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The annual cycle of nanoflagellates in the Central Cantabrian Sea (Bay of Biscay)

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Abstract

The annual cycle of nanoflagellates (NF) including autotrophic (ANF), heterotrophic (HNF) and mixotropic (MNF) flagellates carried out in a temperate sea (Central Cantabrian Sea, southern Bay of Biscay) is presented. Three stations with characteristics ranging from coastal to oceanic conditions were analysed in order to compare NF response to this gradient. Samples were monthly collected at each station at three different depths between February 2002 and December 2002. CTD profiles were also taken at each station. NF were grouped according to their trophic status into ANF, HNF and MNF. Abundance and biomass were determined for each group. The annual cycle showed a general pattern consisting in a maximum in July with secondary maxima in March and October and minimum values in May. ANF were the most important fraction, making a major contribution (nearly 75%) to total NF biomass in all stations. HNF represented over 20% along the cycle, except for a peak in spring found in every station. MNF reached less than 5%, showing low seasonability. Small flagellates (2–5 μm) dominated throughout the cycle. Microplankton community was also analysed in terms of abundance and biomass. A significant positive correlation ($r^2=0.49$) was obtained between 2–5 μm NF and 10–20 μm HNF–MNF biomasses, suggesting a possible trophic relationship between these groups which should be cautiously taken. No significant relationships were found between microplankton and NF or between nutrients and ANF, indicating that the regulation of NF numbers is complex and probably implicates other groups. In addition to this, the unexpected 2002 Chl *a* concentration pattern and the misplacing of upwelling events render necessary to perform additional studies to fully understand the precise behaviour of NF in the Cantabrian Sea. To the best of our knowledge, this is the first study of a NF cycle in a temperate sea that considers all functional groups.

Keywords: Nanoflagellates; HNF; ANF; Mixotrophy; Seasonal variations; Temperate zone; Abundance; Biomass; Spain; Bay of Biscay; Cantabrian Sea

1. Introduction

Nanoflagellates (NF) are a major constituent part of the nanoplankton and their populations provide an important link in the microbial food web (Safi and Hall, 1997). They specifically behave as an important trophic link between the two ends of the bacterial and the herbivorous food webs described by Legendre and Rassoulzadegan (1995). Dominance of this size fraction usually occurs when hydrographic conditions prevent bigger cells from developing, due to shortage of nutrients or light (Azam and Fenchel, 1983; Cushing, 1989). Therefore, marine nanoplanktonic communities

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are commonly associated with low biomass levels and lack of blooms although flagellate blooms have also been reported (e.g. Nogueira and Figueiras, 2005).

Heterotrophic nanoflagellates (HNF) and mixotrophic nanoflagellates (MNF) act as grazers of bacteria and picophytoplankton providing an essential link in the transfer of carbon to higher trophic levels and in turn, autotrophic nanoflagellates (ANF), HNF and MNF are a potential major food resource for omnivorous micro- and mesozooplankton. There are several studies that have addressed the interactions among components of the microbial loop, including NF (e.g. Gasol, 1994; Vaqué et al., 1994). However, precise relationships between different NF groups have not been studied in detail. In addition to this, several investigations have suggested that this compartmentalization might be too broad: for many larger nano-sized colourless flagellates, the optimum prey size is in the nano size fraction (Sherr and Sherr, 1991). Still little is known about the prey size spectrum of the phagotrophic 10–20 μm protists (Havskum and Hansen, 1997). The role of HNF and MNF as controllers of pico and nanoplanktonic communities consists in maintaining their own growth rate close to their preys (Fogg, 1995), generating predator–prey couplings (Quevedo and Anadón, 2001).

The abundance of NF in different environments can be highly variable (Sanders et al., 1992; Tamigneaux et al., 1995). Factors underlying this variability still have to be elucidated, including, for example, light, nutrient concentrations, temperature or predation of ANF populations and the involvement of HNF in nitrogen regeneration through microheterotrophic activity (Safi and Hall, 1997 and references therein). Large increases in NF abundance have been reported during spring and autumn; a considerable part of these findings, however, come from freshwater environments. Taking into account the central role of NF within pelagic systems, it would seem reasonable to expect a great amount of studies focused on them. Seasonal studies that have described NF behavior have mainly focused attention on HNF (e.g. Sanders et al., 1992; Kuuppo, 1994; Solic and Krstulovic, 1994; Tanaka and Rassoulzadegan, 2002), with some cycles from nearby areas in the Bay of Biscay (e.g. Bode et al., 2004). Nonetheless, seasonal approaches that have analyzed both ANF and HNF are rare (e.g. Coats and Dolan, 1990; Mackiewicz, 1991; Ratkova et al., 1999; Verity et al., 1999).

The Central Cantabrian Sea 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 (Serret et al., 1999). Some research addressing NF has been performed in this area (e.g. Bode and Fernández, 1992; Fernández and Bode, 1994), though these works only described ANF using a non-specific methodology which could underestimated its smallest fraction. Only one study has included both ANF and HNF in a nearby area from present work (Barquero, 1999). Finally, there are no comprehensive annual cycles that have addressed NF as a group including ANF, HNF and MNF patterns in temperate waters. Thus far, available information about NF in these waters is still scarce.

The aim of the present study was to describe the cycle of NF in a temperate sea (Central Cantabrian Sea, southern Bay of Biscay) through a monthly basis along a coastal–oceanic gradient. NF abundance and biomass, as well as NF trophic groups in different size classes were also determined. Further objectives included analysis of relative contribution of ANF to phytoplanktonic community and of the relationship between HNF and bigger microzooplankton. A potential trophic coupling among NF size classes was also studied.

2. Materials and methods

2.1. Area of study

The study was conducted in the Central Cantabrian Sea (NW Spain, southern Bay of Biscay), at three stations along a coastal–oceanic gradient. This area is characterized by a narrow continental shelf and the Avilés canyon (Fig. 1). Three stations with characteristics ranging from coastal to oceanic conditions were analysed. Station 1 (St 1) was located near the coast (60 m bottom depth), station 2 (St 2) on the shelf-break (180 m bottom depth) and station 3 (St 3) on the slope of the canyon (800 m bottom depth). The sampling transect 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. A general long term description of the area is available elsewhere (Llope et al., 2006; Llope et al., 2007).

2.2. Sampling

Samples were taken monthly during 2002 on board the BO José Rioja. Vertical profiles of temperature, conductivity, fluorescence and PAR (400 to 700 nm) were obtained with a CTD SBE 25. Water samples were collected from 4 up to 10 discrete depths with 5 L General Oceanics Niskin bottles. Samples for determination of dissolved nitrate concentration were frozen

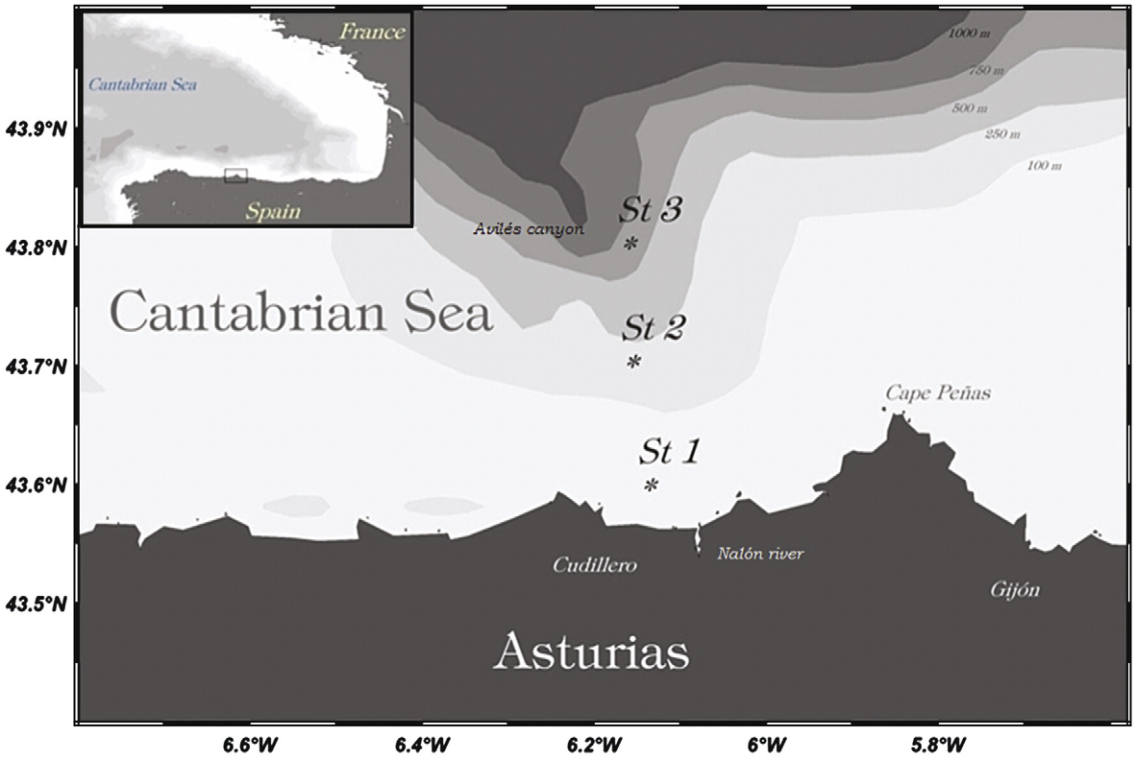

Fig. 1. Area of study in the Cantabrian Sea (southern Bay of Biscay), showing sampling stations. St 1: coastal station; St 2: shelf-break station; St 3: oceanic station.

immediately after collection and stored at −20 °C until analysis. Nitrate was analysed following the method of Grashoff et al. (1983) using a Skalar SanPlus System auto-analyzer.

2.3. Abundance and biomass of microphytoplankton and microzooplankton

Chlorophyll *a* (Chl *a*) concentration was estimated fluorometrically with a Turner Designs 10-AU fluorometer. Water samples (100 ml) were collected and filtered onto GF/F filters. All filters were frozen after collection; pigments were extracted in 5 ml of 90% acetone overnight at 4 °C (Yentsch and Menzel, 1963). Seawater samples for identification and enumeration of microplankton (2–20 μm) were collected in 125 cm^3 glass bottles with Lugol's iodine. Microplankton enumeration and identification was carried out with an inverted microscope following the Utermöhl method at 400× and 650× magnifications. Estimation of microphytoplankton cell biomass was calculated from specific carbon content from Plymouth Marine Laboratory (*pers. comm.*). Ciliates and heterotrophic dinoflagellates biomass was obtained by measuring linear dimensions of all sample cells. For this purpose a calibrated ocular micrometer was used and measurements were converted to biovolume by assuming simple geometric shapes (Stoecker et al., 1989). Carbon content was calculated from biovolume using literature conversion factors: 0.19 pgC μm^{-3} for Lugol's preserved aloricate ciliates and 0.14 pgC μm^{-3} for dinoflagellates (Quevedo and Anadón, 2000). All biomass and abundance values were averaged in the water column.

2.4. Nanoflagellate abundance and biomass

NF samples were collected from the same three previous depths. From each depth 150 ml of water were collected in dark bottles and fixed with 25% Glutaraldehyde to obtain 0.5% final concentration, following the recommendations of Sherr et al. (1993). Fixed samples were stored in the dark at 4 °C and were processed within 12 h after sampling. Thirty milliliters of sample were stained with DAPI (4',6 diamidino-2-fenilindol) and filtered following Sherr and Sherr (1993) methodology. Filters were stored at −20 °C until observation by epifluorescence microscopy. NF were identified and counted with a *Leica* epifluorescence

microscope equipped with UV excitation light and blue emission filter. Appropriate combinations of UV and the blue filter allowed to distinguish between autotrophic and heterotrophic cells by the red autofluorescence of the former under blue light and to classify as mixotrophic those with red autofluorescence and secondary signs of DNA from other organism inside them (Havskum and Riemann, 1996; Havskum and Hansen, 1997). In the present study, NF were grouped according to their trophic status: ANF, which are primarily photosynthetic; HNF, which graze; and MNF, which are capable of both photosynthesis and grazing. Each group was subdivided into three size classes: 2–5 μm, 5–10 μm and 10–20 μm. One or two transect were counted at 400× magnification for 10–20 μm cells and at 1000× for the other ones until reaching a minimum of 80 cells. NF biomass was estimated measuring one or two dimensions of all counted cells. For this purpose a calibrated ocular micrometer was used and measurements were converted to biovolume by assuming simple geometric shapes (Hillebrand et al., 1999). Carbon contents were estimated using the volume–carbon ratio developed by Norland (1993) from the data of Simon and Azam (1989): 0.12 pgC $(\mu m^3)^{0.7}$. Carbon biomass data were combined with abundance to obtain total biomass. All biomass and abundance values were averaged in the water column.

3. Results

3.1. Environmental variables

As the three studied stations followed a similar pattern and the continental shelf station is an intermediate model among coastal and oceanic conditions, only St 2 values are represented. The thermal structure along the year shows a well-mixed and stratified pattern, but two upwelling events could be detected from temperature and increased nitrate concentration in subsurface waters. The first upwelling event took place during March and April, and the second one occurred in July, coinciding

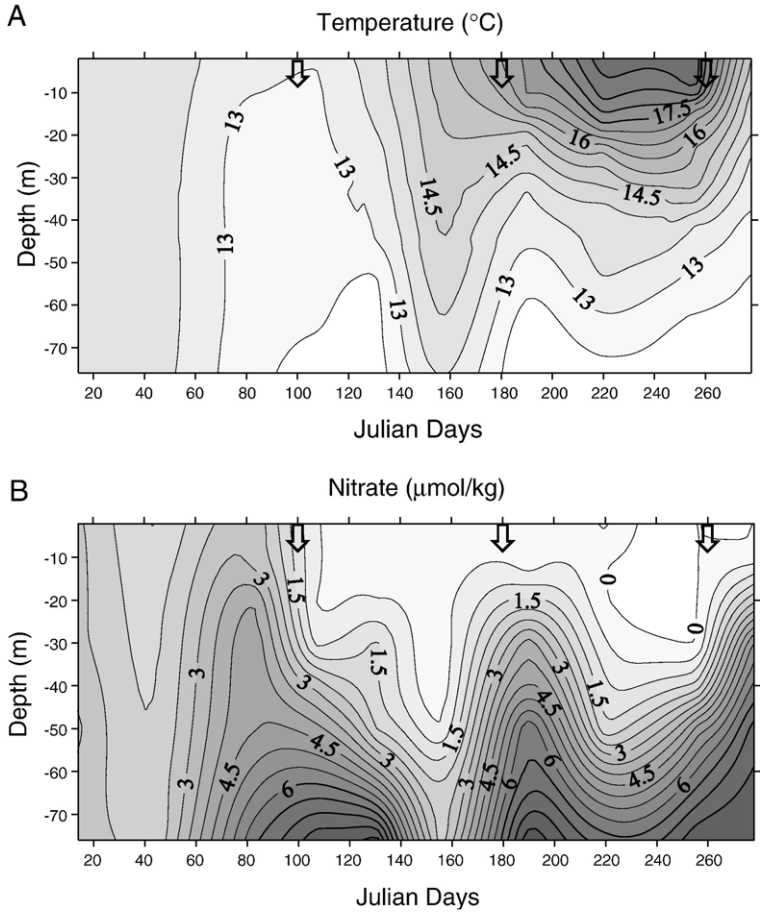

Fig. 2. Depth temporal variation of A) temperature (C) and B) nitrate concentration (μmol kg^{-1}) at St 2. Arrows indicate upwelling events (empty) and autumn mixing period with upwelling (filled).

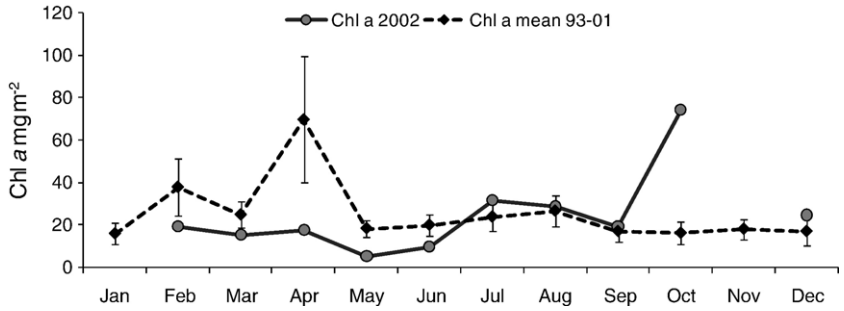

Fig. 3. Annual variation of integrated Chl *a* (mg m^{-2}) at St 2 during 2002 and mean Chl *a* concentration during 1993–2001 period.

with positive Ekman transport (positive upwelling index; see Llope et al., 2006). The last period with high nutrient in subsurface waters corresponded to a phase of eroding thermocline in autumn also coinciding with a positive upwelling index phase. Nitrate concentration was unusually high in summer and autumn (Llope et al., 2006) (Fig. 2). As a result of these hydrographical conditions, the highest values of Chl *a* in the water column were observed in July and October instead of during spring, which has been the trend found in previous years; this unexpected finding lead us to carry out an inter-annual Chl *a* comparison (Fig. 3). The same pattern occurred in the other two sampled stations (data not shown).

3.2. Nanoflagellates

The amount of NF at the three sampled stations was similar but showed an offshore increasing mean abundance (Table 1). ANF dominated this complex group, representing more than 74% of total NF community; percentages of different trophic groups remained almost constant at each station. Pattern of seasonal changes in ANF abundance at different depths showed higher abundances usually occurring in superficial waters at the three sampled stations; again, St 2 pattern is represented as a model (Fig. 4).

Due to dramatic differences in abundance between different NF size classes, biomass expression is used here in order to evaluate relative importance of each metabolic group and its size. Seasonal trend of total NF biomass was similar in all stations (Fig. 5), with maximum values in July and secondary maxima in March and October. Minimum values were found in May. This seasonal behaviour can be related to prevailing hydrographical conditions in the area during summer, besides the input of nutrients to the photic layer.

The smallest fraction (2–5 μm in diameter) dominated total NF biomass as well as both ANF and HNF groups, but size varied between trophic groups (Table 2A). The medium size fraction (5–10 μm) was the commonest

Table 1
NF mean abundance with monthly values according to trophic groups ($\times 10^3$ cells ml^{-1}) at the three stations sampled during the cycle and its mean annual per cent contribution

NF mean abundance	St 1			St 2			St 3		
	1.84			2.12			2.17		
	ANF	HNF	MNF	ANF	HNF	MNF	ANF	HNF	MNF
Feb	0.20	0.04	0.01	1.91	0.20	0.02	0.74	0.22	0.00
Mar	1.24	0.60	0.05	1.82	0.76	0.11	–	–	–
Apr	0.32	0.54	0.02	0.74	0.88	0.14	0.48	0.71	0.04
May	0.33	0.10	0.03	0.60	0.20	0.07	0.63	0.24	0.05
Jun	1.10	0.13	0.04	1.32	0.25	0.06	1.65	0.20	0.08
Jul	4.29	0.32	0.11	3.39	0.30	0.09	3.87	0.44	0.08
Aug	1.90	0.46	0.05	1.51	0.34	0.06	2.22	0.26	0.04
Sep	1.66	0.45	0.07	1.50	0.49	0.09	2.16	0.36	0.14
Oct	1.43	0.43	0.10	1.51	0.64	0.11	2.11	0.45	0.24
Dec	1.37	0.91	0.14	–	–	–	–	–	–
Mean %	75.06	21.64	3.30	74.83	21.24	3.92	79.64	16.50	3.87

(–: not available).

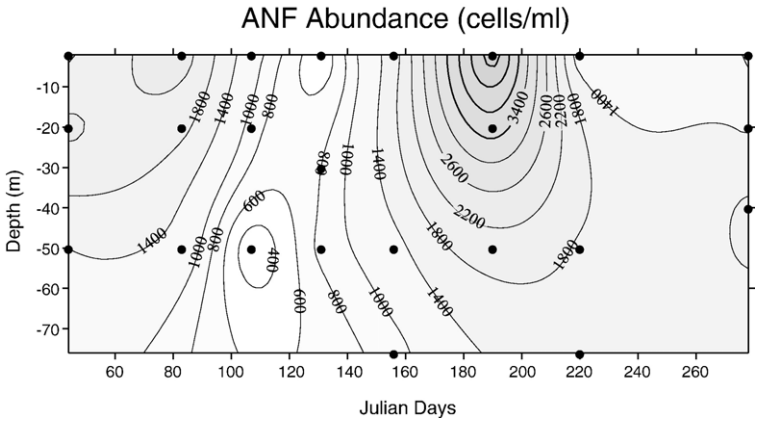

Fig. 4. Vertical distribution of ANF abundance (cells ml^{-1}) during the sampling period at St 2.

within the MNF group. Table 2B highlights that relative importance of ANF decreased from small to large cells, while both HNF and MNF contributions markedly increased with size. ANF dominance over NF community can be expressed as a ratio of ANF/(HNF + MNF) biomasses; however, this ratio is reversed within 10–20 μm size class due to an increased contribution of both HNF and MNF groups at all three sampled stations.

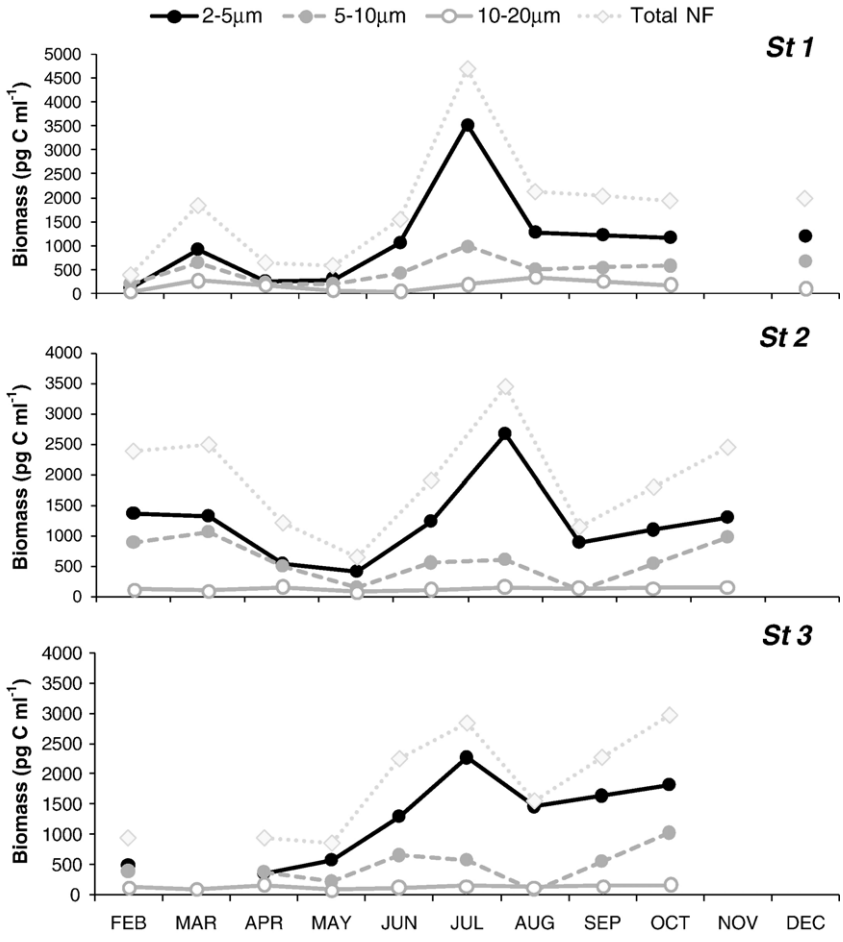

Fig. 5. Annual variation of size fractionated (2–5 μm; 5–10 μm; 10–20 μm) NF biomass (pgC ml^{-1}) at St 1, St 2 and St 3.

Table 2
Mean per cent contribution of A) size classes to total NF biomass by trophic groups, and B) trophic groups to total NF biomass by size classes and stations

A	ANF			HNF			MNF		
Size (μm)	2–5	5–10	10–20	2–5	5–10	10–20	2–5	5–10	10–20
St 1	62,61	28,35	9,05	54,61	22,93	22,46	27,68	49,87	22,46
St 2	62,02	31,07	6,91	46,11	27,45	26,44	27,60	52,56	19,84
St 3	67,53	26,55	5,92	51,05	29,63	19,32	37,31	39,50	23,19
Total	64,05	28,65	7,29	50,59	26,67	22,74	30,86	47,31	21,83
B	2−5 μm			5–10 μm			10–20 μm		
Size (μm)	St 1	St 2	St 3	St 1	St 2	St 3	St 1	St 2	St 3
ANF	75.29	74.52	77.85	69.61	64.99	66.99	48.69	35.40	41.78
HNF	21.46	21.71	17.49	18.40	22.50	22.21	39.49	53.04	40.50
MNF	3.26	3.77	4.66	11.99	12.51	10.80	11.83	11.56	17.72
Ratio	3.05	2.92	3.52	2.29	1.86	2.03	0.95	0.55	0.72

Ratio: ANF/(HNF+MNF).

Organisms classified as MNF represent a minor fraction all over the year within all size classes. The most evident changes over NF biomass throughout the annual cycle were mainly caused by 2–5 μm cells, but the rate of change decreased from coast to ocean (Fig. 5). This size fraction showed a small contribution during winter at St 1 and St 3, decreasing to very low biomass levels during April and May. Both 5–10 and 10–20 μm size classes also displayed seasonability, with an increase in biomass in July and October which was more apparent at St 2 and St 3.

3.3. Microphytoplankton and microzooplankton

Large phytoplankton (microphytoplankton >20 μm) analysis was carried out only at St 2. Phytoplankton >20 μm were mainly composed by Dinophyceae, which accounted for about 65% of abundance, Diatomophyceae and Criptophyceae. Microphytoplankton abundance ranged from 34 to 316 cell ml^{-1} in September and June respectively (mean of 179 cell ml^{-1}). ANF was more important numerically than microphytoplankton, contributing a mean of 88.3% to total (2–200 μm) phytoplankton abundance. This relationship displayed little seasonal variation, ranging from a minimum ANF contribution of 73.7% in April to a maximum of 97.8% in September. Microphytoplankton biomass ranged from 1.11×10^4 to 4.95×10^5 pgC ml^{-1} in February and July respectively (mean of 8.93×10^4 pgC ml^{-1}); it represented the bulk of total phytoplankton (92.7%), with a maximum contribution of 99.3% in July and a minimum of 82.3% in February, displaying low seasonal variability. Patterns in Chl *a* concentration were similar to those of ANF, with an analogous seasonal trend. A significant positive linear correlation was observed between ANF abundance and Chl *a* ($r^2=0.86$, $p<0.001$).

Ciliate abundance ranged from 1 to 14 cell ml−1 in July and October respectively (mean of 5 cell ml−1). HNF were more important numerically than ciliates, contributing on average to 98.8% of total (2–200 μm) microzooplankton abundance with almost no seasonal variation. Ciliate biomass ranged from 3.53×103 to 5.42×104 pgC ml−1 in June and May respectively (mean of 2.38×104 pgC ml−1). However, in terms of biomass, ciliates represented the most significant part of total microzooplankton (94.6%), with a maximum contribution of 99.2% in May and a minimum of 86.3% in June.

3.4. Trophic interactions

The regression line performed between 2–5 μm NF and 10–20 μm HNF–MNF biomasses found a highly significant ($p=0.0001$) positive correlation ($r^2=0.49$) (Fig. 6A). This finding could support the idea of a general trophic relationship between these groups. When MNF are excluded from this analysis, correlation coefficient clearly falls ($r^2=0.30$). However, taking into account specific dynamic relationships at each station, positive correlation between both groups become less apparent (Fig. 6B); no clear evidences of interactions affecting densities of either preys or predators can be deducted from these relationships. All three stations showed a heterogeneous dynamic behaviour, with mayor changes in 2–5 μm NF at intermediate densities of 10–20 μm HNF+MNF. There was a positive relationship between microzooplankton abundance and Chl *a* concentration ($r^2=0.62$), but these positive relationship is not observed between HNF and Chl *a*.

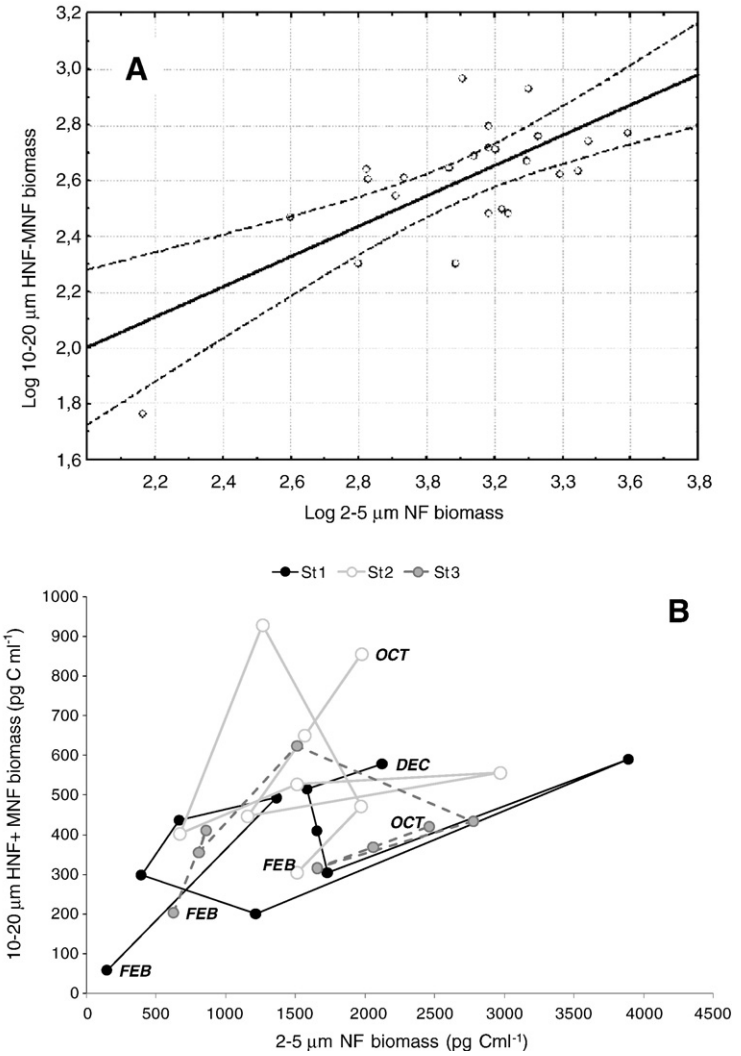

Fig. 6. A) Biomass relationship between small 2–5 µm NF (preys) and large 10–20 µm HNF+MNF (predators) in all depths of the three sampled stations. Fit: log (10–20 µm HNF–MNF biomass)=0.915+0.544 * log (2–5 µm NF biomass); r^2=0.49; p=0.0001. B) Annual variation of integrated biomass of small NF (preys) and large HNF+MNF (predators) at St 1, St 2 and St3. Initial and final sampled months at each station are shown.

However, there was no relationship neither between microzooplankton and NF nor between the former and microphytoplankton, suggesting that cells consumed by ciliates may be smaller than the 2–200 µm fraction. Relationships between nutrients and ANF were not significant, indicating that regulation of NF numbers is a complex mechanism that probably involves other groups to some extent, intricate competition for nutrients with other organisms or predation of bigger HNF cells by meso or macrozooplankton.

4. Discussion

Despite relative little knowledge available about NF, their abundance in different environments has been already reported to be highly variable. Both ANF and HNF abundance in this study are consistent with previous studies carried out in a nearby area, the Northwest Iberian Peninsula (Barquero, 1999; Bode et al., 2004), showing maximum values in superficial waters and during summer, and a greater contribution of the 2–5 µm size class. Previous studies in the area has also shown dominance of ANF in the phytoplankton community (Bode and Fernández, 1992; Fernández and Bode, 1994), although abundances were lower than in present study probably due to a methodology not specifically focussed on NF. Higher ANF abundances towards offshore have also been reported by Férnandez (1990) in the same area. Available data of NF behaviour from the Cantabrian Sea and nearby areas apparently follow a

similar pattern. Besides, the seasonal cycle of NF biomass in the Central Cantabrian Sea was similar to that reported by Mackiewicz (1991) in cold waters of the Southern Baltic, with maximums in July and secondary peaks in spring and late-summer, but diverge from Verity et al. (1999) results; this latter work found that HNF mimicked ANF behaviour. However, both studies differ from NF abundances of present study, being lower in Mackiewicz (1991) and higher in Verity et al. (1999). In contrast with general distribution of NF in a coast ocean gradient, both surveys found higher abundances at inshore than at offshore stations. All these findings suggest that NF do no necessary follow the same dynamics in different marine environments, although dominance of small (2–5 μm) NF appears to be constant in diverse areas (Christaki et al., 1999; Christaki et al., 2001).

ANF seasonal pattern parallelise total NF due to their greater abundance as well as the less pronounced seasonal trend of both HNF and MNF. This result replicates the finding that ANF often show more pronounced seasonal variations in response to changes in light and temperature (Safi and Hall, 1997 and references therein). Analysis of NF according to trophic groups clearly showed ANF dominance over HNF and MNF, with the latter making the lowest contribution to total NF; data available from literature support that this finding represent a general trend (Havskum and Hansen, 1997; Safi and Hall, 1997; Christaki et al., 1999, 2001) although information concerning MNF should be carefully interpreted as available recognition methodology could underestimate their abundance; nevertheless, even considering this point, it is unlikely that MNF became as important as other NF nutritional groups.

Phytoplankton >20 μm were less important numerically than photosynthetic nanoplankton, contributing a mean of 11.7% to total (>2 μm) phytoplankton abundance. This relationship coincides with previous studies in temperate and cold areas (Fernández and Bode, 1994; Verity et al., 1999). In contrast, phytoplankton >20 μm biomass represented the most significant part of total phytoplankton (92.7%) due to their larger cell size and the fact that dominant ANF belong to the smallest analysed size fraction (2–5 μm). However, this pattern cannot be considered as a general one because Safi and Hall (1997) and Verity et al. (1999) found that ANF also dominated in terms of biomass. Anyhow, patterns in Chl *a* concentration were similar to those of ANF abundance, with a significant positive linear correlation between both variables in coincidence with previous results of Fernández and Bode (1994) and Verity et al. (1999). In general, ANF must be considered as a significant part of the autotrophic phytoplankton. The observed dominance of HNF over 2–200 μm microzooplankton reversed in like manner when analysing biomass, being ciliates dominant due to both their larger cell size and dominance of smaller (2–5 μm) HNF cells in the study area. In cold waters of the North Atlantic, ciliate biomass represented approximately 5% of HNF due to small (8–15 μm) ciliates dominance (Verity et al., 1999). Relation between ciliates and HNF in different hydrographical conditions or distinct pelagic ecosystems dynamics remains unclear.

The annual cycle of NF presented here shows a similar pattern to that expected for small organisms in a temperate sea, with maxima values in summer. Following the idea of a continuum of trophic structures and mechanisms in the pelagic environment (Legendre and Rassoulzadegan, 1995), NF play an important role as part of both the microbial and the multivorous food web, which link two successive phytoplankton blooms. Relationships between both ANF-Phytoplankton >20 μm and HNF-Ciliates remained almost unchanged during the cycle. This finding links to the idea that the development of different trophic webs, with subsequent alternation of dominant organisms, takes place within a different time scale from the one analysed in our study.

The highly significant positive correlation between 2–5 μm NF and 10–20 μm HNF–MNF biomasses ($r2=0.49$) observed in present work could support the idea of a trophic relationship between these groups. A coupled relationship ($r_2=0.44$) is also reported between authotrophic and heterotrophic nanoplankton as well as the finding of clearly recognizable phototrophic pico- and nanoplankton in HNF digestive vacuoles (Verity et al., 1999). Both Havskum and Riemann (1996) and Havskum and Hansen (1997) have observed 10–20 μm HNF to prey on nanoplankton. Furthermore, Safi et al. (2002) showed that ANF and HNF growth rates are similar, and so are their seasonal variations too. Besides, these findings may indicate that the main factor controlling NF grazing is size instead of trophic status. Trophic significance of MNF is considerable due to the fact that they are mainly composed by >5 μm cells, which can be proved by the fall in the correlation coefficient when MNF are excluded; nonetheless, a more general perspective could be developed if MNF are considered as bacterivorous predators (Unrein et al., 2007). Although linear relationships can be interpreted as direct trophic interactions, these are not fully understood in multi-species communities. Differences observed in small NF—larger HNF+MNF biomasses relationships along a coastal ocean gradient suggest that numerical interactions must occur at smaller temporal

scales than observed in this study. High growth rates of these organisms (Safi et al., 2002) and their rapid response to environmental changes could account for such dynamical behaviour.

To the best of our knowledge, no annual cycle including ANF, HNF and MNF carried out in a temperate sea has been previously reported. Due to unexpected 2002 Chl *a* concentration pattern (see Fig. 3) and the misplacing of upwelling events (see Fig. 2), it seems reasonable to wonder whether this cycle could be considered as the standard pattern of NF in the area. Additional studies would be necessary to fully understand the precise behaviour of NF in the Central Cantabrian Sea.

5. Conclusion

To the best of our knowledge, we have presented the first annual cycle focused on NF including autotrophic (ANF), heterotrophic (HNF) and mixotropic (MNF) flagellates carried out in a temperate sea (Central Cantabrian Sea, southern Bay of Biscay). Findings concerning NF abundance and biomass, trophic and size class distribution and seasonability, alongside results from both microphytoplankton and microzooplankton analysis and trophic interactions, will contribute to a more comprehensive and accurate understanding of NF role within pelagic systems.

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