

# Nanoflagellates in the Gdańsk Basin: coexistence between forms belonging to different trophic types

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**KEY WORDS**  
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KRZYSZTOF RYCHERT  
Sea Fisheries Institute,  
Kołłątaja 1, PL-81-332 Gdynia, Poland;  
e-mail: krychert@pap.edu.pl

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## Abstract

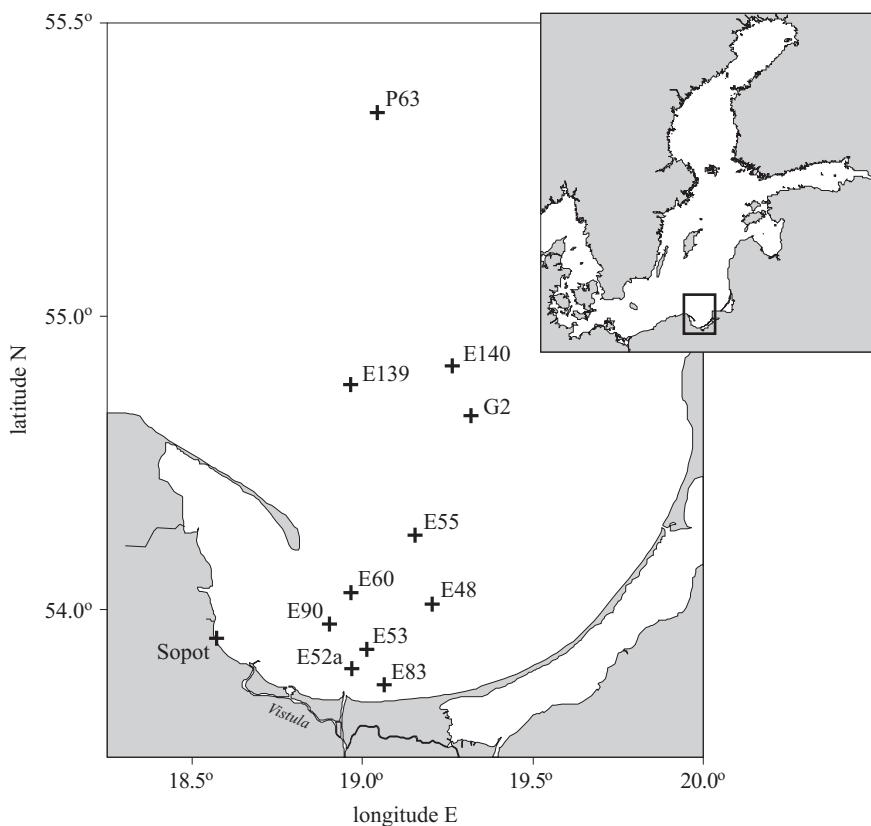
Nanoflagellates are important bacterivores, but their role is often underestimated because forms capable of phagotrophy and containing chlorophyll (mixotrophs) are excluded from analyses. Research conducted in the coastal zone of the Gdańsk Basin (Baltic Sea) revealed seasonal changes in the relationship between the biomasses of small (1–8  $\mu\text{m}$ ) nanoflagellates with (NFChl+) and without chlorophyll (NFChl−). Three distinct patterns were distinguished: (i) spring, when the biomass of NFChl+ was lower than that of NFChl−, (ii) summer and autumn, when NFChl+ were dominant (about 90%), and (iii) late autumn and winter, when the prevalence of NFChl+ was lower (71–79%). Additional studies showed the existence of spatial differences in the composition of the nanoflagellate community. These spatial differences were not shaped by freshwater input. The possible importance of mixotrophic forms in the Gdańsk Basin is discussed.

Nanoflagellates are recognised as being a very important component of the microbial food web (Azam et al. 1983) because of their significance as consumers of bacteria (e.g. Fenchel 1982, McManus & Fuhrman 1988, Sanders et al. 1992, Cho et al. 2000). During the last c. 25 years, many studies on their biomass and impact on bacterioplankton have been carried out, but they have usually taken only obligate heterotrophic nanoflagellates (HNF, cells without chlorophyll) into account. In planktonic communities, however, there are also mixotrophic nanoflagellates (Bird & Kalff 1986, Porter 1988, Caron 2000) capable of both phototrophy and phagotrophy.

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These protozoan organisms have turned out to be important consumers of bacteria in both freshwater and marine environments (Estep et al. 1986, Hall et al. 1993, Safi & Hall 1999, Pålsson & Daniel 2004) and have even been introduced to ecological models (Tett & Wilson 2000).

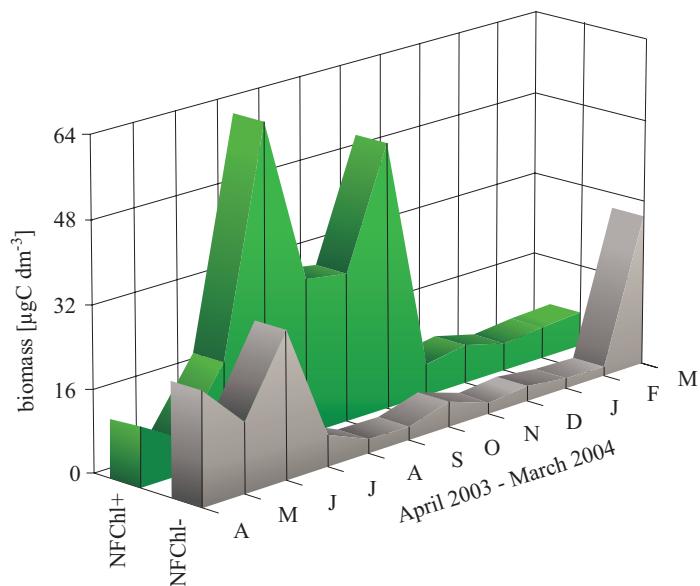
A few authors have simultaneously studied nanoflagellates with and without chlorophyll and reported suspiciously stable relationships between the two forms (Andersson-Nordström 1989, van Duyl et al. 1990, Kuuppo-Leinikki 1993). The results presented in this communication describe seasonal and spatial changes in the relationship between nanoflagellates with (NFChl+) and without chlorophyll (NFChl-) in planktonic communities in the Gdańsk Basin. These results also form the basis for a discussion of the importance of mixotrophic forms. Only flagellates smaller than 1–8  $\mu\text{m}$  have been taken into account. In the Gdańsk Basin they form the absolute majority as regards the abundance and biomass of nanoflagellates (unpublished observation).



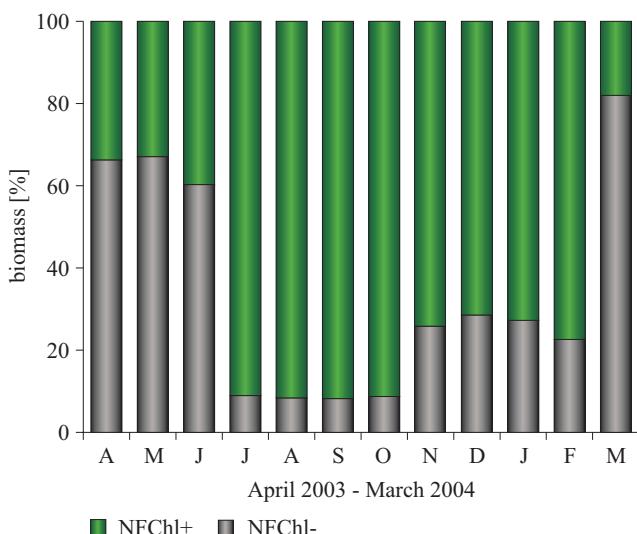
**Fig. 1.** Gdańsk Basin (Baltic Sea). Location of sampling stations

The research was conducted from April 2003 to March 2004. Samples were taken once a month at the end of the 450-metre-long wooden pier in Sopot. Water was collected about half a metre above the bottom (depth approx. 7 metres). The water column was typically well mixed and only in autumn was a slight stratification observed. Additional samples were taken during a cruise of r/v 'Baltica' (June 2005). Sampling was carried out in the surface zone (0–5 m) at eleven stations located in different parts of the Gdańsk Basin. All sampling sites are presented in Fig. 1. After each sampling, the water collected was fixed with glutaraldehyde (0.5%). Three (in a few cases only two) replicates of water were immediately filtered and the flagellates deposited on the filters were stained with primulin (Caron 1983). Filters were kept frozen ( $-20^{\circ}\text{C}$ ) and analysed under an epifluorescence microscope within a period of two weeks. Only flagellates in the size range of 1–8  $\mu\text{m}$  were analysed. Cells with and without chlorophyll were distinguished on the basis of the red autofluorescence of chlorophyll (Caron 1983). The biomasses of both groups were calculated with the factor  $220 \text{ fgC } \mu\text{m}^{-3}$  (Børshøj & Bratbak 1987). The values obtained for the replicates were averaged.

Analysis of the samples taken in Sopot revealed (Fig. 2) the existence of a spring peak of NFChl- and a summer prevalence of NFChl+. These observations are rather typical (Mackiewicz 1991). More interesting, however, were comparisons between the biomasses of NFChl+ and NFChl- (Fig. 3.), where three distinct seasonal patterns were distinguished: (i) spring, when the biomass of NFChl+ was lower than that of NFChl-, (ii) summer and autumn, when NFChl+ were dominant (91–92%) and (iii) late autumn and winter, when the prevalence of NFChl+ was lower (71–79%). The differences between these three seasons are statistically significant (variances were homogeneous – Levene test,  $p = 0.051$ ; ANOVA,  $p < 0.0001$ ; *post-hoc* Tukey test, differences between comparisons, i–ii  $p = 0.0002$ , ii–iii  $p = 0.0002$ , i–iii  $p = 0.004$ ). The pattern observed during summer and early autumn (91–92% of biomass contributed by NFChl+) cannot be generalised over the whole coastal zone of the Gulf of Gdańsk, because in summer 2004 at Orłowo (about 4 kilometres from the pier in Sopot) the observed proportion of NFChl+ was lower (65%). At Orłowo, the Kacza Stream enters the sea, and freshwater input could have had an impact on the nanoflagellate community. To test this hypothesis, additional samples were taken in June 2005 along a transect from the mouth of the largest river entering the Gdańsk Basin (the River Vistula) to the open sea (Fig. 1). As Fig. 4 shows, the sample analyses did not reveal a clear trend. The composition of the nanoflagellate community was quite similar to that of the summer and early autumn community observed previously in

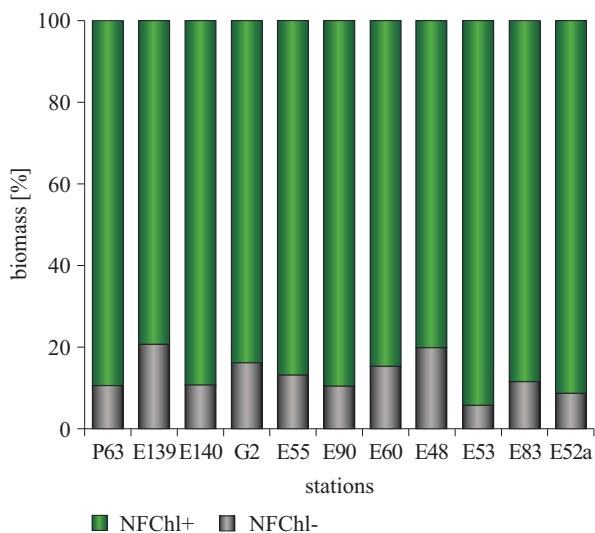


**Fig. 2.** Annual changes in flagellate biomasses in the 1–8  $\mu\text{m}$  size fraction. Cells with (NFChl+) and without chlorophyll (NFChl-) were distinguished. The research was carried out in the near-bottom zone at the end of the wooden pier in Sopot (Gulf of Gdańsk)\*



**Fig. 3.** Relationship between the biomasses of two groups of flagellates (1–8  $\mu\text{m}$ ) with (NFChl+) and without chlorophyll (NFChl-). The annual research cycle was conducted in the near-bottom zone at the end of the wooden pier in Sopot (Gulf of Gdańsk)

\* In the printed version of *Oceanologia* 48 (2) the scale factor on the vertical axis of this figure was given incorrectly. This error has now been rectified in the Internet version. We apologise to our readers for any inconvenience this error may have caused.



**Fig. 4.** Relationship between the biomasses of two groups of flagellates (1–8 µm): with (NFChl+) and without chlorophyll (NFChl−). Samples were taken at stations located in different parts of the Gdańsk Basin (see Fig. 1, page 324)

Sopot – NFChl+ prevalent (79–91% biomass, average 87%). To the author's knowledge, the present study is the only one that has revealed three distinct patterns in the seasonal changes of the nanoflagellate community.

Other studies conducted in the Baltic Sea have not revealed such patterns (Boikova 1984, Andersson-Nordström 1989). In the northern Bothnian Sea (Baltic Sea) NFChl- and NFChl+ were about equally distributed throughout the year (Andersson-Nordström 1989). On the other hand, analysis of data presented by Sherr et al. (2003) from the central Arctic Ocean demonstrated seasonal changes in the nanoflagellate community structure but without any distinct patterns. It is possible that the patterns of NFChl- and NFChl+ coexistence described here are characteristic of the Gdańsk Basin.

The most interesting aspect of this work was the summer and autumn prevalence of NFChl+. Many of the NFChl+ were probably mixotrophic. Two points support this claim: part of the NFChl+ readily turned to heterotrophy (and lost chlorophyll) after being isolated from light (unpublished observation), and the observation that the majority of NFChl+ belonged to the *Prymnesiophyceae*, which are known mixotrophs (Parke et al. 1955, Sherr & Sherr 2000). According to general ecological knowledge, strong competition (as is the case in a planktonic community during summer) should result in the prevalence of specialised organisms (strictly autotrophic or heterotrophic). However, a few advantages at least of the nanoflagellates'

mixotrophy were demonstrated during the summer. Two modes of nutrition (phototrophy and phagotrophy) could allow mixotrophs to grow when there are not enough nutrients for obligate autotrophs, and the concentration of food particles is too low for heterotrophs (Havskum & Riemann 1996, Tittel et al. 2003). Additionally, mixotrophs could (through phagotrophy) obtain more nutrients than autotrophs (Nygaard & Tobiesen 1993). Finally, the consumption of bacteria by mixotrophs relaxes the grazing pressure of predators that feed simultaneously on bacterivorous mixotrophs and bacteria (Rothhaupt 1996). Studies done in the Bay of Aarhus (Baltic Sea) in June (Havskum & Riemann 1996) demonstrated that mixotrophic nanoflagellates were responsible for 86% of the entire flagellate bacterivory in nutrient-depleted water. An observation consistent with this (dominance of mixotrophs during periods with low nutrient concentrations) was also reported in the northern Baltic Sea (Samuelsson 2003). The evaluation of the importance of mixotrophic flagellates in the Gdańsk Basin needs further studies.

In view of the existence of patterns in the relationships between the biomasses of NFChl+ and NFChl-, the Gdańsk Basin is potentially a very convenient area for research into the factors shaping the composition of nanoflagellate communities. Such studies will certainly extend knowledge of the competitiveness of nanoflagellates of different trophic types. Useful methods for further studies could be the detection of bacterivorous flagellates with the use of FLB (Sherr et al. 1987) and, following its application in environmental studies, the labelling of nanoplankton by means of fluorescence *in situ* hybridisation (Beardsley et al. 2005).

## References

- Andersson-Nordström A., 1989, *Flagellates in the marine microbial food web: The ecology of a mixotrophic nanoflagellate, Ochromonas sp.*, Ph.D. thesis, Umeå Univ., 43 pp.
- Azam F., Fenchel T., Field J. D., Gray J. S., Meyer-Reil L. A., Thingstad F., 1983, *The ecological role of water-column microbes in the sea*, Mar. Ecol. Prog. Ser., 10, 257–263.
- Beardsley Ch., Knittel K., Amann R., Pernthaler J., 2005, *Quantification and distinction of aplastidic and plastidic marine nanoplankton by fluorescence in situ hybridization*, Aquat. Microb. Ecol., 41, 136–169.
- Bird D. F., Kalf J., 1986, *Bacterial grazing by planktonic lake algae*, Science, 231, 493–495.
- Boikova E., 1984, *Ecological character of protozoans (Ciliata, Flagellata) in the Baltic Sea*, Ophelia, 3 (Suppl.), 23–32.

- Børshem K. Y., Bratbak G., 1987, *Cell volume to carbon conversion factors for a bacteriovorous Monas sp. enriched from seawater*, Mar. Ecol. Prog. Ser., 36, 171–175.
- Caron D. A., 1983, *Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures*, Appl. Environ. Microbiol., 46 (2), 491–498.
- Caron D. A., 2000, *Symbiosis and mixotrophy among pelagic microorganisms*, [in:] *Microbial ecology of the oceans*, D. L. Kirchman (ed.), Wiley-Liss, 495–523.
- Cho B. Ch., Na S. Ch., Choi D. H., 2000, *Active ingestion of fluorescently labeled bacteria by mesopelagic heterotrophic nanoflagellates in the East Sea, Korea*, Mar. Ecol Prog. Ser., 206, 23–32.
- Estep K. W., Davis P. G., Keller M. D., Sieburth J. McN., 1986, *How important are oceanic algal nanoflagellates in bacterivory?*, Limnol. Oceanogr., 31 (3), 646–650.
- Fenchel T., 1982, *Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers*, Mar. Ecol. Prog. Ser., 9, 35–42.
- Havskum H., Riemann B., 1996, *Ecological importance of bacterivorous, pigmented flagellates (mixotrophs) in the Bay of Aarhus, Denmark*, Mar. Ecol. Prog. Ser., 137, 251–263.
- Hall J. A., Barret D. P., James M. R., 1993, *The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment*, J. Plankton Res., 15 (9), 1075–1086.
- Kuuppo-Leinikki P., 1993, *Horizontal distribution of photo- and heterotrophic microorganisms on the coastal area of the northern Baltic Sea – a case study*, J. Plankton Res., 15 (1), 27–35.
- Mackiewicz T., 1991, *Composition and seasonal changes of nanoflagellates in the Gdańsk Basin (Southern Baltic)*, Acta Ichthyol. Piscat., 21 (Suppl.), 125–134.
- McManus G. B., Fuhrman J. A., 1988, *Clearance of bacteria-sized particles by natural populations of nanoplankton in the Chesapeake Bay outflow plume*, Mar. Ecol. Prog. Ser., 42, 199–206.
- Nygaard K., Tobiesen A., 1993, *Bacterivory in algae: a survival strategy during nutrient limitation*, Limnol. Oceanogr., 38 (2), 273–279.
- Pålsson C., Daniel C., 2004, *Effects of prey abundance and light intensity on nutrition of a mixotrophic flagellate and its competitive relationship with an obligate heterotroph*, Aquat. Microb. Ecol., 36, 247–256.
- Parke M., Manton I., Clarke B. J., 1955, *Studies on marine flagellates II. Three new species of Chrysochromulina*, J. Mar. Biol. Ass. UK, 34, 579–609.
- Porter K. G., 1988, *Phagotrophic phytoflagellates in microbial food webs*, Hydrobiologia, 159, 89–97.
- Rothhaupt K. O., 1996, *Utilization of substitutable C- and P-sources by the mixotrophic chrysophyte Ochromonas sp.*, Ecology, 77 (3), 706–715.

- Safi K. A., Hall J. A., 1999, *Mixotrophic and heterotrophic nanoflagellates grazing in the convergence zone east of New Zealand*, Aquat. Microb. Ecol., 20(1), 83–93.
- Samuelsson K., 2003, *Mechanisms structuring the pelagic microbial food web*, Ph. D. thesis, Umeå Univ., 35 pp.
- Sanders R. W., Caron D. A., Berninger U., 1992, *Relationships between bacteria and heterotrophic nanoplankton in marine and freshwaters: an interecosystem comparison*, Mar. Ecol. Prog. Ser., 86, 1–14.
- Sherr E. B., Sherr B. F., 2000, *Marine microbes (an overview)*, [in:] *Microbial ecology of the oceans*, D. L. Kirchman (ed.), Wiley-Liss, 13–46.
- Sherr B. F., Sherr E. B., Fallon R. D., 1987, *Use of monodispersed, fluorescently labelled bacteria to estimate in situ protozoan bacterivory*, Appl. Environ. Microbiol., 53 (5), 958–965.
- Sherr B. E., Sherr B. F., Wheeler P. A., Thompson K., 2003, *Temporal and spatial variations in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean*, Deep-Sea Res. Pt. I, 50 (5), 557–571.
- Tett P., Wilson H., 2000, *From biogeochemical to ecological models of marine microplankton*, J. Marine Syst., 25, 431–446.
- Tittel J., Bissinger V., Zippel B., Gaedke U., Bell E., Lorke A., Kamjunke N., 2003, *Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs*, PNAS, 100, 12776–12781.
- van Duyl F. C., Bak R. P. M., Kop A. J., Nieuwland G., 1990, *Bacteria, auto- and heterotrophic nanoflagellates and their relations in mixed, frontal and stratified waters of the North Sea*, Neth. J. Sea Res., 26, 97–109.