Seasonal succession of tintinnids in the Nervión River estuary, Basque Country, Spain

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The seasonal succession of tintinnids was examined in the outer part of the Nervión River estuary. Sampling was carried out at monthly intervals from March 2000 to March 2002. In this period, 21 species, belonging to 12 genera, were recorded, among which Tintinnopsis was the most abundant genus, contributing up to 86% of the total ciliate abundance. The maximum abundance was recorded in summer, with $7.4 \times 10^4$ individuals L$^{-1}$ in July 2001, while the lowest value occurred in winter. A significant and positive correlation was found between temperature and tintinnid abundance. Most of the species showed a distinct seasonal occurrence and on this basis five different groups were differentiated. Two main changes in the species composition were recorded, one in March–April and the second in October–November. In this paper, the seasonal dynamics and the spatial distribution, as well as remarks on the morphology and ecology, of the most important tintinnid species in the estuary are given.

INTRODUCTION

Microzooplankton play an important role in transferring nanoplankton production to higher trophic levels. Although tintinnids comprise a minimal portion (5–10%) of the total ciliate abundance or biomass (Dolan and Marrasé, 1995; Dolan, 2000), they are an important component of the planktonic microprotozoan community as they can exert a large grazing pressure on nanophytoplankton assemblages. There have been many studies which have quantified the significance of tintinnids as grazers on nano- and picoplankton (Capriulo et al., 1991, 2002). Field investigations carried out by different authors [e.g. (Verity, 1987)] suggest that tintinnids can remove large portions (10–27%) of the daily primary production in some coastal waters.

Tintinnids are ideal organisms for the study of changes in the structure or composition of microzooplankton communities because changes in the composition of their populations can easily be detected since species identification is based upon morphological characteristics of preservable loricae (Dolan and Gallegos, 2001). There are many reports in the literature on tintinnid taxonomy as well as on the abundance of common species (Balech, 1962; Marshall, 1969; Bakker and Phaff, 1976; Krsinic, 1987; Verity, 1987; Pierce and Turner, 1992). However, in recent years interest in the ecology of tintinnids has apparently decreased.

A basic knowledge of the diversity of the microbial community is required to assess the changes that could occur in different microbial populations as a result of changes in the environment. Very few data exist on the diversity and distribution of microzooplankton in the eastern Cantabrian Sea, and quantitative data have not been reported from this area. The Nervión River estuary is a eutrophic estuary, which receives large amounts of nutrients and organic compounds from a Wastewater Treatment Plant located in the middle estuary. However, the water quality has undergone a remarkable improvement since 1996.

The aim of the present study is to clarify seasonal and interannual variability of the tintinnid population on a local scale. The distribution and seasonal dynamics of the tintinnid population are described over 25 months in the outer zone of the Nervión River estuary.

METHOD

Surface-water samples were collected monthly from eight stations located along the Nervión River estuary,
Bay of Biscay (43°20'N, 3°00'W), from March 2000 to March 2002 (Figure 1). However, as tintinnids were restricted to stations 1–3, only data corresponding to this area are reported here. At each sampling site, salinity and temperature were measured. The depth of the estuary ranged between 9 and 20 m. Samples were preserved with 0.1% glutaraldehyde and stored in the dark at 4°C. A total of 225 samples were analysed. For ciliate enumeration, 10–50 ml subsamples were allowed to settle in an Utermöhl chamber; larger volumes were not settled because of the high seston content of the water. The whole chamber area was examined at 100× magnification for most species. For the smallest individuals, several transects were analysed at 400×. An additional water sample was taken with a net (45 µm mesh size) to analyse the complete tintinnid diversity present in the outer estuary. Tintinnids were identified using the morphology of the lorica following Marshall (Marshall, 1969) and Bakker and Phaff (Bakker and Phaff, 1976). When possible, at least 30 individuals were measured.

For chlorophyll a (Chl a) quantification an additional 1 L water sample was immediately filtered through Whatman GF/F filters and extracted in 90% acetone for each station. Chl a concentration was determined using a Shimadzu UV-160A spectrophotometer.

Statistical analyses were performed using the SPSS 7.5.1 statistical package. The relationship between environmental factors and the log-transformed abundance of each tintinnid species was analysed with bivariate correlations.

RESULTS

Environmental factors
Surface salinity ranged between 5.2 and 34.8 p.s.u. during the study period (Figure 2a). Salinity remained >20 p.s.u. during late spring and summer, decreasing markedly in autumn and winter. The lowest value was recorded in November 2000 at station 3, as a result of heavy rains and the resultant river run-off.

Surface temperature showed a typical seasonal cycle, with values up to 22°C in summer and close to 10°C in winter (Figure 2b).

Chl a concentration remained relatively low during the autumn–early spring period but increased in late spring and summer, reaching a peak of 10 mg L⁻¹ at station 2 (Figure 2c). Another peak was observed in April 2000 at station 2. The highest values were generally registered at the innermost stations.

Seasonal dynamics and distribution of the tintinnid community
Maximum ciliate abundance in the estuary was ~3 × 10⁵ individuals L⁻¹. Tintinnids contributed to total ciliate abundance with a mean of 1.5%, being higher at station 1 and decreasing towards the inner estuarine zone. In July and October 2001, the tintinnid contribution was ~20%.

Twenty-one tintinnid taxa were found, 20 of them identified to species level (Table I). Taxa were assigned to 12 different orders, according to Small and Lynn (Small and Lynn, 1985). Tintinnid abundance ranged between total absence and 7.4 × 10³ individuals L⁻¹, the latter being observed in July 2001 at station 1. Tintinnids had a seaward distribution, being restricted to the outer estuary (stations 1–3), where salinity was normally >25 p.s.u. (Figures 2 and 3). Tintinnid abundance showed marked seasonal changes, with the highest cell concentrations observed in July of both years and in September 2000 and October 2001.

The agglutinated genus Tintinnopsis was the most common and diverse, accounting for up to 86% of the total tintinnid abundance. Other relevant taxa were Stenosmella, Eutintinnus and Favella. Hyaline species were more common during 2001, but they were normally observed in low densities.

Seasonal succession of tintinnid species
The distribution of the different taxa of tintinnids was characterized by a clear seasonality (Figure 4). Five different groups of species were discerned depending on their seasonal behaviour.
The first group was composed of autumn–winter species, such as *Tintinnopsis parva* and *Stenosemella nivalis*. The former was present from November to January and the latter from November to April. A second group was formed by winter–early spring species, such as *Stenosemella ventricosa*, which was the most abundant species within the group, appearing from January to April. These two groups were composed only of agglutinated species and appeared when temperatures were $<16^\circ C$ and salinity values were relatively low, especially at station 3.

The third group was present in spring when water temperatures were $>15^\circ C$. The hyaline species *Eutintinnus latus* and *Undella subcaudata* subsp. *subcaudata* were the most noticeable species of this group. From May and June the spring–summer species appeared, some of them lasting until October. *Tintinnopsis beroidea* was the most abundant species during this period. The last group was composed of summer species, such as *Eutintinnus* sp. and *Tintinnopsis campanula*. The fourth and fifth groups appeared during the period of greater stability, when the highest tintinnid abundances were recorded, and when temperatures and salinity values were high.

During the sampling period a clear succession of species was recorded with two main changes in species composition, the first in March–April and the second in October–November.

**Table I: List of tintinnid species encountered in the Nervión River estuary**

<table>
<thead>
<tr>
<th>Tintinnid species</th>
<th>Metacylis joergensenii</th>
<th>Favella ehrenbergii</th>
<th>Eutintinnus latus</th>
<th>Salpingella decurtata</th>
<th>Steenstrupiella steenstrupii</th>
<th>Undella claparedei</th>
<th>Undella subcaudata subsp. subcaudata</th>
<th>Undella subcaudata subsp. subcaudata</th>
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<td>Codonella aspera</td>
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<td>Tintinnopsis baltica</td>
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<td>Tintinnopsis beroidea</td>
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<td>Tintinnopsis campanula</td>
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<td>Tintinnopsis cylindrica</td>
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<td>Tintinnopsis levigata</td>
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<td>Tintinnopsis parva</td>
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<td>Stenosemella nivalis</td>
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<td>Stenosemella ventricosa</td>
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<td>Dictyocysta elegans</td>
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<td>Helicotomella subulata</td>
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Morphological and ecological remarks on the most relevant species

*Stenosemella nivalis*

*Stenosemella nivalis* was one of the smallest tintinnids present in the estuary, with a length ranging from 35 to 60 μm and an oral diameter of ~20 μm, ranging from 15 to 25 μm (Figure 6e). Some individuals had particles attached to the hyaline collar.

This tintinnid was present during autumn, winter and early spring, reaching the highest densities, ~300 individuals L⁻¹, in March 2002 (Figure 5a). At this time the water temperature ranged between 10 and 17.8°C. *Stenosemella nivalis* was observed in a broad range of salinity (from 6.8 to 33 p.s.u.) but the highest cell concentrations were registered when salinities were >17 p.s.u.

*Stenosemella ventricosa*

The length of the lorica varied from 75 to 90 μm, and the oral diameter was between 32 and 50 μm. The mean width of the bowl was 67 μm. As in *S. nivalis*, some individuals of *S. ventricosa* had an enlargement in the oral region formed by small particles attached to the hyaline collar (Figure 6f and g).

*Stenosemella ventricosa* only appeared in winter and early spring, when the temperature was between 11 and 16°C. The highest numbers were recorded in February 2001 but they were not >300 individuals L⁻¹ (Figure 5b). Although *S. ventricosa* was observed in salinity values ~9 p.s.u., normally it was more abundant at salinities >17 p.s.u.

*Tintinnopsis beroidea*

The length of the lorica of the agglutinated ciliate *T. beroidea* ranged between 35 and 125 μm (mean 73 μm) but was normally between 60 and 80 μm. The length of the lorica varied throughout the year, depending on the season. The smallest loricae were observed in summer when the temperature was ~22°C, ranging from 40 to 88 μm. In autumn, with temperatures ~18°C, the length of the lorica ranged between 50 and 105 μm. Oral
diameter was very variable, ranging between 20 and 50 μm. The lorica was formed by small-sized particles (2–10 μm) and sometimes showed an enlargement around the oral region (Figure 6c and d).

*Tintinnopsis beroidea* was the most abundant species in the estuary, reaching values >7 × 10^3 individuals L^{-1} in July 2001 at station 1, and contributing up to 78% of the total tintinnid abundance. It was especially abundant in 2001. This species was observed from May to October, when temperatures ranged from 12 to 22°C, although its highest abundances were registered in summer and autumn, with peaks in July 2000 and July and October 2001 (Figure 5c). Despite the fact that this species appeared within a wide range of salinities (between 18 and 35 p.s.u.) it was more abundant at salinities >25 p.s.u.

*Tintinnopsis cylindrica* was generally observed in late summer and early autumn, although it also appeared in May 2001 (Figure 5d), at temperatures between 16.9 and 21.3°C. The highest densities, ~400 individuals L^{-1}, were registered at station 3, when salinity values were >22 p.s.u.

*Favella ehrenbergii*

This was the largest species encountered in the estuary. Its length varied from 150 to 450 μm (mean 250 μm). The largest individuals were observed in June 2001. Oral diameter ranged between 50 and 102 μm but remained relatively constant within each period, being 65–90 μm for individuals in September 2000, 95–102 μm for those in June 2001, and 85–95 μm for those in July 2001. The length of the pedicel ranged between 20 and 55 μm (Figure 6a).

It was present from June to September, but there were always <100 individuals L^{-1} (Figure 5e). During this period the temperature was >17°C and salinity was between 25 and 34 p.s.u.

*Eutintinnus sp.*

Individuals of this species ranged in length from 72 to 100 μm, but were most frequently ~90 μm. Oral
Fig. 6. The most important tintinnid species in the Nervión River estuary, (a) *Favella ehrenbergii*, (b) *Tintinnopsis cylindrica*, (c) and (d) *T. beroidea*, (e) *Stenosemella nivalis*, (f) and (g) *S. ventricosa* and (h) and (i) *Eutintinnus* sp.
diameter varied from 18 to 23 μm, and aboral diameter was between 15 and 19 μm. 

*Eutintinnus* sp. was the second most abundant species in the estuary. It was observed in July 2001 and September of both years, reaching the highest concentration ($1.7 \times 10^3$ individuals L$^{-1}$) in September 2000 at station 3 (Figure 5), coinciding with temperatures between 17.6 and 21.6°C, and salinity $>30$ p.s.u. Some individuals appeared partially covered with small particles (Figure 6h and i).

**DISCUSSION**

The estuary showed a typical seasonal pattern of warm temperate waters, with the maximum temperature $\sim 22^\circ$C in summer, and the lowest values $<10^\circ$C in winter. Salinity values ranged between 5.2 and 34.8 p.s.u. and mainly depended on the river flow conditions. These were a good reflection of the residence time of the water, which was much longer in summer.

The genus *Tintinnopsis* was the most numerous in terms of number of species and abundance. This genus comprised seven of the 21 tintinnid species encountered in the estuary and contributed up to 86% of the total tintinnid abundance. Similar species richness has been reported by some authors in other estuaries and coastal waters (Krsinic, 1987; Sanders, 1987; Verity, 1987; Barria de Cao, 1992; Kamiyama and Tsujino, 1996; Cordeiro et al., 1997). The maximum abundance ($\sim 7.5 \times 10^{-3}$ individuals L$^{-1}$) was also comparable with those observed in similar environments (Sanders, 1987; Barria de Cao, 1992; Pierce and Turner, 1992, 1994; Kamiyama and Tsujino, 1996).

Maximum tintinnid abundance was observed in summer and early autumn as in other coastal waters (Krsinic, 1987; Sanders, 1987; Verity, 1987). A significant and positive correlation was observed between tintinnid abundance and temperature ($0.46$, $P < 0.01$), but not with Chl a. Similar trends were observed by Kamiyama and Tsujino (Kamiyama and Tsujino, 1996). Barria de Cao, however, did not observe any clear correlation between temperature and tintinnid abundance (Barria de Cao, 1992). Meanwhile tintinnid distribution seemed to be restricted by salinity and water turbidity. The low salinity values and the high turbidity observed in the middle and inner estuary precluded the intrusion of tintinnids. The high concentration of small suspended particles in the water may interfere with the filter-feeding mechanism of the tintinnids (Laybourn-Parry et al., 1992).

Most of the tintinnids found in the estuary are considered cosmopolitan or neritic species (Pierce and Turner, 1993). The community was dominated by tintinnids with an agglutinated loria, as they seemed to be more abundant than those with a hyaline loria with decreasing distance from shore, as has been stated by Pierce and Turner (Pierce and Turner, 1992).

Some authors have observed different seasonal dynamics for the same tintinnid species. In this study, *S. rivulais* was present from December to April. A similar distribution pattern was observed by Kamiyama and Tsujino (Kamiyama and Tsujino, 1996) in Hiroshima Bay, and by Krsinic (Krsinic, 1987) in the eastern Adriatic. The presence of *S. ventricosa* in the Nervión River estuary was restricted to winter and early spring, when temperatures were $<16^\circ$C. Krsinic registered the highest abundance of this tintinnid in winter, but it was also present in August (Krsinic, 1987). However, *S. ventricosa* was present from January to December in Narragansett Bay, showing the highest abundances in summer (Verity, 1987). *Tintinnopsis beroidea* was the most abundant species in the estuary and was observed during spring, summer and autumn, reaching highest concentrations in July and October. Similar seasonal dynamics have been observed in Narragansett Bay (Verity, 1987) and Bahia Blanca estuary (Barria de Cao, 1992). Kamiyama and Tsujino found that *T. beroidea* dominated the tintinnid community numerically in Hiroshima Bay (Kamiyama and Tsujino, 1996). It was present throughout the year, although with low densities in summer. *Favella ehrenbergii* showed a spring-summer distribution, as was observed by Krsinic (Krsinic, 1987) and Kamiyama and Tsujino (Kamiyama and Tsujino, 1996).

The unidentified species *Eutintinnus* sp. resembled *Eutintinnus tubulosus*, although the individuals observed in the estuary were smaller (72–100 μm) than those reported by other authors (Balech, 1962; Marshall, 1969; Cariou et al., 1999). However, data reported by Nakamachi and Iwasaki are in the same range as that observed in the estuary (Nakamachi and Iwasaki, 1998). However, the *Eutintinnus* sp. observed in this study lacks the slight brim at the oral end that is typical in *E. tubulosus*.

Most of the tintinnid species observed in the estuary were in the same size range as those observed by Marshall (Marshall, 1969) and by Bakker and Phaff (Bakker and Phaff, 1976). However, differences were observed in the dimensions of some species with respect to data reported in the literature. The maximum lengths observed for *F. ehrenbergii* and *E. latu* in the estuary, 470 and 450 μm, respectively, were greater than those observed by other authors (Silva, 1950; Marshall, 1969; Gold and Morales, 1975; Laval-Perou, 1981). By contrast, *Helicostomella subulata* was shorter (60–235 μm) than most of those reported from other coastal waters (Marshall, 1969; Gold and Morales, 1975; Verity, 1987), while the oral diameter was similar (18–25 μm).
We observed seasonal changes in the lorica length of some species. Thus, *T. beroidea* produced longer loricae in colder waters, lorica length varied from 40–88 μm at 22°C to 50–105 μm at 18°C. *Favella ehrenbergii* showed a similar trend, the largest loricae (250–470 μm) were observed at 18°C, while the smallest loricae (182–265 μm) corresponded to warmer waters. Some authors (Gold and Morales, 1975; Bakker and Phaff, 1976) observed the same tendency in some *Tintinnopsis* species, which showed longer loricae in colder waters. This difference in the length of the lorica between winter and summer specimens could be explained if physiological processes were accelerated as a function of elevated temperature (Gold and Morales, 1975).

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