# Spring microzooplankton composition, biomass and potential grazing in the central Cantabrian coast (southern Bay of Biscay)

**Mario QUEVEDO *, Ricardo ANADÓN**

Dept. Biología de organismos y sistemas, Unidad de Ecología, Universidad de Oviedo, C/ Catedrático R. Uría s/n 33071 Oviedo, España

Received 4 November 1999; revised 14 January 2000; accepted 14 January 2000

**Abstract** – Microzooplankton abundance, biomass and composition were investigated in the coastal waters of Asturias (southern Bay of Biscay) in May 1996. Abundance ranged from $0.7 \times 10^3$ to $8.5 \times 10^3$ cell·L$^{-1}$. The protists community was dominated by aloricate ciliates averaging 82 % of microheterotrophs. Small aloricate ciliates, below 20 µm in size, contributed 63 % to total ciliate abundance. Carbon biomass ranged from 2.4 to 15.4 mgC·m$^{-3}$, averaging 23 % of phytoplankton biomass. Aloricate ciliates were also the dominant component of biomass (56.2 %), but the importance of copepod nauplii increased in terms of carbon, averaging 28.5 % of total biomass. Microzooplankton biomass was significantly correlated with Chl *a* concentration in the water column. Theoretical estimates of the grazing impact of the microzooplankton community on phytoplankton were calculated and resulted in an average value of 28.3 % of phytoplankton standing stock potentially consumed per day. Choreotrich ciliates were the most important potential grazers in the study (13.7 % of standing stock) followed by metazoan nauplii (6.3 %). The potential microzooplankton impact on phytoplankton was consistent with the large size of primary producers during diatom spring blooms, like the one found in this study. The validity of theoretical estimates of microzooplankton grazing impact, as well as the importance of including metazoan larvae in coastal microzooplankton community studies are discussed. © 2000 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

**microzooplankton / ciliates / biomass / grazing / southern Bay of Biscay**

**Résumé – Composition, biomasse et broutage potentiel du microzooplancton au printemps sur la côte centrale cantabrique (golfe de Gascogne).** L'abondance, la biomasse et la composition du microzooplancton ont été analysées dans les eaux côtières des Asturies (golfe de Gascogne) en mai 1996. L'abondance variait entre $0,7 \times 10^3$ et $8,5 \times 10^3$ cellules·L$^{-1}$. La communauté des protistes est dominée par des ciliés aloricates, représentant, en moyenne, 82 % des microhétérotrophes. De petits ciliés choréotriches, d'une taille inférieure à 20 µm, forment 63 % de l'abondance des ciliés. La biomasse de carbone varie de 2,4 à 15,4 mgC·m$^{-3}$ correspondant, en moyenne, à 23 % de la biomasse du phytoplancton. Cette biomasse est encore dominée par les ciliés aloricates (56 %), mais l'importance des nauplii de copépodes augmente, représentant, en moyenne, 28,5 % de la biomasse totale. La biomasse du microzooplancton est correlée significativement avec la concentration de Chl *a* dans la colonne d'eau. Des estimations théoriques de l'impact du broutage donnent une valeur moyenne de 28,3 % de la biomasse du phytoplancton consommée par jour. Les ciliés choréotriches sont les consommateurs les plus importants (13,7 % de le biomasse), suivis par les nauplii métazoaires (6,3 %). L'impact du microzooplancton sur le phytoplancton est dû à la grande taille des producteurs primaires pendant la floraison printanière de diatomées. Nous discutons la validité des estimations théoriques de l'impact de broutage, ainsi que l'importance d'inclure les larves métazoaires dans les études sur le microzooplancton côtier. © 2000 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

**microzooplancton / ciliés / biomasse / broutage / golfe de Gascogne**

* Correspondence and reprints: mquevedo@sci.cpd.uniovi.es


PII: S0399-1784(00)00128-6/FLA

## 1. INTRODUCTION

The term microzooplankton in marine pelagic environments commonly comprises both phagotrophic protozoa and larval stages of metazoan organisms. Within the protozoan assemblage, heterotrophic dinoflagellates and ciliates of the orders Choreotrichia and Oligotrichida [15, 33] are ubiquitous and usually dominate the community. Metazoan organisms in the microplankton size range are often dominated by copepod nauplii, whose biomass might sometimes be close to that of the Protozoa [32].

The importance of microzooplankton within pelagic food webs resides in its role as both consumer of phytoplankton and as prey for metazoans. Here, phagotrophic protists are considered as grazers of both bacteria and phytoplankton in pelagic systems [39], as well as a nutritionally important food source for many invertebrate zooplankton and fish larvae at the onset of exogenous feeding [41]

Although some work has been done in the Cantabrian area regarding microplankton community structure and its biochemical composition [2, 10, 11], little specific attention has been focused on its phagotrophic components. The area of study has been shown to be characterized by spring to summer transition from autotrophy to heterotrophy in the production–respiration balance, and by high contribution of non-phytoplanktonic respiration (ca. above 80 %) to total respiration rate on an annual basis [37]. In this context, our study might be theoretically framed in a coastal ecosystem as defined by Longhurst [23], during a net autotrophic period according to the data recently presented by Serret et al. [37]. Therefore, the aim of this study is to assess the composition and biomass of the microzooplankton

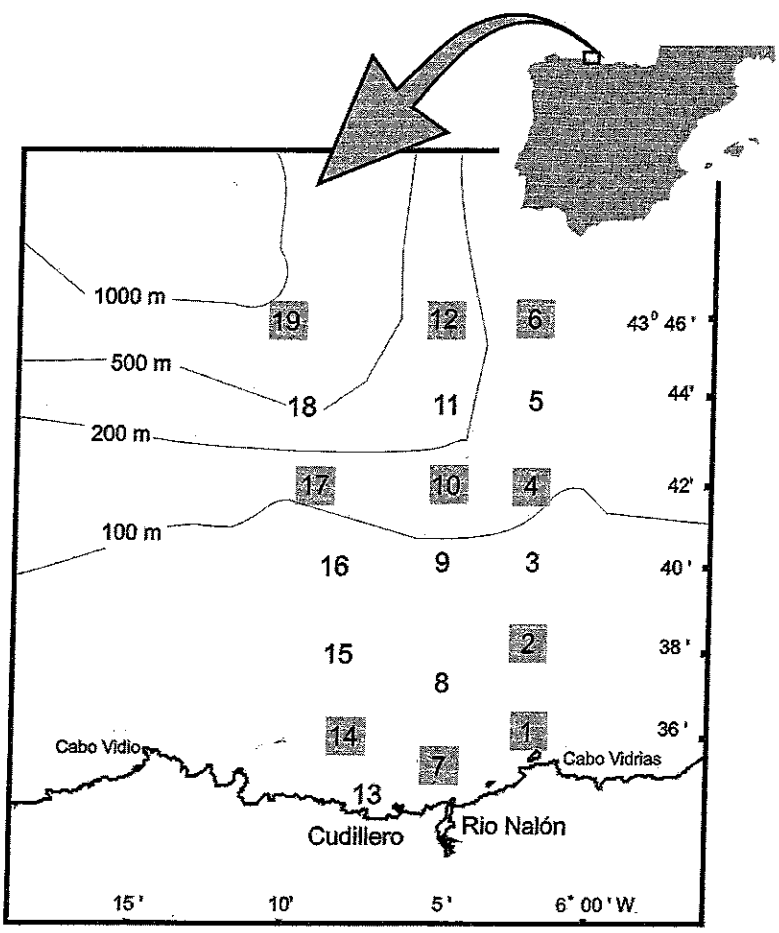


**Figure 1.** Study area. Shading indicates hydrographic and biological station.

community of the central Cantabrian Sea during a productive phase of the annual cycle. Estimates of the potential grazing impact on phytoplankton standing stock are also presented and discussed.

## 2. METHODS

### 2.1. Area of study

The study was carried out in the coastal waters of Asturias (central Cantabrian Sea, NW Spain) between 5 and 15 May 1996. This area is characterized by a narrow continental shelf and the presence of the Avilés canyon, resulting in a maximum depth of 800 m at station 19 (*figure 1*). During the spring, eastward currents and intrusions of high salinity water dominate the hydrography and saline stratification develops close to the coast as a consequence of freshwater inputs [3]. The sampling grid occupied the area of influence of the Nalón river, which is the most important Cantabrian river in terms of allochthonous nutrient fluxes to the Bay of Biscay [34].

### 2.2. Hydrography and phytoplankton

Sampling took place aboard R.V. José Rioja along three coast-ocean transects and a total number of 19 stations were sampled. Vertical profiles of temperature, salinity and density were obtained with a SBE 25 CTD at every station (except stations 18 and 19 due to a CTD failure). At selected stations (*figure 1*), water samples for analysis of environmental variables and plankton were collected from 10 m depth intervals using 5 litre Niskin bottles. In order to estimate phytoplankton biomass, 200 mL water samples were filtered through Whatmann 25 mm GF/F filters that were subsequently frozen. Chlorophyll *a* concentration was determined using a Turner Designs fluorometer after extraction in 90 % acetone for 24 h at 4 °C [43]. For the determination of carbon uptake by phytoplankton, triplicate 70 mL acid-washed polycarbonate bottles were filled from each depth, inoculated with 370 Kbq (10 μCi) of $NaH^{14}CO_3$, and placed in outdoor water-cooled incubators. Samples were incubated for 24 h (14/10 light/dark) at an irradiance that simulated in situ conditions. When the incubation had finished, samples were filtered through Whatman GF/F filters under low vacuum pressure. The filters were then frozen and stored at −20 °C until analysis. Samples were counted in a liquid scintillation Packard counter, after addition of Optiphase Hi-safe scintillation liquid.

Phytoplankton composition was qualitatively analysed by settling 100 mL for 24 h and identifying the most abundant species from five acidic Lugol's samples, corresponding to the surface at station 1 plus surface and mid depths of stations 4 and 6.

### 2.3. Microzooplankton abundance and biomass

Microzooplankton samples were collected from three depths, which were 0, 10 and 40 m (or maximum depth available) at shallow shelf stations. At the shelf-break or oceanic stations, sampling depths were fixed at 0, 20 and 50 m. These depths were chosen according to previous records on the seasonal average depths of the euphotic layer (1 % of incident irradiance) in the area. These were 35, 42 and 47 m respectively for coastal, slope and oceanic stations [37]. Different sampling strategies were used for common protozoan and larger or rarer protozoan and metazoans. One litre samples were fixed in pre-added acid Lugol's solution, in order to estimate abundance and biomass of common species. Samples were kept at 5 °C in the dark, and counted within 2 months of collection. To estimate the abundance and biomass of larger and rarer organisms (tintinnids, foraminiferans and metazoan nauplii), 5 litres from Niskin bottles were sieved through a 30 μm mesh. The retained material was washed with GF/F filtered seawater to a final volume of 125 mL. These samples were preserved in 2.5 % tetraborate-buffered formaldehyde and also kept cool in the dark until analysis.

For the abundance estimates of common groups, subsamples of 50 to 200 mL (counting at least 100 individuals of the main taxon) were settled for a minimum of 16 h in sedimentation chambers. The entire chamber bottom was processed under an inverted microscope at a magnification of × 100 or × 300. Ciliates were identified to genus level according to Lynnet et al. [24–27]. Since fixation with Lugol solution prevents detection of chloroplasts, only *Laboea strobila* and *Tontonia* spp were counted as

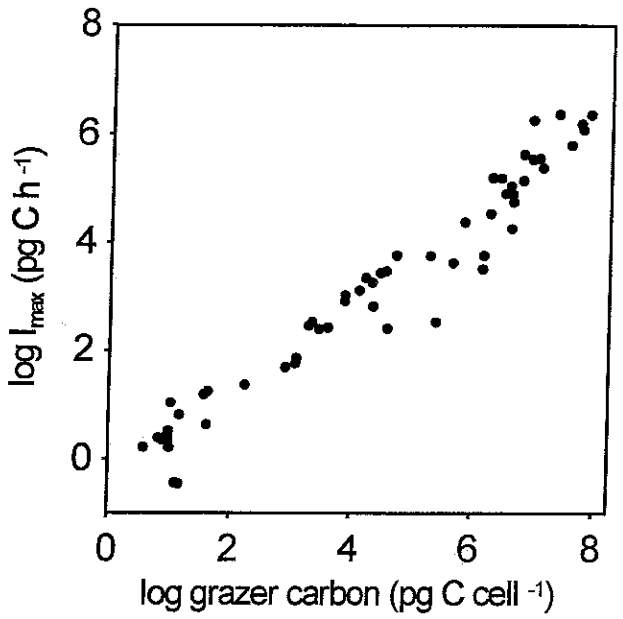


**Figure 2.** Log–log relationship between $I_{max}$ and predator body carbon. Data from Hansen et al. [16].

mixotrophic ciliates. Dinoflagellates were identified to genus level according to Dodge [8]. Though the capability of several photosynthetic dinoflagellates to perform phagotrophy or extracellular digestion is becoming better known [12, 45], only dinoflagellates described as phagotrophs or mixotrophs according to [12, 21, 22] were counted and measured.

Cell biomass was calculated by measuring the linear dimensions of 10–20 individuals of the common groups and of all the rare ones. For this purpose a calibrated ocular micrometer was used and measurements were converted to biovolumes by assuming simple geometric shapes [42]. Carbon content was estimated from biovolume using literature conversion factors: 0.19 pgC·$\mu m^{-3}$ for Lugol-preserved aloricate ciliates [35] and 0.14 pgC·$\mu m^{-3}$ for dinoflagellates [21]. Carbon biomass data were combined with abundance data to obtain total biomass. To estimate biomass of tintinnids, foraminiferans and metazoans, whole formalin-preserved samples were counted using an inverted microscope at a magnification of × 100. The carbon content of tintinnids was estimated by applying a factor of 0.053 pgC·$\mu m^{-3}$ of lorica volume [46]. The volume-carbon conversion factor used for foraminiferans was 0.089 pgC $\mu m^{-3}$ [28]. In the case of copepod nauplii, a dry weight–carapace length relationship was applied [17], and a dry weight–carbon factor of 0.42 $\mu gC\ \mu g^{-1}$ was assumed [20] to give a conversion equation:

$$C\ (\mu g) = ((2.17 \times \text{carapace length (mm)}) - 0.13) \times 0.42$$

### 2.4. Potential grazing impact

Estimates of the potential grazing impact of microzooplankton were based both on ingestion data from the extensive compilation of Hansen et al. [16], and from individual carbon content of each taxa or morphotype obtained from our samples. Hansen's ingestion rate data were adjusted to 14 °C with a $Q_{10}$ value of 2.8, as reported in the same paper. Maximum ingestion rate ($I_{max}$) data were plotted against body carbon to provide an across-taxa log-log equation, $\log I_{max} = 0.84 \log \text{body carbon} - 0.56$, where $n = 65$, $R^2 = 0.93$, $F = 999$ and $P < 0.001$ (*figure 2*). This equation was used to estimate $I_{max}$ from the body carbon of each species or morphotype found in our samples.

Two different estimates of potential grazing impact on phytoplankton standing stock were calculated. The first was calculated from the $I_{max}$ values of each species, as described above. This represents the theoretical maximum grazing impact. To obtain the second estimate, the median $K_m$ (half saturation constant) value for each major group was calculated from Hansen et al. [16]. Then $K_m$, $I_{max}$ and the actual concentration of phytoplankton in pgC $L^{-1}$ were entered into a Michaelis-Menten equation for type II functional response, which yields the estimate $I_{Km}$. According to this, $I_{Km}$ is related to the functional response of each group.

Values for the carbon/chlorophyll ratio have been reported to vary between 30 and 60 [1, 9]. For the estimates in this study, we have used a fixed ratio of 50:1 to reduce the complex data presentation which would result if a range of C:Chl *a* ratios is used. The fluctuation of this ratio would only influence $I_{Km}$ estimates since $I_{max}$ ones depend only on grazer body carbon and temperature. Then, $I_{Km}$ estimates for the ingested phytoplankton standing stock would be 82 % lower than the presented ones if the upper

C:Chl *a* ratio of 60 is used. On the contrary, estimates would be 165 % greater than the ones presented if a ratio of 30 were used

### 2.5. Correlation analysis

A multiple correlation analysis was performed between environmental variables including Chl *a* and primary production and the biomass and abundance of different groups. Individual significance tests for each correlation coefficient were corrected using the Bonferroni sequential technique [36] to prevent type 1 error

## 3. RESULTS

### 3.1. Environmental variables

The hydrographic structure during the survey was characterized by slight stratification and the presence of a smooth thermal shelf-break front. Transects 1 and 2 (stations 1 to 6 and 7 to 12, respectively) showed a band of slightly colder surface temperature and increased vertical mixing above the 100 m isobath. Both coastal and oceanic waters appeared slightly warmer and more stratified. The temperature gradient in the upper 60 m of the water column was less than 1 °C, within the range between 13.4 and 14.2 °C. Salinity showed a minimum value of 30 7 at the surface of station 1, because of the freshwater discharge from the river Nalón. This station also showed a peak in the values of nitrate, which at 9 μmol $L^{-1}$ was almost an order of magnitude higher than the other surface values. Evidently, this station is an exception within the study area, and showed sharp haline stratification (30.7 to 35.3 in the upper 5 m of the water column) and allochthonous inputs of inorganic nutrients. Salinity in the rest of the study area ranged from 35.30 to 35.65‰.

Chl *a* distribution showed maxima at the surface in every station with the exception of stations 19 and 10 The highest value of 2 74 mg·$m^{-3}$ was observed at stations 1 and 17 (*figure 3*). Primary production showed a similar pattern, with peaks of 123 8 and 108 7 mgC·$m^{-3}$·$day^{-1}$ in transect one, i.e., over the continental shelf. Maximum values in transects two and three, over the slope and beyond, were notably lower· 53.8 and 47.4 mgC·$m^{-3}$·$day^{-1}$, respectively (*figure 4*). However, the water column productivity did not show significant differences between the three transects (Kruskal-Wallis ANOVA; $H_{2,45} = 4\ 02$; $P = 0\ 134$)

### 3.2. Phytoplankton composition

Chain-forming diatoms dominated the phytoplankton The dominant species were *Skeletonema* sp. (5 × 3 μm), *Chaetoceros* sp. (5 × 5 μm), *Thalassiosira delicatula* (20 × 15 μm), *Navicula transitans* f. *delicatula* (40 × 8 μm), *Nitzchia seriata* (90 × 10 μm), *Rhizosolenia imbricata* and *R. hebetata* (90 × 18 μm). There were also a few large dinoflagellates like *Ceratium* spp and *Prorocentrum* sp.

### 3.3. Microzooplankton abundance and composition

Total abundance of microzooplankton ranged from $0.68 \times 10^3$ to $8.5 \times 10^3$ cells $L^{-1}$ (*table I*). The highest value was found in surface waters of station 1, coinciding with peaks in Chl *a* (*figure 3*) and primary production (*figure 4*). However, the correlation between total microzooplankton abundance and Chl *a* was not significant after applying Bonferroni correction for multiple correlation tests ($r = 0.48$; $P = 0.018$), and the same occurred with primary production ($r = 0.53$; $P = 0.008$).

During the survey the microzooplankton community was thoroughly dominated by aloricate ciliates of the orders Choreotrichia and Oligotrichia, with concentrations ranging from $6\ 3 \times 10^2$ to $3.7 \times 10^3$ cells·$L^{-1}$ and averaging 81.9 % of overall microheterotrophs abundance (*table I*) Aloricate ciliates clearly determined total abundance profiles in the water column, except for surface water in station 1 (*figure 3*). An important proportion of the abundance of ciliates consisted of cells smaller than 20 μm (the upper limit of nanoplankton size class), which contributed an average of 63 % of total ciliate abundance (*table II*). In the larger size fraction, *Strombidium* and *Strobilidium* were the most common genera (*table III*). These included more than one species or morphotypes that could not be identified because of shrinkage after fixation. The mixotrophic choreotrichs *Laboea stro-*

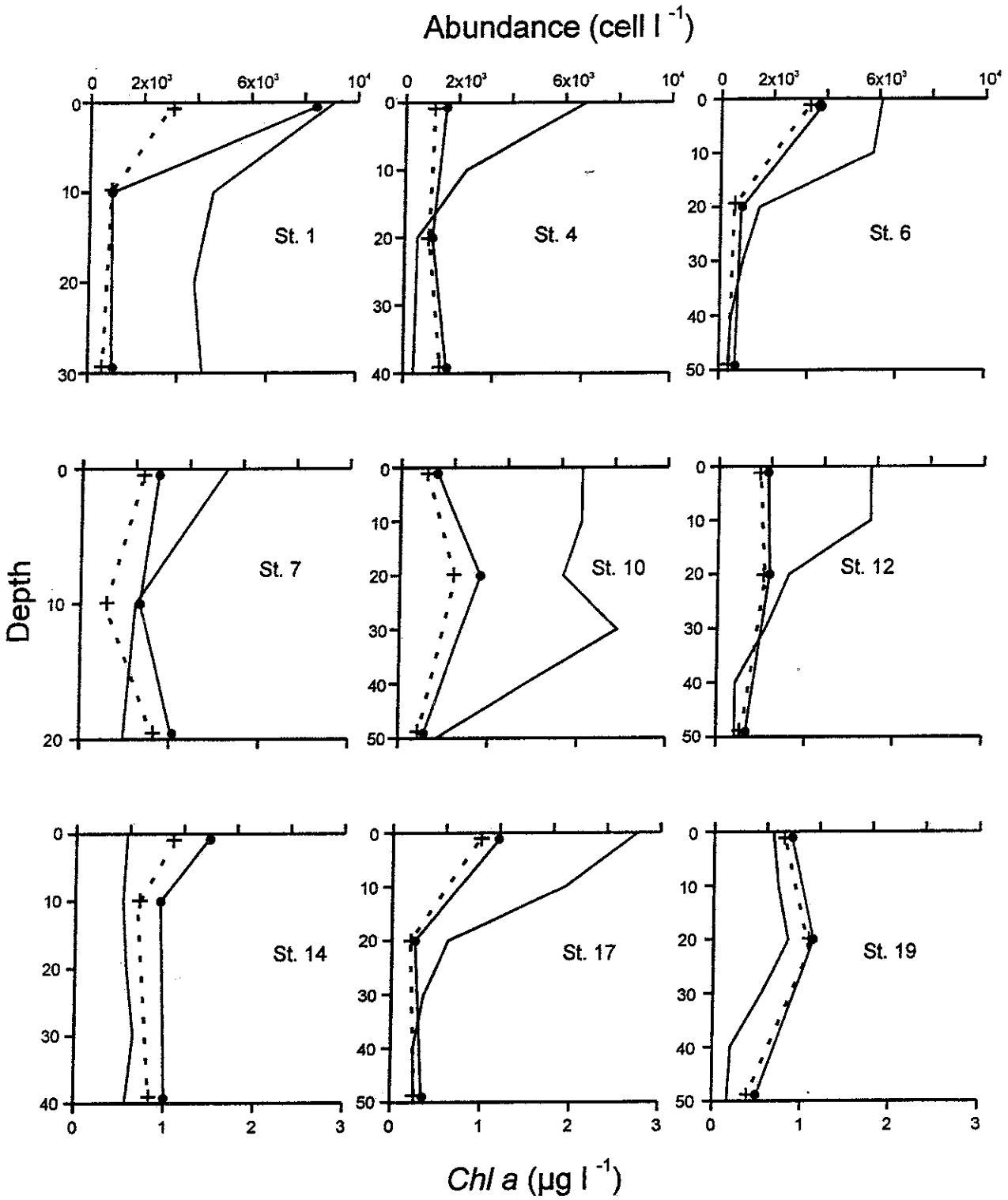


**Figure 3.** Abundances: total microzooplankton (●) and aloricate ciliates (+). Chl *a* concentration profiles at each station (—)

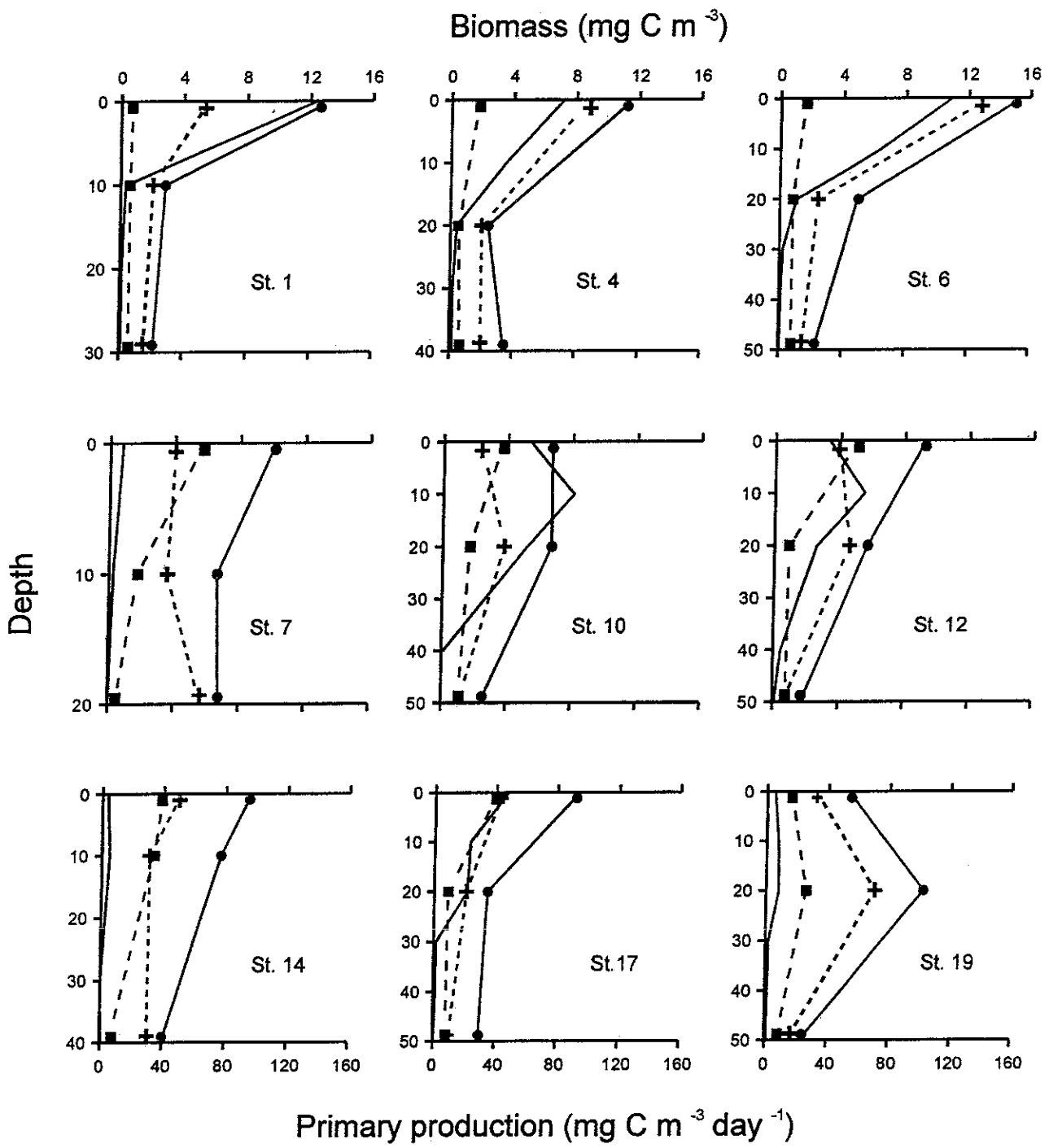


**Figure 4.** Profiles of major groups biomass: total microzooplankton (●), aloricate ciliates (+) and nauplii (■) biomass. Primary production profiles at each station (—).

*bila* and *Tontonia* spp were present in almost all the samples, with the exception of some deep ones. These mixotrophs have comparatively larger size and carbon content than other aloricate ciliates Their abundance range was 0 to 520 cell $L^{-1}$ and contributed an average of 9 % of ciliate abundance (*table II*). The presence of *Laboea strobila* was limited to some surface samples.

Tintinnids (Choreotrichia, Tintinnina) were very rare in all the samples in this study. Although comprising a large number of genera, they appeared always as isolated cells (or simply empty loricas) and did not contribute substantially to the numerical composition of the microzooplankton community. Besides choreotrichs, two ciliates belonging to the order Haptorida, *Rhabdoaskenasia* sp. and *Myrionecta rubra*, were also found in some of the samples. The former is a predatory ciliate and the latter an obligate autotroph.

Dinoflagellates were markedly less abundant (*table I*) than their major protist competitors, the aloricate ciliates. An exception to this was given by the surface water of station 1, where a bloom of a small *Gymnodinium* sp. (probably *G. minus*) reached concentrations of $5.4 \times 10^3$ cells·$L^{-1}$ versus $3.1 \times 10^3$ cells $L^{-1}$ of aloricate ciliates. At the other stations, dinoflagellate abundance was much less, with the most abundant taxa being *Gymnodinium* spp., *Gyrodinium fusiforme* and *Gyrodinium* spp. All of these, except *Gyrodinium fusiforme*, were within $< 20$ µm size fraction (*table III*). Also in the $< 20$ µm fraction there were some *Amphidinium* sp. and *Oxyrrhis marina*. The only heterotrophic thecate dinoflagellate found in most of the samples was *Protoperidinium depressum* This species, however, never contributed substantially to standing stocks.

Foraminiferans were also found in the samples, with juvenile individuals of *Globigerina* spp. and *Globigeri-*

**Table I.** Biomass (mgC $m^{-3}$), abundance (indv $L^{-1}$) and percentage contribution to overall carbon and abundance of each microzooplanktonic major group (only groups with presence in every sample are considered).

| Microzooplankton community | | Mean | Max. | Min. | Percent overall |
|---|---|---|---|---|---|
| Total | Biomass | 6.7 | 15.4 | 2.4 | - |
| | Abundance | 2 450 | 8 500 | 680 | - |
| Ciliates | Biomass | 3.9 | 13.3 | 0.7 | 56.2 |
| | Abundance | 1 930 | 3 720 | 630 | 81 9 |
| Dinoflagellates | Biomass | 0 5 | 6.1 | 0 | 6.3 |
| | Abundance | 557 | 5 380 | 0 | 16.4 |
| Foraminifers | Biomass | 0 5 | 1 7 | 0 1 | 8.7 |
| | Abundance | 5 0 | 9 | 1 6 | 0 3 |
| Nauplii | Biomass | 1 9 | 5 7 | 0.4 | 28 5 |
| | Abundance | 31 | 128 | 5 | 1 5 |

**Table II.** Biomass (mgC $m^{-3}$), abundance (indv $L^{-1}$), cell carbon (ngC $cell^{-1}$) and percent contribution to ciliate carbon of heterotrophic (above and below 20 µm) and mixotrophic ciliates (tintinnids excluded from computations).

| Ciliate community | | Mean | Max. | Min. | Percent overall |
|---|---|---|---|---|---|
| <20 µm | Biomass | 0 5 | 1.4 | 0.1 | 16 |
| | Abundance | 1 152 | 2 500 | 270 | 63 |
| | Cell carbon | 0.4 | 0 8 | 0.1 | |
| >20 µm | Biomass | 1 7 | 11 9 | 0 1 | 44 |
| | Abundance | 500 | 2 530 | 80 | 28 |
| | Cell carbon | 3.4 | 9.6 | 0 7 | |
| Mixotrophs | Biomass | 1 5 | 5.4 | 0 | 39 |
| | Abundance | 135 | 520 | 0 | 9 |
| | Cell carbon | 11 | 26 | 3 | |

**Table III.** Composition and biometric characters of microzooplanktonic protozoan community Mean length ± s.e (µm). Mean biovolume ± s.e. ($\mu m^3 \times 10^3$) Occur = Frequency of occurrence (1, rare; 2, <50 % of the samples; 3, >50 % of samples; 4, widespread). % ab. = percentage of overall abundance

| | Taxa | Mean length | Mean Bv | Occur | % ab. |
|---|---|---|---|---|---|
| Class | SPIROTRICHEA | | | | |
| Order | Oligotrichida | | | | |
| | *Strombidium* spp. | 56 ± 5 | 38 ± 12 | 4 | 9 |
| | *Tontonia spp* | 76 ± 5 | 61 ± 8 | 3 | 5 |
| | *Laboea strobila* | 89 ± 4 | 45 ± 6 | 2 | 1 |
| Order | Choreotrichida | | | | |
| So | Strobilidiina | | | | |
| | *Strobilidium* spp. | 38 ± 2 | 34 ± 5 | 4 | 7 |
| | Naked ciliates<20 µm | 16 ± 0.5 | 2.2 ± 0.2 | 4 | 48 |
| So | Tintinnina | | | | |
| | *Acanthostomella norvegica* | 26 | 9 | | |
| | *Amphorides* sp. | 105 | 113 | 1 | <1 |
| | *Codonella aspera* | 88 | 139 | 1 | <1 |
| | *Dyctiocista speciosa* | 63 | 75 | 1 | <1 |
| | *Petalotricha major* | 110 | 445 | 1 | <1 |
| | *Proplectella ovata* | 69 | 113 | 1 | <1 |
| | *Salpingella* sp. | 233 | 36 | 1 | <1 |
| | *Stenosemella ventricosa* | 35 | 18 | 1 | <1 |
| | *Xistonella* sp. | 154 | 81 | 1 | <1 |
| Class | LITOSTOMATEA | | | | |
| Order | Haptorida | | | | |
| | *Myrionecta rubra* | 20 ± 2 | 4.1 ± 1 | 1 | <1 |
| | *Rhabdoaskenasia* sp. | 28 ± 2 | 13 ± 2 | 2 | 1 |
| Class | DYNOPHICEAE | | | | |
| Order | Gymnodiniales | | | | |
| | *Gyrodinium* sp. | 19 ± 2 | 4 ± 1 | 2 | 2 |
| | *Gyrodinium fusiforme* | 46 ± 4 | 14 ± 6 | 2 | 1.5 |
| | *Gymnodinium* sp. | 19 ± 1 | 3.8 ± 0.5 | 3 | 18 |
| | *Protoperidinium depressum* | 74 | 190 | 3 | <1 |
| | *Protoperidinium brevipes* | 40 | 33 | 1 | <1 |
| | *Dinophysis rotundata* | 40 | 33 | 2 | <1 |
| | *Oxyrrhis marina* | 12 | 1,5 | 1 | <1 |
| Class | FORAMINIFERA | | | | |
| | *Globigerinoides ruber* | 68 ± 1 | 180 ± 7 | 3 | <1 |
| | *Globigerina* spp. | 130 ± 14 | 2211 ± 619 | 4 | <1 |

*noides ruber* present in all the samples, although in low abundance (*tables 1* and *III*).

Metazoans were mainly represented by copepod nauplii, which exhibited concentrations ranging from 5 to 128 indv·$L^{-1}$ and always less than 5 % of total abundance. Copepod nauplii were more abundant in surface samples, except at station 19. The highest value was found at station 7 with 128 nauplii·$L^{-1}$ in surface water, while the lowest was found in the surface waters of the station 1 with 5 nauplii·$L^{-1}$.

### 3.4. Standing Stock

Total carbon ranged from 2.4 to 15.4 mgC·$m^{-3}$ and generally decreased with depth (*figure 4*), in a similar way to phytoplankton biomass. Microzooplankton biomass corresponded on average to 23 % (± 3 s.e.) of the phytoplankton biomass (at a C:Chl *a* ratio of 50:1). The highest values were recorded in surface water samples along transect 1.

As was the case with abundance, biomass was dominated by aloricate ciliates in most of the samples

(*figure 4*). These accounted for 0 7 to 13 3 mgC·m$^{-3}$, averaging 56 % of total community carbon (*table I*). Within the ciliates, mixotrophs constituted the largest fraction of the biomass averaging 39 % (*table II*) due to their high cell carbon content (more than 3 times the cell carbon of choreotrichs larger than 20 µm). Heterotrophic choreotrichs of $< 20$ µm contributed with 0.06 to 1.46 mgC·m$^{-3}$, averaging 16 % of the total ciliate carbon. The cell carbon content of these small ciliates averaged 0.4 ngC cell$^{-1}$. The contribution of tintinnids to standing stock was negligible, always below 0.5 %.

The relative importance of metazoans increases when considering carbon estimates. For instance, copepod nauplii accounted for 0 43 to 5 75 mgC m$^{-3}$ and averaged 28.5 % of total microzooplankton carbon, thus exceeding the biomass of ciliates in some surface samples (*figure 4*). The individual carbon content of copepod nauplii averaged 61.3 ngC indv$^{-1}$. The contribution of the other groups was much less important, except for the heterotrophic dinoflagellates in the surface water of station 1. Here, a *Gymnodinium* bloom reached concentrations of 6 1 mgC·m$^{-3}$ (49 % of total carbon). Biomass values of dinoflagellates at the other stations were never above 0.49 mgC·m$^{-3}$.

Total microzooplankton carbon was positively correlated with Chl *a* concentration ($r = 0.71$, $P < 0.001$; $n = 24$) and with primary production ($r = 0\ 68$, $P < 0.001$; $n = 24$). The biomass of the most abundant group in the samples, i.e. ciliates, was correlated with primary production ($r = 0.64$, $P < 0.001$; $n = 26$). Conversely, the correlation between aloricate ciliates carbon and Chl *a* concentration ($r = 0.48$; $P = 0.016$) was not significant after applying Bonferroni correction for multiple correlation tests.

## 4. DISCUSSION

Our study was conducted during the onset of a vertical temperature gradient and with medium to large diatoms dominating the producer's community. The values of microzooplankton abundance and biomass that were found fall within known average ranges for temperate nearshore waters [33, 44]. In addition, a dominance of choreotrich ciliates on the microzooplankton community has also been widely reported [33 and references therein].

The abundance of ciliates in our study was on average more than three-fold higher (1830:510 cell L$^{-1}$) than that reported for the same area in early spring 1987 by Fernández et al. [11]. They also reported smaller peak values (3700:2120 cell L$^{-1}$). This may reflect an increase in ciliates abundance due to the advanced spring season during our study, as is generally expected for ciliates [33]. Large mixotrophic ciliates (*Laboea strobila* and *Tontonia* spp.) contributed little to the ciliate community abundance (9 %). These ciliates exhibited lower abundance than those reported from other temperate and subtropical neritic areas, where spring values are around 40 % of the total community [42 and references therein] The values found in our study probably are an underestimation due to our assumption that no *Strombidium* species is mixotrophic. In contrast to this, mixotrophic choreotrichs on average accounted for an important portion of total ciliate carbon in our study (39 %) The contrast between contribution to abundance and biomass of mixotrophic ciliates is due to the elevated cell carbon per individual that was found in our study (11 ng C cell$^{-1}$). The lack of mixotrophic ciliates in some of the deepest samples, and the association of *Laboea strobila* with surface waters, are consistent with the findings of Stoecker et al. [42], who reported that ciliates with chloroplasts were found higher up in the euphotic zone. Within the ciliate assemblage, the numeric importance of choreotrich ciliates $< 20$ µm in size was remarkable. They accounted for an average of 51 % of total phagotrophs abundance and 9 % of total biomass. Sherr and Sherr [38] reported high cell-specific clearance rates for bacterial-sized particles in spirotrich ciliates ranging from $2 \times 10^2$ to $3 \times 10^3$ µm$^3$. Hence, the average carbon and biovolume of nanociliates found in our study (0.4 ng C cell$^{-1}$, $2.1 \times 10^3$ µm$^3$ cell$^{-1}$), and their typical predator-prey size ratio close to 8:1 [14], extend the range of available prey for small ciliates down to picoplankton-sized preys.

The abundance of metazoan nauplii in spring was previously reported for the area by Fernández et al. [11]. Similarly to what was found for aloricate ciliates, the values reported in their study in terms of average (31:12 indv·L$^{-1}$) and maximum values (128:59 indv·L$^{-1}$) are lower than those in ours. This again suggests that in our study microzooplankton was in a more advanced phase of response to the phytoplankton spring bloom. The study area exhib-

ited a rather high abundance of metazoan nauplii within its microplankton community, when compared with spring values for other temperate areas [4, 6, 30]. Both highest and lowest values of nauplii abundance were found in the surface waters of two adjacent stations, i.e. 7 and 1. Between these stations, a sharp mesoscale front in salinity and nutrients is normally present as a consequence of runoff from the river Nalón. This is consistent with increased naupliar concentrations at stations with a stratified water-column [18]. The contribution of copepod nauplii to community carbon (28.5 %) and ingestion (6.3 %) in our study shows the importance of including complementary sampling methods for metazoan larvae in microzooplankton community studies. Despite its importance, this kind of information is rather scarce [32].

In this study we find a number of significant positive correlations between microzooplankton and phytoplankton. These correlations might be attributed to an active response to changes in phytoplankton stock. However, correlations vanish when the variables are integrated through the water column, suggesting that the positive correlations might be reflecting the seasonal evolution of biological data. Nevertheless, the generation time and the rates of movement of microzooplankton on the same scales as phytoplankton, particularly in the case of ciliates [39, 40], permit a high degree of coupling between the two components of the food web.

Despite the theoretically poor edibility of diatoms to ciliates [15], their abundance was rather high. Indeed, the mean yearly abundance of ciliates in neritic waters is about 1000 cells $L^{-1}$ [33]. This situation might support the idea that herbivorous planktonic ciliates are seldom controlled by food scarcity but rather by mesozooplanktonic predators [31], and that in these unfavourable conditions ciliates might be making use of small patches of unevenly distributed edible prey [29].

The estimated grazing of the microzooplankton community on phytoplankton carbon ranged from 2.1 to 26.9 $mgC\,m^{-3}\,day^{-1}$ at the maximum rate ($I_{max}$) and from 0.1 to 11 $mgC\,m^{-3}\,day^{-1}$ at half saturation rate ($I_{Km}$). Maximum values of grazing impact were always associated with surface waters, for both estimates. This pattern applied not only to total grazing but also to the estimates for each major group (*table IV*). Total community grazing impact averaged 28.3 % of phytoplankton standing stock consumed per day, when estimated using $I_{max}$. Using $I_{Km}$, grazing estimated averaged a much lower 5.2 % of phytoplankton standing stock consumed per day. The most important grazers in both estimates were choreotrich ciliates bigger than 20 µm, and averaged 13.7 and 3 % of phytoplankton standing stock for $I_{max}$ and $I_{Km}$ respectively. Copepod nauplii were the second largest group in terms of grazing impact when calculated using $I_{max}$ (6.3 %). When calculated using $I_{Km}$, ciliates smaller than 20 µm averaged 0.8 % ver-

**Table IV.** Estimated grazing impact on phytoplankton standing stock for each major group ($mgC\,m^{-3}\,day^{-1}$); in brackets follows station and depth. % AISS = Averaged percentage of ingested phytoplankton standing stock; in brackets follows the 95 % confidence interval. See Methods for the influence of the variability of the C:Chl *a* ratio on AISS estimates.

| Group | | Ingestion | | | |
|---|---|---|---|---|---|
| | | Median | Max. | Min. | AISS |
| Total community | $I_{max}$ | 8.8 | 26.9 (6–0) | 2.1 (12–50) | 28.3 (7.4) |
| | $I_{Km}$ | 1.7 | 11 (6–0) | 0.1 (12–50) | 5.2 (1.5) |
| Oligotrichs > 20 µm | $I_{max}$ | 4.5 | 22.5 (6–0) | 0.8 (12–50) | 13.7 (3.5) |
| | $I_{Km}$ | 1.1 | 9.7 (6–0) | 0.1 (12–50) | 3 (0.8) |
| Oligotrichs < 20 µm | $I_{max}$ | 0.9 | 3.6 (14–0) | 0.2 (6–20) | 4 (1.5) |
| | $I_{Km}$ | 0.3 | 1.6 (17–0) | 0 (6–20) | 0.8 (0.2) |
| Dinoflagellates | $I_{max}$ | 0.5 | 14.1 (1–0) | 0 (1–10) | 2.5 (1.2) |
| | $I_{Km}$ | 0.1 | 3.2 (1–0) | 0 (1–10) | 0.2 (0.2) |
| Nauplii | $I_{max}$ | 1.6 | 7.4 (7–0) | 0.6 (7–20) | 3.6 (1.8) |
| | $I_{Km}$ | 0.2 | 1.1 (7–0) | 0 (7–20) | 0.5 (0.1) |

sus 0.5 % of copepod nauplii With the exception of the surface water at station 1, heterotrophic dinoflagellates accounted for a small portion of the total grazing impact, averaging 2.5 % ($I_{max}$) or 0 2 % ($I_{Km}$) Maximum ingestion estimates were found at the surface for each group, both when using the $I_{max}$ or the $I_{Km}$ approach

Given the phytoplankton composition and the levels of microzooplankton biomass present at the time of the study, it seems reasonable to assume that grazing impact was closer to the $I_{max}$ than to the $I_{km}$ estimates. The latter are in fact too low to sustain the levels of microzooplankton biomass To test this assumption, gross growth efficiency of 40 % might be assumed on the basis that reported efficiency for protozoa is high [5] Then, ingestion values calculated using $I_{max}$ account on average for 65 % ( $\pm$ 1.5 s.e.) of microzooplankton standing stock Anyway, herbivory impact on phytoplankton standing stock is rather low according to the estimates calculated in our study This is consistent with the poor edibility of the dominant phytoplankton found in the study [13]. The impact on phytoplankton standing stock might be greater at any other time of the year, when phytoplankton is dominated by smaller cells such as microflagellates and energy flows through the microbial loop [7], with the result of a shifting of the ecosystem to an heterotrophic phase [37]. For the period of the study, both the presence of high abundance of nanociliates and the importance of copepod nauplii to standing stock suggest the existence of a metazoan-protozoan-bacteria food chain in the area [19]

**Acknowledgements**

We wish to thank the crew of R.V *José Rioja* from the I.E.O. From the B.O.S. department, we thank Rafael González-Quirós, Jorge A. Sostres, Esteban Cabal, Natalia González and Leticia Viesca. Jesus Cabal encouraged this study as the leader of the survey and Florentina Álvarez allowed its inclusion in the project. José Luis Acuña and Markus Kiefer helped with ingestion estimates María José Bañuelos reviewed previous drafts of the manuscript. Critical reviews by Télesphore Sime-Ngando and an anonymous referee greatly improved the manuscript. We also thank Anne I. Jolly for correcting the English and Beat Gasser for checking the French abstract. The survey was funded by FICYT project PB-REC95-05 At the time of writing this work, M. Quevedo was funded by a grant from the Universidad de Oviedo within the European Commission project CE-96-MAS3-CT96-0060.

## REFERENCES

[1] Antia N.J., McAllister C.D., Parsons T.R., Stephens R., Strickland J.D.H., Further measurements of primary production in coastal sea water using a large volume plastic sphere, Limnol. Oceanogr. 6 (1963) 237–258.

[2] Bode A., Fernández E., Variability of biochemical composition and size distributions of seston in the euphotic zone of the Bay of Biscay: implications for microplankton trophic structure, Mar. Biol. 114 (1992) 147–155.

[3] Botas J.A., Fernández E., Bode A., Anadón R., Water masses off the central Cantabrian Coast, Sci. Mar. 53 (1989) 755–761.

[4] Burkart C.A., Kleppel G.S., Brander K., Holliday D.V., Pieper R.E., Copepod and barnacle nauplius distributions in the Irish Sea: relation to springtime hydrographic variability, J. Plankton Res. 17 (1995) 1177–1188.

[5] Caron D.A., Goldmann J.C., Protozoan nutrient regeneration, in: Capriulo G.M. Jr (Ed.), Ecology of Marine Protozoa, Oxford University Press, New York, 1990, 366 p.

[6] Checkley D.M. Jr., Raman S., Maillet G.L., Mason K.M., Winter storm effects on the spawning and larval drift of a pelagic fish, Nature 335 (1988) 346–348.

[7] Cushing D.H., A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified, J. Plankton Res. 11 (1989) 1–13.

[8] Dodge J.D., Marine dinoflagellates of the British Isles (Ed.), Her Majesty's Stationery Office, London, 1982, p. 303.

[9] Eppley R.W., Harrison W.H., Chisholm S.W., Stewart E., Particulate organic matter in surface waters of southern California and its relationship to phytoplankton, J. Mar. Res. 35 (1977) 671–696.

[10] Fernández E., Bode A., Botas A., Anadón R., Microplankton assemblages associated with saline fronts during a spring bloom in the central Cantabrian Sea: differences in trophic structure between water bodies, J. Plankton Res. 13 (1991) 1239–1256.

[11] Fernández E., Cabal J.A., Acuña J.L., Bode A., Botas J.A., García-Soto C., Plankton distribution across a slope current-induced front in the southern Bay of Biscay, J. Plankton Res. 15 (1993) 619–641.

[12] Gaines G., Elbrächter M., Heterotrophic nutrition, in: Taylor F.J.R. (Ed.), The Biology of Dinoflagellates, Blackwell, Oxford, 1987, pp. 224–269.

[13] Gifford D.J., Impact of grazing by microzooplancton in the Northwest Arm of Halifax Harbour, Nova Scotia, Mar. Ecol. Prog. Ser. 47 (1988) 249–258.

[14] Hansen B., Bjørnsen P.K., Hansen P.J., The size ratio between planktonic predators and their prey, Limnol. Oceanogr. 39 (1994) 395–403.

[15] Hansen P.J., Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web, Mar. Ecol. Prog. Ser. 73 (1991) 253–261.