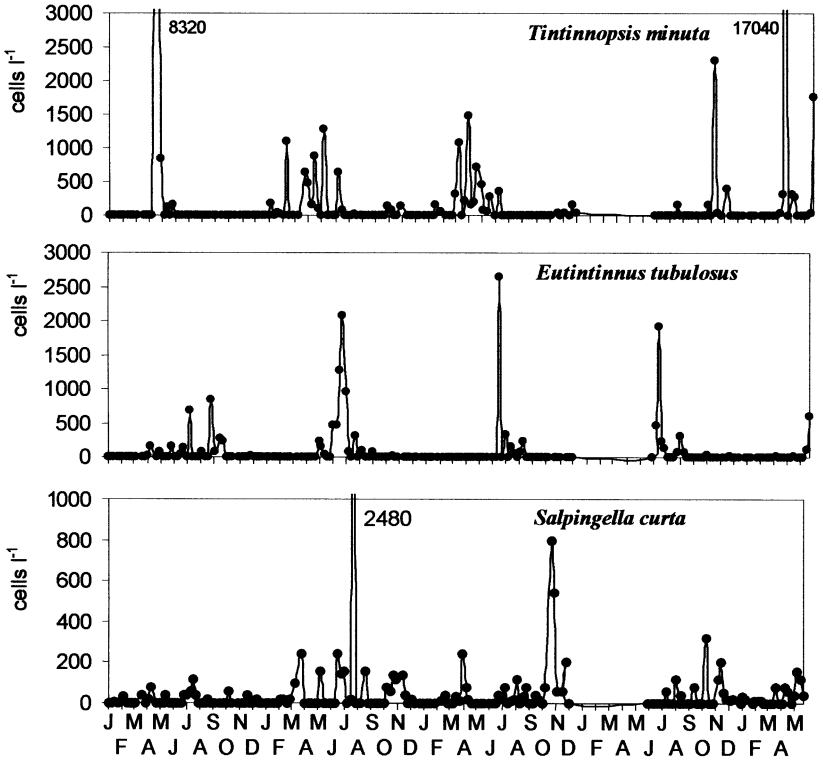

Fig. 2. Variation in abundance for some of the most abundant tintinnid species, 1997 to 2001

Table 2. Tintinnid species at Stn MC, 1997–2001

	No. of observations	Maximum abundance (cells l^{-1})
Acanthostomella minutissima	1	5
Amphorella amphora	1	20
Amphorella quadrilineata[a] var. minor	15	80
Amphorides quadrilineata	1	11
Ascampbeliella sp.	1	280
Canthariella sp.	2	1140
Codonella apicata	1	440
Codonella galea	1	5
Codonellopsis schabi	1	15
Coxliella annulata	1	40
Coxliella sp.	1	15
Craterella torulata[a]	32	80
Dadayiella ganymedes[a]	32	140
Dictyocysta elegans	5	40
Dictyocysta entzii	1	40
Dictyocysta mitra	3	120
Dictyocysta sp.	1	80
Epiplocylis acuminata	1	23
Eutintinnus apertus	6	160
Eutintinnus elegans	2	73
Eutintinnus fraknoi	9	80
Eutintinnus lusus-undae	9	208
Eutintinnus tubulosus[a]	50	2640
Eutintinnus sp.	2	80
Favella azorica	4	80
Favella campanula	3	140
Favella ehrenbergii	2	80
Favella serrata	3	160
Helicostomella subulata[a]	51	28 720
Metacylis annulifera[a]	16	160
Metacylis jörgensenii[a]	24	1120
Petalotricha sp.	1	80
Proplectella claparèdei	3	20
Proplectella columbiana	5	344
Proplectella ostenfeldi	1	10
Proplectella urna	1	160
Protorhabdonella curta	8	66
Rhabdonella conica	1	200
Rhabdonella cornucopia	1	20
Rhabdonella spiralis	1	40
Salpingacantha sp.	1	20
Salpingella acuminata	2	10
Salpingella curta[a]	85	2480
Salpingella decurtata[a]	50	4240
Salpingella glockentögeri	1	5
Salpingella rotundata[a]	10	1760
Steenstrupiella gracilis	1	40
Steenstrupiella steenstrupii[a]	41	320
Stenosemella nivalis	4	40
Stenosemella pacifica	1	5
Stenosemella ventricosa	1	40
Stenosemella sp.	2	480
Tintinnopsis beroidea[a]	88	6240
Tintinnopsis campanula	4	48
Tintinnopsis compressa	1	8
Tintinnopsis cylindrica	10	560
Tintinnopsis levigata[a]	27	1120
Tintinnopsis minuta[a]	55	14 880
Tintinnopsis nana[a]	17	720
Tintinnopsis radix	1	20
Tintinnopsis rotundata	1	5
Tintinnopsis sinuata	1	5
Undella clevei[a]	14	240
Undella declivis	1	10
Xystonella longicauda	1	20

[a]Common species

ences in mean annual abundance for the most abundant species were not significant (ANOVA), with the exception of *Salpingella decurtata*, which showed a high variability between years. Each of these species showed maximum abundance in different periods of the year (Fig. 3). Species of the genus *Tintinnopsis* showed maximum occurrence in early spring, *Helicostomella subulata* in late spring, *Metacylis annulifera* and *Eutintinnus tubulosus* in summer, *S. decurtata* in late summer and *S. curta* from late summer to autumn. Maximum annual abundances, 13.9×10^3 and 30.5×10^3 cells l^{-1}, occurred in early spring and were due to the sharp increment of *T. minuta*, *T. beroidea* or both.

To test for differences in species composition between seasons and between years, a cluster analysis (Bray-Curtis similarity index) was performed for the species observed in 5% or more of the samplings. The seasonal occurrence of these species was evident when the cluster analysis was performed on all samples (data not shown), but a more detailed interpretation was difficult due to the large number of final branches. We thus pooled the abundance of each species month by month (Fig. 4) and, again, a seasonal pattern in tintinnid species composition was clearly shown. Four main clusters were recorded. Cluster I collected several winter samples characterised mainly

Table 3. Lorica dimensions of the 7 most abundant tintinnid species; range (mean). LOD: lorica oral diameter

	Length (µm)	LOD (µm)
Eutintinnus tubulosus	80–110 (90)	18–28 (20)
Helicostomella subulata	80–190 (90)	16–20 (18)
Metacylis annulifera	55–75 (65)	14–18 (16)
Salpingella decurtata	110–150 (120)	12–15 (13)
Salpingella curta	40–90 (60)	9–15 (12)
Tintinnopsis beroidea	35–70 (45)	19–25 (21)
Tintinnopsis minuta	25–35 (30)	12–15 (14)

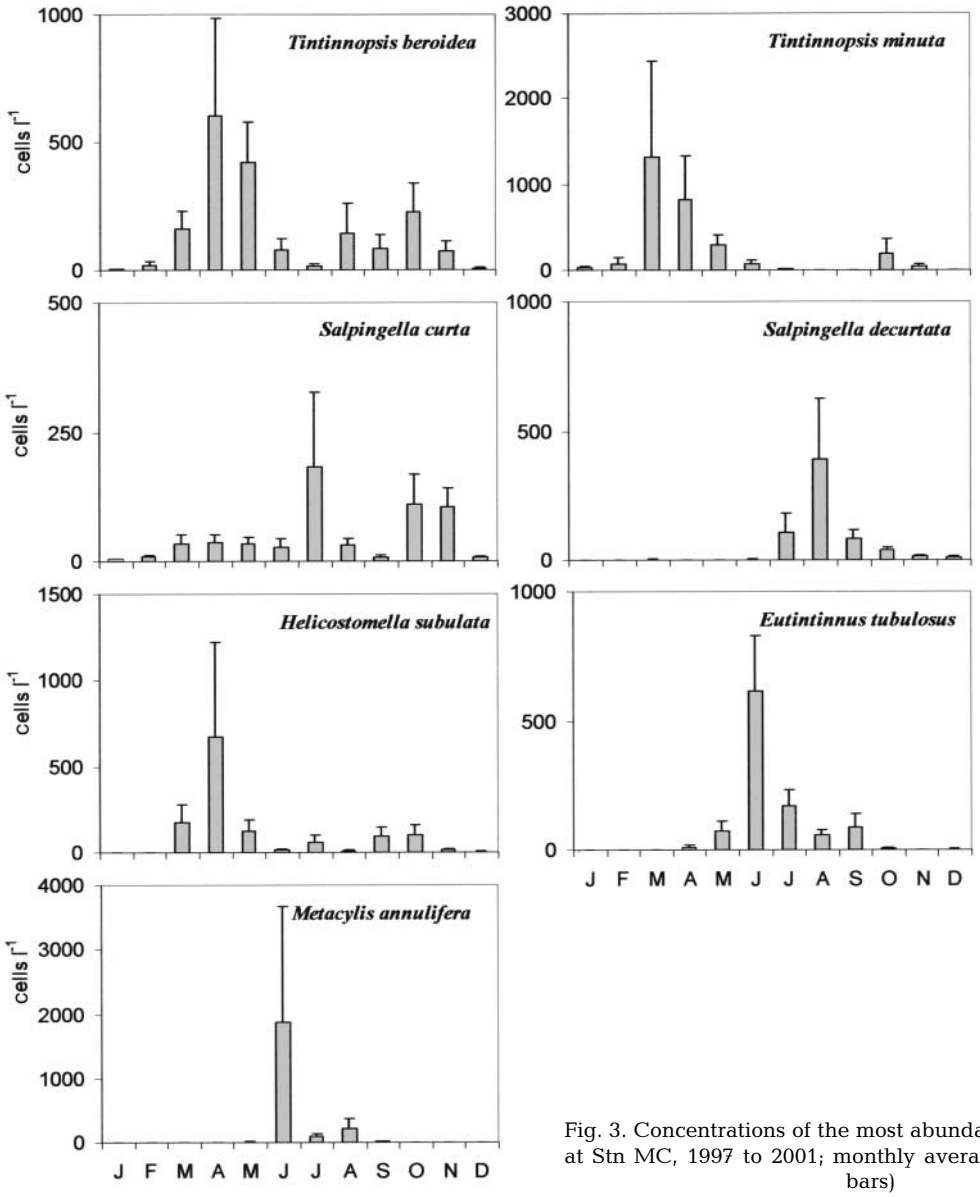

Fig. 3. Concentrations of the most abundant tintinnid species at Stn MC, 1997 to 2001; monthly averages (standard error bars)

by 2 species, alternatively, *Tintinnopsis minuta* and *Steenstrupiella steenstrupii*. *Craterella torulata* was present in all samples but in low abundances. Cluster II, which included almost all summer samples, was characterised by the presence in all samples of *Eutintinnus tubulosus* and by the occasional presence in high numbers of *Helicostomella subulata*, *Salpingella decurtata* and *E. tubulosus*. Cluster III included mainly spring samples in which *Tintinnopsis* spp. prevailed. In particular, *T. beroidea* was observed in all samples and *Metacylis annulifera*, *H. subulata*, *T. beroidea* and *T. minuta* were abundant during their periods of seasonal maxima. Cluster IV included winter samples characterised by several species of similar importance, with some prevalence of *S. curta*.

A jack-knife analysis for the study period resulted in 98 predicted species as compared to the 65 taxa observed (Fig. 5). We also included the tintinnids determined to the level of genus.

The Shannon diversity index ranged between 0 and 3.2, with a mean of 1.5 ± 0.7; no seasonal patterns were discernible (Fig. 6). Oscillations in the diversity index were sharp and closely tracked the number of species encountered ($r = 0.8$, $p < 0.01$). Both the Shannon diversity index and the number of species recorded showed slightly higher values with time, but differences between annual means for both parameters were not significant (ANOVA). Undetermined tintinnids, given a code as outlined above, were included in the calculations. The increase in both

species number and diversity index was thus not due to a better definition of tintinnid taxa during the course of this study. The number of tintinnid species was positively correlated to total tintinnid abundance ($r = 0.31$, $p < 0.01$) and to the relative contribution of tintinnids to total ciliate numbers ($r = 0.47$, $p < 0.01$). On the other hand, no correlation was found between the diversity index and tintinnid abundance and relative contribution. This implies that the number of species increased at higher tintinnid concentrations but equitability, i.e. the numerical dominance of any single species, was similar at very different levels of total tintinnid abundance.

DISCUSSION

Tintinnid abundance in relation to the trophic characteristics of the study site

Tintinnid numbers were considerably higher than those reported from other Mediterranean coastal sites (Margalef 1963, Rassoulzadegan 1979, Abboud-Abi Saab 1989) but similar to the findings for coastal eutrophic areas (Sanders 1987, Verity 1987, Kamiyama & Tsujino 1996).

In contrast to the notable change in phytoplankton populations observed since 1984 (Fig. 7), the charac-

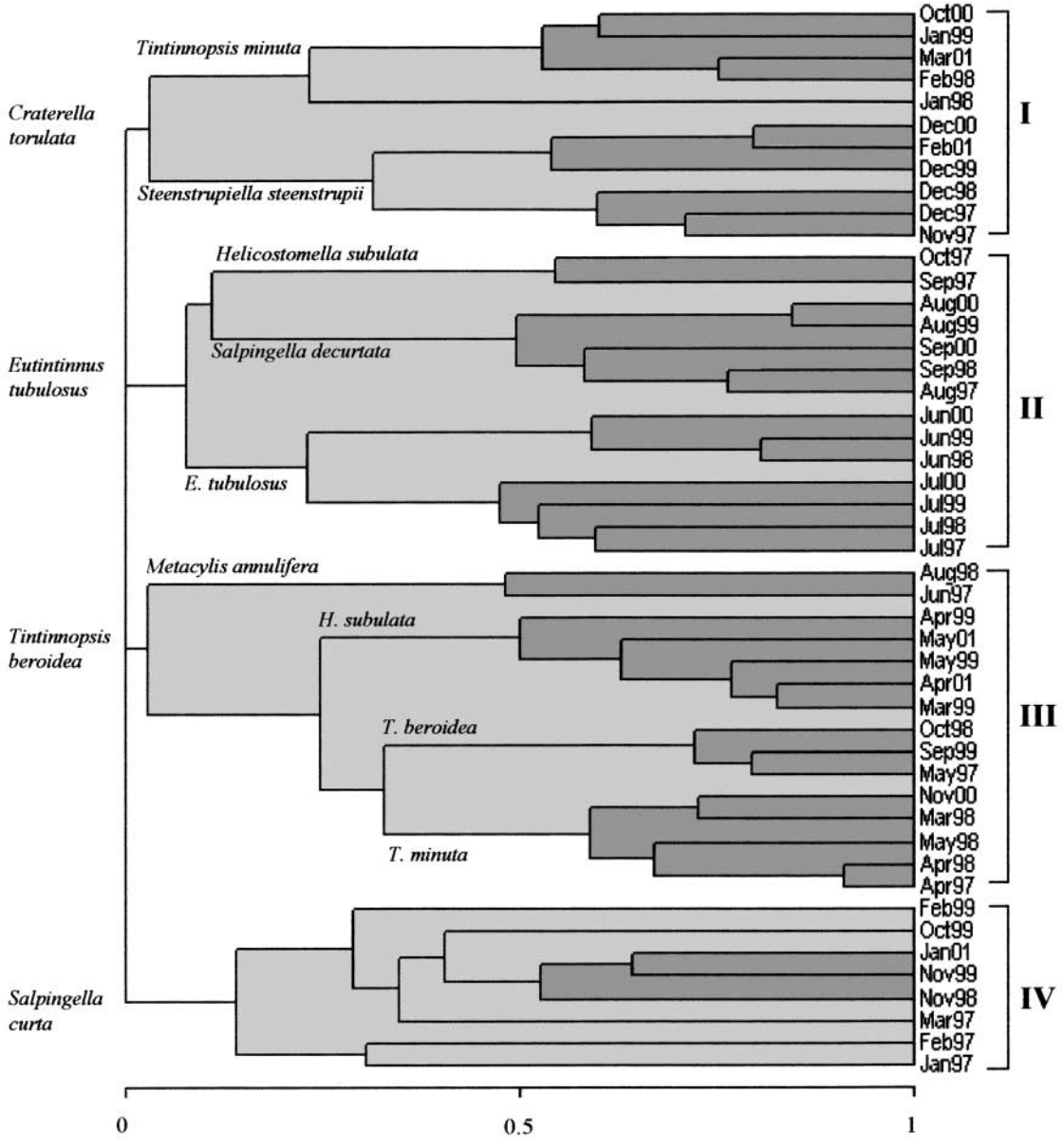

Fig. 4. Bray-Curtis cluster analysis of tintinnid species occurrence, 1997 to 2001

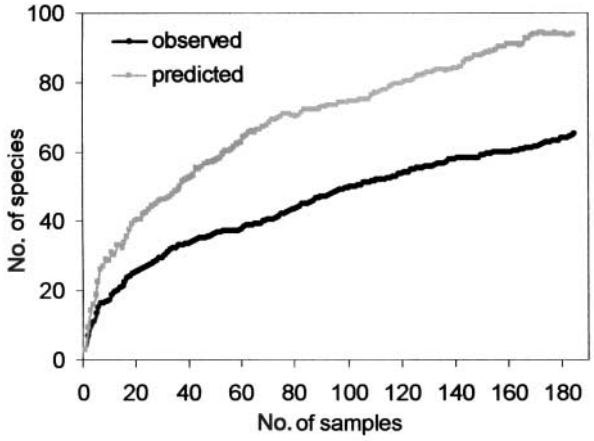

Fig. 5. Jack-knife estimation of expected species versus observed species in 190 samples at Stn MC

teristics of tintinnid assemblages were the same over this 4 yr study and in the 1984-85 annual cycle in terms of total abundance, species composition and timing of the dominant species (Scotto di Carlo et al. 1985). Increments in tintinnid abundance and number of species were recorded at high phytoplankton abundance, which has been related to food availability (Verity 1987). Conversely, the increase in nanoplanktonic cell numbers since 1984 (Ribera d'Alcalà et al. 2002) did not result in an increase in tintinnid numbers. This lack of change may be due to tintinnids being part of the microbial community, where constant biomass and numbers are a common feature (Lynn & Montagnes 1991). Also, mesozooplankton communities have not shown any major changes either in biomass or in species composition over the 17 yr sampling at Stn MC (Mazzocchi & Ribera d'Alcalà 1995, Ribera d'Alcalà et al. 2002). Mesozooplankton grazing pressure rather than resource availability have been reported to control ciliate communities in coastal waters (Nielsen & Kiørboe 1994), and grazing on tintinnid assemblages would have been maintained at fairly constant levels at the study site. In contrast to the notable variability in environmental characteristics reported (Ribera d'Alcalà et al. 2002), the persistence of tintinnid and mesozooplankton assemblages is noteworthy. The relatively constant numbers and timing of occurrence of the dominant species in both compartments highlights the role of biological processes at the single species level in regulating the dynamics of the communities.

Phytoplankton biomass was highest in the surface layer at Stn MC (Scotto di Carlo et al. 1985, Modigh et al. 1996, this study), and highest tintinnid numbers have generally been reported to occur at maximum chlorophyll concentrations along the water column (e.g. Verity 1987, Thompson et al. 1999, Pitta et al. 2001). Below the first 5 to 10 m of the water column at our study site, both tintinnid numbers and variability were considerably lower, as reported in short-term intensive samplings (0 to 40 m) during this study (Castaldo 2000, Castaldo & Modigh 2001) and over an annual cycle of samples at 30 m (Scotto Di Carlo et al. 1985).

However, in this study only surface samples were considered and total tintinnid abundance was correlated to phytoplankton biomass, with decreasing correlation with increasing algal size. The small lorica oral diameter of most of the tintinnids (Table 3) suggests that microplankton was not a suitable prey. Large phytoplankton, mainly chain-forming diatoms, occurred in particular during the late winter bloom

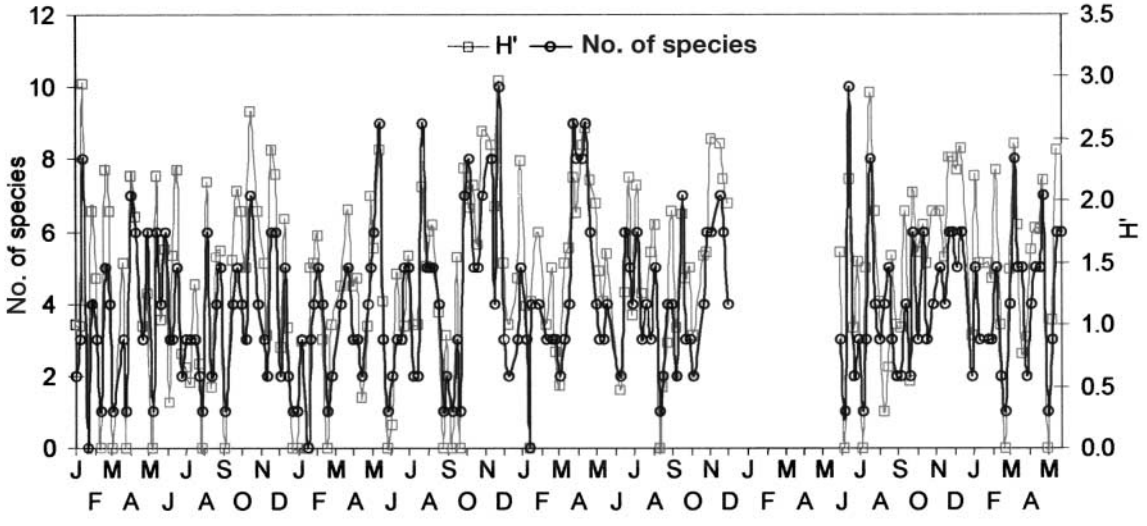

Fig. 6. Number of tintinnid species and Shannon diversity index (*H'*) for each sample, 1997 to 2001

and at times in autumn (Ribera d'Alcalà et al. 2002); this was not accompanied by an increment in tintinnid numbers. On the other hand, a rise in tintinnid abundance and number of species occurred with peaks in nano- and picoplankton biomass during the period of stratification of the water column. These peaks were associated with the arrival of low salinity coastal waters at the study site. A lack of correlation for tintinnid species numbers, and a lower correlation for tintinnid abundance and the 2 to 10 µm chlorophyll fraction than for the <2 µm fraction may be explained by a major grazing pressure on the nanoplanktonic algae. In fact, tintinnids have been shown to feed more efficiently on nanoplankton than on picoplankton (Pitta et al. 2001).

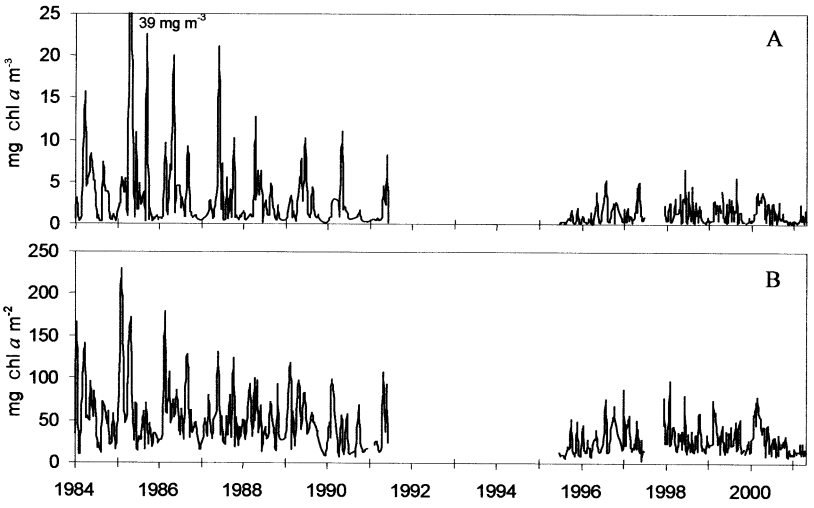

Fig. 7. Chlorophyll *a* (chl *a*) concentrations at Stn MC from 1984 to 1991 and from 1995 to 2001. (A) At surface; (B) integrated over 0 to 6 m

Tintinnid abundance was not correlated to temperature. In fact, the tintinnid annual maximum was observed before the warming of surface waters. The lack of correlation between temperature and microbial assemblages is commonly found in the Mediterranean Sea due to high (>13°C) winter temperatures (e.g. Satta et al. 1996).

Tintinnid species diversity

The bulk of tintinnid numbers was due to only 7 species accompanied by a large number of species at low or very low concentrations. The estimated number of species had the sampling been more intensive (Fig. 5) indicates a considerable underestimation of the total number of species; bottle sampling and the examination of only 100 ml of sample are not sufficient for collecting very rare species. When large volumes of water were examined, high species diversity in tintinnid assemblages was found (Cariou et al. 1999). Moreover, we present data on surface samples, which may exclude species having a subsurface distribution. While most tintinnid species do not show any vertical preferences, some species concentrate at specific depths (e.g. Verity 1987, Abboud-Abi Saab 1989). For the Mediterranean Sea, no differences in tintinnid species composition along the water column have been reported (Pitta et al. 2001), at least down to below the deep chlorophyll maximum (Dolan 2000). Furthermore, only minor differences in tintinnid species composition were found between 0 and 30 m at Stn MC (Scotto di Carlo et al. 1985). However, this study was focused on the definition and occurrence of tintinnid key species rather than a complete species list.

The comparison between reports on species composition of tintinnid communities present some difficulties due to differences in sampling methods (bottles, horizontal or vertical net tows, using nets of different mesh size, etc.) and to the problems related to lorica polymorphism. Due to this last point, the estimation of species diversity and the comparison between different sets of data of tintinnid assemblages are necessarily accompanied by some degree of uncertainty (Pierce & Turner 1993). In particular, information may be lost on the real extension of species distribution and similarities in the tintinnid species composition between different study sites due to species synonyms.

Persistence of the dominant tintinnid species

Several seasonal cycles of tintinnid assemblages have been recorded for coastal sites, and a recurrent pattern of the same most abundant species was recorded in each of these studies (e.g. Navarro & Massuti 1940, Margalef et al. 1957, Margalef & Morales 1960, Rassoulzadegan 1979 and references therein, Verity 1987, Abboud-Abi Saab 1989, Kamiyama & Tsujino 1996). Furthermore, the persistence of the same dominant tintinnid species has been reported for open ocean areas, which have been re-visited in different years (for the SW Atlantic Ocean: Thompson et al. 1999; for the Arabian Sea: Stelfox et al. 1999 and references therein). Thus, the finding of a recurrent pattern in the most abundant tintinnid species for each year of this study seems to be a general characteristic, i.e.

Table 4. References, annual cycle examined, sampling methods, tintinnid abundance and number of tintinnid species reported for the study sites shown in Fig. 8

	Study site	Duration (yr)	Method	Sampling depth	Abundance (cells l^{-1})	No. of species	Source
1	Gulf of Naples (Italy)	3	Net (m.s. = nd)	Surface	nd	40	Issel (1934)
2	Coast of Castellon (Spain)	1.6	Net (m.s. = nd)	3–8 m	nd	34	Margalef et al. (1957)
3	Coast of Blanes (Gerona, Spain)	3	Net (m.s. = nd)	Surface	nd	33	Margalef & Morales (1960)
4	Baja of Palma de Mallorca (Spain)	6	Net (m.s. = nd)	Surface	nd	64	Navarro & Massuti (1940)
5	Annaba Gulf (Algeria)	1	Net (m.s. = 63 µm)	Surface	1–283	16	Ounissi & Frehi (1990)
6	San Remo (Italy)	1	Net (m.s. = nd)	Surface	nd	68	Rampi (1948)
7	Rade of Villefranche-sur-Mer (France)	1	Bottles, net (m.s. = 50 µm)	Surface	10–1000	16	Rassoulzadegan (1979)
8	Gulf of Naples (Italy)	1	Bottles	Surface, 30 m	Maximum surface 5295 Mean surface 1054	56	Scotto di Carlo et al. (1985)
9	Marseilles (France)	3	Bottles	0–60 m	Maximum 2000	68	Travers & Travers (1971)
10	Marseilles (France)	1	Bottles	0–70 m	Mean 600	15	Travers (1973)
11	Algeri (Algeria)	0.6	Bottles	0–50 m	Maximum surface 16 700	42	Vitiello (1964)
12	Gulf of Naples (Italy)	4	Bottles	Surface	Maximum 30 480 Mean 1062	56	This study

there is a specific 'fingerprint' in dominant tintinnid species for each area. An exception to this general 'rule' seems to be provided by 2 studies on longitudinal patterns in tintinnid distributions in offshore waters of the Mediterranean Sea (Dolan 2000, Pitta et al. 2001). The dominant tintinnid species differed between the 2 studies carried out in spring (May to June) of different years. An explanation may be found in the timing in peak abundance of tintinnid species, which vary within approximately 1 mo (e.g. Scotto di Carlo et al. 1985, Verity 1987, Abboud-Abi Saab 1989, this study). Alternatively, the most abundant species contributed on average only 16% to total tintinnid numbers (Pitta et al. 2001); thus, a real dominance was not the case and the 'fingerprint' concept could not be applied to such highly diverse tintinnid assemblages.

Comparison with other Western Mediterranean coastal sites

Comparisons between tintinnid assemblages from adjacent sites have shown that some of the most abundant tintinnid species are common over fairly large areas (Navarro & Massuti 1940, Rassoulzadegan 1979, Verity 1987, Pierce & Turner 1994). To check for similarities within the western Mediterranean basin, we analysed the data on annual cycles of tintinnid assemblages over this area (Fig. 8, Table 4). All studies were carried out in coastal waters, within the 100 m isobath, and all, except the studies off Marseilles (Travers & Travers 1971, Travers 1973) and a study off Algeria (Vitiello 1964), were limited to the surface layer. Different sampling methods were employed, but the strong similarities in species composition (Fig. 8) found between bottle samples and the net samples collected at different sites around the basin validate the comparison of the different sets of data.

Tintinnid abundances, where available, were lower at the other Mediterranean study sites than those found in this study for the Gulf of Naples (Table 4), presumably related to a major influence of land runoff at our study site. Relatively high tintinnid numbers were reported also for a study at an Algerian coastal site carried out, however, over only 7 mo, due to tintinnid numbers being too low during summer months (Vitiello 1964). Independently of sampling method (bottles or nets), the dominant tintinnid species reported along the western Mediterranean coasts (see references in Table 4) were generally larger than those reported in this study. Larger size is associated with the capability to exploit lower minimum food concentrations, as well as with lower maximum growth rates (Perez et al. 1997). Large tintinnids at low concentrations seem to be typical of oligotrophic conditions (Abboud-Abi Saab 1989, Gilron et al. 1991, Dolan 2000), and smaller tintinnids at higher concentrations are reported for more eutrophic sites (Sanders 1987, Verity 1987, Kamiyama & Tsujino 1996).

As regards the characteristic tintinnid species reported along the western Mediterranean coasts, a small number of abundant species was reported for each site (Fig. 8), and most of these key species were common to several of the study sites. Furthermore,

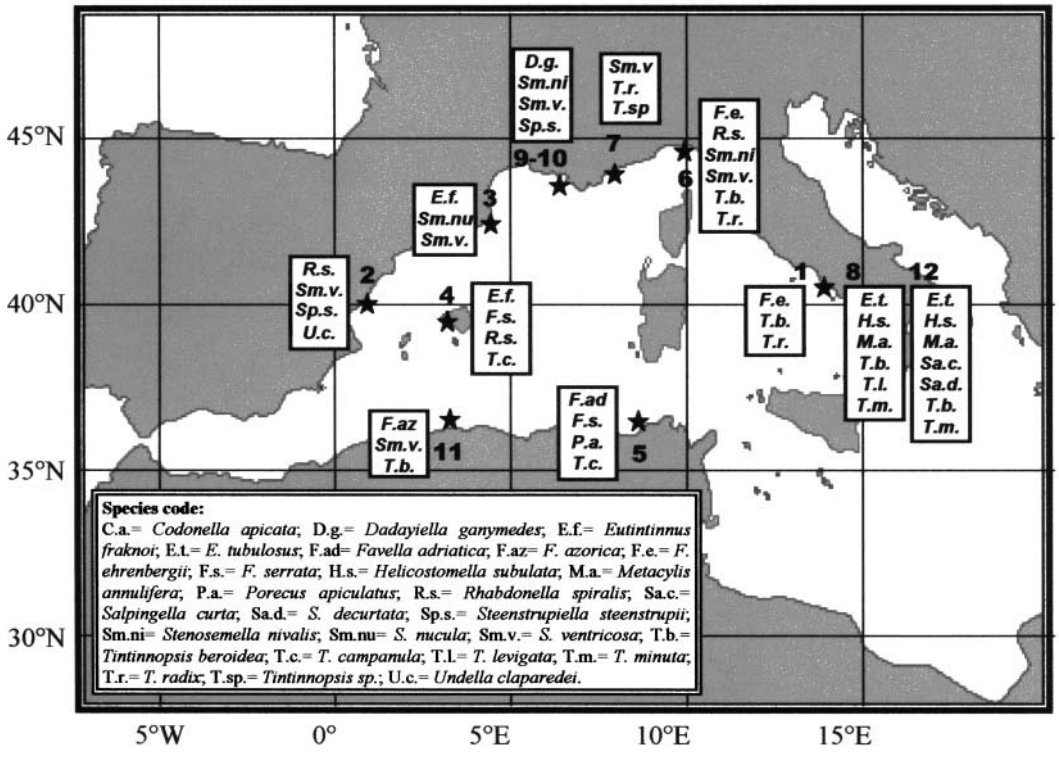

Fig. 8. Location of study sites of tintinnid assemblages in the western Mediterranean basin. The most abundant tintinnid species are shown

Issel (1934) reported 3 yr of weekly surface net sampling for the study of phytoplankton and tintinnids in an area close to Stn MC. The dominant species of this 1929 to 1931 data set were also reported at other sites around the western Mediterranean basin. Large tintinnid species and low concentrations suggest that oligotrophic conditions prevailed at most of the study sites, including the Gulf of Naples at the time of Issel (1934). Similar trophic conditions may explain the presence of the same dominant tintinnid species at several of the coastal sites. On the contrary, at present a different tintinnid fingerprint characterises our study area (Scotto di Carlo et al. 1985, this study), with the notable exception of *Tintinnopsis beroidea*.

Tintinnopsis beroidea was the most frequently observed and abundant species, with maximum numbers occurring in April at the time of Issel (1934), in the study of Scotto di Carlo et al. (1985) and in the present study. The prominent role of *Tintinnopsis* is a feature common to several coastal sites (e.g. Verity 1987, Abboud-Abi Saab 1989, Ounissi & Frehi 1990, Gilron et al. 1991, Pierce & Turner 1994). The persistence of *T. beroidea* as the most abundant and frequently observed species in the Gulf of Naples shows its capacity to adjust to changing environmental conditions, whereas the other dominant tintinnid species reported by Issel (1934) have been replaced.

Are there ongoing changes in the Gulf of Naples tintinnid assemblages?

There are some indications that the tintinnid community at Stn MC is also changing. The complete 1984-85 data set of the tintinnid community (Petrillo 1988) was analysed by means of ANOVA for total tintinnid numbers and for the abundance of the dominant species compared to the findings of the present study. Differences in annual mean numbers were not significant for the tintinnid community as a whole, as well as for 5 species (*Tintinnopsis minuta*, *T. beroidea*, *Eutintinnus tubulosus*, *Helicostomella subulata* and *Metacylis annulifera*) reported to be the most abundant in the 1984-85 annual cycle (Scotto di Carlo et al. 1985). In addition to these 5 species, the present study reported *Salpingella curta* and *S. decurtata* among the key species at Stn MC. Numbers of these 2 species were higher in all 4 yr of this study, and differences in abundance were significant for all or 3 out of 4 annual cycles, respectively, as compared to the 1984-85 data. The increment in the number of dominant species, as well as the observation of a small but gradual increase in both the number of tintinnid species and the diversity index over the 4 yr of this study, may suggest that the tintinnid community in the Gulf of Naples is gradually changing.

Differences in the occurrence of less abundant tintinnid species may also suggest an adjustment in the tintinnid community, as seems to be the case for *Stenosemella ventricosa.* Observations over several decades have reported *S. ventricosa* to be among the most abundant tintinnid species in the western Mediterranean Sea (Rampi 1948, Margalef et al. 1957, Margalef & Morales 1960, Vitiello 1964, Travers & Travers 1971, Rassoulzadegan 1979, Dolan 2000). In 1984-85, *S. ventricosa* was found in low abundances at –30 m at Stn MC; it occurred at the surface from December till March (when the water column is well mixed) at concentrations up to 750 cells l^{-1} (Scotto di Carlo et al. 1985, Petrillo 1988). Rassoulzadegan (1979) reported maximum concentrations of 300 cells l^{-1} at Villefranche, but due to low total tintinnid numbers, *S. ventricosa* resulted as one of the dominant tintinnid species. In our surface samples, only single specimens of *S. ventricosa* were encountered, suggesting a retreat from our study area of this very widely distributed species.

Conclusions

In conclusion, each area seems to be characterised by a specific fingerprint in dominant tintinnid species, which persist in time. Our data provide evidence that tintinnid assemblages are able to cope with environmental changes such as the ones observed in phytoplankton composition and biomass in the Gulf of Naples since 1984, maintaining the same annual mean in total numbers and seasonal cycles of the most abundant species. On the other hand, essential changes in the tintinnid community have occurred since the study of Issel (1934). Seventy years ago the city of Naples was not yet industrialised, and the quality and quantity of land runoff have changed considerably over this long time span. To our knowledge, a change in dominant tintinnid species, such as the one that has occurred in the Gulf of Naples, has not been reported for other sites. It would be of great interest to verify whether the tintinnid species composition has changed at the sites where previous information on tintinnid assemblages is available. Furthermore, past reports on tintinnid species in the western Mediterranean have shown that dominant species are very similar at different coastal sites around the basin. On the other hand, differences in plankton populations between Mediterranean coastal sites, in particular as regards mesozooplankton, have been discussed in a recent study (Calbet et al. 2001). It would be worthwhile to see whether the similarities in key tintinnid species reported along the north Italian, French, Spanish and Algerian coasts are still valid or whether a major differentiation has occurred among these sites.

Acknowledgements. Thanks are due to Dr. Adrianna Ianora for very helpful criticism and discussion, and to 3 anonymous referees for constructive suggestions on an earlier draft of this manuscript.

LITERATURE CITED

Abboud-Abi Saab M (1989) Distribution and ecology of tintinnids in the plankton of Lebanese coastal waters (eastern Mediterranean). J Plankton Res 11:203–222

Bakker C, Phaff WJ (1976) Tintinnida from coastal waters of the S.W.-Netherlands I. The genus *Tintinnopsis* Stein. Hydrobiologia 50:101–111

Balech E (1959) Tintinnoinea del Mediterráneo. Trab Inst Esp Oceanogr 28:1–88

Calbet A, Garrido S, Saiz E, Alcaraz M, Duarte CM (2001) Annual zooplankton succession in coastal NW Mediterranean waters: the importance of the smaller size fractions. J Plankton Res 23:319–331

Capriulo GM, Carpenter EJ (1983) Abundance, species composition and feeding impact of tintinnid microzooplankton in Central Long Island Sound. Mar Ecol Prog Ser 10: 277–288

Capriulo GM, Sherr EB, Sherr BF (1991) Trophic behavior and related community feeding activities of heterotrophic marine protists. In: Reid PC, Turley CM, Burkill PH (eds) Protozoa and their role in marine processes. NATO ASI Ser G25. Springer-Verlag, Berlin, p 219–279

Cariou JB, Dolan JR, Dallot S (1999) A preliminary study of tintinnid diversity in the NW Mediterranean Sea. J Plankton Res 21:1065–1075

Castaldo S (2000) Diversità e struttura trofica dei ciliati planctonici del Golfo di Napoli. Thesis, University of Naples Parthenope

Castaldo S, Modigh M (2001) Trophic groups and short-term variations in a coastal ciliate community. Rapp Comm Int Mer Medit 36:368

Dolan JR (2000) Tintinnid ciliate diversity in the Mediterranean Sea: longitudinal patterns related to water column structure in late spring-early summer. Aquat Microb Ecol 22:69–78

Finlay BJ, Fenchel T (1999) Divergent perspectives on protist species richness. Protist 150:229–233

Foissner W (1999) Protist diversity: estimates of the near-imponderable. Protist 150:363–368

Gilron GL, Lynn DH, Roff JC (1991) The annual cycle of biomass and production of tintinnine ciliates in a tropical neritic region near Kingston, Jamaica. Mar Microb Food Webs 5:95–113

Issel R (1934) Ciclo annuale del microplancton di superficie nel Golfo di Napoli (golfo interno). Pubbl Staz Zool Napoli 14:1–50

Jörgensen E (1924) Mediterranean Tintinnidae. Rep Danish Oceanographical Expeditions 1908–1910 to the Mediterranean and adjacent seas II(J3). Host AF & Son, Copenhagen, p 1–110

Kamiyama T, Tsujino M (1996) Seasonal variation in the species composition of tintinnid ciliates in Hiroshima Bay, the Seto Inland Sea of Japan. J Plankton Res 18:2313–2327

Kofoid CA, Campbell AS (1929) A conspectus of the marine and freshwater 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

Kofoid CA, Campbell AS (1939) The Ciliata: the Tintinnoinea. Bull Mus Comp Zool Harvard Coll 84:1–473

Laval-Peuto M (1981) Construction of the lorica in Ciliata Tintinnina. *In vivo* study of *Favella ehrenbergii*: variability of the phenotypes during the cycle, biology, statistics, biometry. Protistologica 17:249–272

Laval-Peuto M (1982) Methods of taxonomy and selection of criteria for determination of marine planktonic protozoa. Ann Inst Oceanogr Paris 58(Suppl):151–168

Laval-Peuto M, Brownlee DC (1986) Identification and systematics of the Tintinnina (Ciliophora): evaluation and suggestions for improvement. Ann Inst Oceanogr Paris 62: 69–84

Laval-Peuto M, Rassoulzadegan F (1988) Autofluorescence of marine planktonic Oligotrichina and other ciliates. Hydrobiologia 159:99–110

Lynn DH, Montagnes DJS (1991) Global production of heterotrophic marine planktonic ciliates. In: Reid PC, Turley CM, Burkill PH (eds) Protozoa and their role in marine processes. NATO ASI Ser G25. Springer-Verlag, Berlin, p 281–307

Magurran AE (1988) Ecological diversity and its measurement. Chapman and Hall, London

Margalef R (1963) Rôle des ciliés dans le cycle de la vie pélagique en Méditerranée. Rapp Comm Int Mer Medit 17: 511–512

Margalef R, Duran M (1953) Microplancton de Vigo, de octubre de 1951 a septiembre de 1952. Publ Inst Biol Appl 13:5–78

Margalef R, Morales E (1960) Fitoplancton de las costas de Blanes (Gerona), de julio de 1956 a junio de 1959. Invest Pesq 16:3–31

Margalef R, Muñoz F, Herrera J (1957) Fitoplancton de las costas de Castellón de enero de 1955 a junio de 1956. Invest Pesq 7:3–31

Mazzocchi MG, Ribera d'Alcalà M (1995) Recurrent patterns in zooplankton structure and succession in a variable coastal environment. ICES J Mar Sci 52:679–691

Modigh M (2001) Seasonal variations of photosynthetic ciliates at a Mediterranean coastal site. Aquat Microb Ecol 23:163–175

Modigh M, Saggiomo V, Ribera d'Alcalà M (1996) Conservative features of picoplankton in a Mediterranean eutrophic area, the Bay of Naples. J Plankton Res 18:87–95

Navarro P, Massuti M (1940) Composición y ciclo anual del plancton superficial de la bahía de Palma de Mallorca. Inst Esp Oceanogr Notas Resum 2:1–62

Neveux J, Panouse M (1987) Spectrofluorometric determination of chlorophylls and phaeophytins. Arch Hydrobiol 109:567–581

Nielsen TG, Kiørboe T (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol Oceanogr 39:508–519

Ounissi M, Frehi H (1990) Variabilité du microphytoplancton et des Tintinnida (Protozoaires Ciliés) d'un secteur eutrophe du golfe d'Annaba (Mediterranée sud-occidentale). Cah Biol Mar 40:141–153

Paffenhöfer GA (1998) Heterotrophic protozoa and small metazoa: feeding rates and prey-consumer interactions. J Plankton Res 20:121–133

Perez MT, Dolan JR, Fukai E (1997) Planktonic oligotrich ciliates in the NW Mediterranean: growth rates and consumption by copepods. Mar Ecol Prog Ser 155:89–101

Petrillo L (1988) Ciclo annuale dei ciliati in un'area interna del Golfo di Napoli. Thesis, University of Naples Federico II

Pierce RW, Turner JT (1992) Ecology of planktonic ciliates in marine food webs. Rev Aquat Sci 6:139–181

Pierce RW, Turner JT (1993) Global biogeography of marine tintinnids. Mar Ecol Prog Ser 94:11–26

Pierce RW, Turner JT (1994) Plankton studies in Buzzards Bay, Massachusetts, USA. IV. Tintinnids, 1987–1988. Mar Ecol Prog Ser 112:235–240

Pitta P, Giannakourou A, Christaki U (2001) Planktonic ciliates in the oligotrophic Mediterranean Sea: longitudinal trends of standing stocks, distribution and analysis of food vacuole contents. Aquat Microb Ecol 24:297–311

Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097–1103

Rampi L (1948) I tintinnoidi delle acque di San Remo. Boll Pesca Piscic Idrobiol 24:50–56

Rassoulzadegan F (1979) Évolution annuelle des ciliés pélagiques en Mediterranée nord-occidentale II. Ciliés oligotriches. Tintinnides (Tintinnina). Invest Pesq 43:417–448

Ribera d'Alcalà M, Conversano F, Corato F, Licandro P and 9 others (2002) Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. Sci Mar (in press)

Sanders RW (1987) Tintinnids and other microzooplankton: seasonal distributions and relationships to resources and hydrography in a Maine estuary. J Plankton Res 9:65–77

Satta MP, Agusti S, Mura MP, Vaque D, Duarte CM (1996) Microplankton respiration and net community metabolism in a bay on the NW Mediterranean coast. Aquat Microb Ecol 10:165–172

Scotto di Carlo B, Tomas CR, Ianora A, Marino D and 7 others (1985) Uno studio integrato dell'ecosistema pelagico costiero del Golfo di Napoli. Nova Thalassia 7:99–128

Sherr EB, Sherr BF, Paffenhöffer GA (1986) Phagotrophic protozoa as food for metazoans: a missing trophic link in the marine pelagic food webs. Mar Microb Food Webs 1:61–80

Stelfox CE, Burkill PH, Edwards ES, Harris RP, Sleigh MA (1999) The structure of zooplankton communities, in the 2 to 2000 µm size range, in the Arabian Sea during and after the SW monsoon, 1994. Deep Sea Res 46:815–842

Thompson GA, Alder VA, Boltovskoy D, Brandini F (1999) Abundance and biogeography of tintinnids (Ciliophora) and associated microzooplankton in the Southwestern Atlantic Ocean. J Plankton Res 21:1265–1298

Travers A, Travers M (1971) Catalogue des Tintinnides (Cilies Oligotriches) récoltés dans le Golfe de Marseille de 1962 à 1964. Tethys 2:639–646

Travers M (1973) Le microplancton du Golfe de Marseille: variations de la composition systématique et de la densité des populations. Tethys 5:31–53

Verity PG (1987) Abundance, community composition, size distribution, and production rates of tintinnids in Narragansett Bay, Rhode Island. Estuar Coast Shelf Sci 24: 671–690

Verity PG, Langdon C (1984) Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J Plankton Res 6:859–868

Vitiello P (1964) Contribucion à l'étude des Tintinnides de la baie d'Alger. Pelagos II:5–42

Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: August 17, 2001; Accepted: February 19, 2002
Proofs received from author(s): July 2, 2002