Contribution of herbivory to the diet of *Temora longicornis* (Müller) in Belgian coastal waters

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Abstract

The contribution of herbivory to the diet of *Temora longicornis* (Müller), an omnivorous calanoid copepod, and the degree of food limitation to its production were investigated in relation to microplankton availability during 2001 in Belgian coastal waters. The gut fluorescence method was combined with egg production measurements to estimate herbivorous and total feeding, respectively. Diatoms were the main phytoplankton component during the sampling period and constituted, with the colonial haptophyte *Phaeocystis globosa,* the bulk of phytoplankton biomass during the spring bloom. HPLC gut pigment analysis showed that diatoms were the main phytoplankton group ingested, whereas no evidence for ingestion of *P. globosa* and nanoflagellates was found. Further, our results showed higher phytoplankton ingestion by *T. longicornis* in spring, when small, chain-forming diatom species such as *Thalassiosira* spp. and *Chaetoceros* spp. were abundant, than in summer, when larger species such as *Guinardia* spp. and *Rhizosolenia* spp. dominated the diatom community. We showed that *T. longicornis* could be regarded as mainly herbivorous during fall and winter, while during spring and summer they needed heterotrophic food to meet their energetic demands for egg production. The phytoplankton spring bloom, either during diatom dominance or during *P. globosa* dominance, did not enhance the contribution of herbivory to the diet. We argue that when *T. longicornis* carbon requirements for egg production increase the contribution of non-phytoplankton food sources (microzooplankton and/or detritus) to total carbon ingestion becomes more important. Except during the early spring diatom bloom of *Chaetoceros socialis*, the egg production rates never reached the production potential of this species, including during the *P. globosa* bloom. This suggests that *T. longicornis* egg production was limited not only by food quantity but also by food quality and that ingestion of non-phytoplankton food sources did not allow the females to compensate for food limitation.

1. Introduction

The coastal waters of the southern North Sea are a well-known eutrophicated ecosystem due to anthropogenic sources of nutrients brought by the discharge of major west-European rivers (Lancelot 1995, Lancelot et al. 1998). The unbalanced nutrient environment of these coastal waters, characterized by an excess of nitrate over silicate and phosphate, induces an increase of spring algal blooms, with a major and sudden change in phytoplankton dominance from diatoms to the colonial flagellate *Phaeocystis globosa* (Lancelot 1995, Peperzak et al. 1998). This is also accompanied by a change in the species composition of the remaining diatoms, characterized by a shift from small chain-forming species toward an assemblage of larger diatom species throughout the spring period (Philippart et al. 2000, Rousseau et al. 2002).

The seasonal changes in phytoplankton abundance, cell size and taxonomic composition discussed above may affect the feeding behaviour of *Temora longicornis* (Müller), an omnivorous calanoid copepod that is present all year round in the coastal waters of the North Sea, with maximum abundances in spring (Daro 1988, Fransz et al. 1992, Williams et al. 1993). Several studies have demonstrated that *P. globosa* is not an adequate food source for *T. longicornis* (Daro 1985, Hansen and van Boekel 1991, Bautista et al. 1992, Breton et al. 1999, Gasparini et al. 2000), and it has been hypothesized that copepods switched to heterotrophic food to compensate for low phytoplankton ingestion (Daro 1985, Hansen and van Boekel 1991). Although field studies confirmed a preferential predation on microzooplankton by copepods during the *Phaeocystis* bloom (Brussaard et al. 1995, Gasparini et al. 2000), this predation seemed insufficient in terms of carbon to cover the copepods' nutritional needs and avoid food shortage (Gasparini et al. 2000). This food shortage could impact negatively not only on the next copepod generation (Bautista et al. 1992), but also on the energy transfer to higher trophic levels (Rousseau et al. 2000).

The general objectives of this study were (1) to examine the possible linkages between seasonal changes in phytoplankton availability and the *in situ* herbivorous feeding of adult *T. longicornis*, (2) to estimate the fraction of *T. longicornis* carbon requirements sustained by herbivory, and (3) to determine whether omnivorous feeding was sufficient to avoid the food limitation of egg production by *T. longicornis*. One approach to estimate omnivory is to measure both total carbon ingestion and algal ingestion and to quantify the degree of omnivory as the difference between the two. As outlined by Dam et al. (1994) and Peterson and Dam (1996) we used the gut fluorescence method combined with egg production measurement to estimate herbivorous and total feeding, respectively. We further analysed gut

pigment content by high-performance liquid chromatography (HPLC), and compared the results to the pigments and microzooplankton present in the water column in order to provide taxonomic information about the composition of *T. longicornis* diet (Kleppel and Pieper 1984, Buffan-Dubau et al. 1996).

2. Materials and methods

Sampling and egg production experiments were carried out in 2001 during field campaigns aboard the RVs *Belgica* and *Zeeleeuw* in Belgian coastal waters (southern North Sea) as part of the AMORE (Advanced MOdelling and Research on Eutrophication) project. Three stations, 230 (depth ~ 13 m), 330 (~24 m) and 435 (~32 m), were monitored fortnightly during spring and monthly for the rest of the year, weather permitting (Fig. IV-1). Vertical profiles of temperature and salinity were obtained using a Seabird Conductivity-Temperature-Depth (CTD) profiler system.

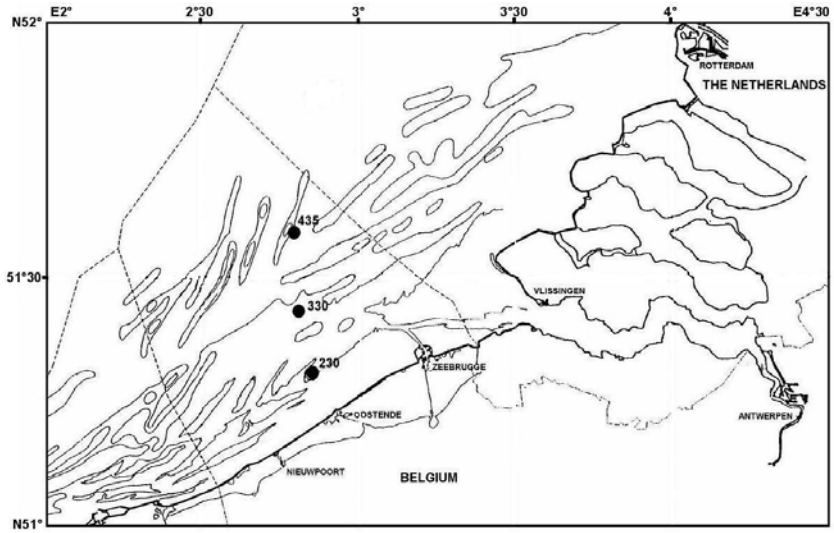

Figure IV-1: Belgian coastal zone, showing location of the three stations sampled during the AMORE 2001 cruises.

Water samples were taken with a 10 litre Niskin bottle from the subsurface, middle and near the bottom of the water column. Seawater subsamples (250-1500 ml) from the different depths were immediately filtered in triplicate using GF/F glass-fibre filters (47 mm diameter, 0.7 µm nominal pore size). All filters were folded in aluminium foil and immediately stored in liquid nitrogen for HPLC pigment analysis. Additional water subsamples (250 ml) were taken in triplicate, after mixing the water from the three depths, and were fixed with 1% (final concentration) glutaraldehyde for algal cell counting. HPLC pigment analysis and algal cell identification and counting followed the procedure described in Antajan and Gasparini (2004).

WP-2 plankton nets (200 µm) were towed obliquely between the surface and bottom (tow duration < 5 min) to collect animals for gut pigment content analysis and egg production experiments. From each zooplankton sample, a subsample was collected onto a piece of 200 µm gauze, wrapped in aluminium foil and frozen immediately in liquid nitrogen to prevent defecation losses. In the laboratory, three replicates of 50 *T. longicornis* adults were sorted under a binocular microscope, placed into 500 µl of 90 % cold acetone and macerated with a tissue grinder. After two hours of extraction in the dark at 4°C, the replicates were filtered on a syringe filter (Acrodisc CR PTFE 0.45 µm) to remove suspended particles, and pigments in the filtrates were analysed by HPLC (Gasparini et al. 2000, Antajan and Gasparini 2004). Chlorophyll-*a* (chl-*a*) and chl-*a* derivatives in gut contents were used as an index of phytoplankton ingestion, whereas carotenoid pigments were used as taxonomic indicators in order to characterize the ingested prey. The amounts of phaeopigments and chl-*a* were summed and the results were expressed as ng chl-*a* equivalents per copepod. No correction was made for possible pigment destruction during the gut passage. Phytoplankton ingestion rates were estimated by multiplying the gut chl-*a* equivalent by the gut clearance rate which was estimated from in situ temperature (Dam and Peterson 1988, Irigoien 1998). Algal carbon ingestion rates were calculated by applying a C:chl-*a* ratio of 50 (Banse 1977). *Temora longicornis* body carbon weight was calculated from the temperature-carbon weight relationships given by Halsband-Lenk (2001).

For egg production measurements, six to twelve replicates of five freshly caught *T. longicornis* females were transferred into 250 ml of filtered seawater (0.7 µm) inside a Plexiglas tube with 200 µm mesh false bottom to prevent egg cannibalism. It has been demonstrated that 24 h of incubation in filtered seawater does not affect egg production rates and reflects the feeding history of the females in the field prior to capture (Peterson and Bellantoni 1987, Tester and Turner 1990, Laabir et al. 1995, Hirche et al. 1997). All replicates were incubated in on-deck incubators and maintained at *in situ* temperature by immersion in a water bath with continuously circulating surface seawater for 24 hours. At the end of the incubation, female survival was visually checked, and eggs spawned during incubation were sieved on a 20 µm mesh and preserved with formalin (4 % final concentration). Into the laboratory eggs were counted and their diameters were measured to calculate their volume assuming a perfectly spherical shape. Then, the volume was converted into carbon content by applying a carbon/volume ratio of 0.14×10^{-6} µgC μm^{-3} (Kiørboe et al. 1985). Egg production rates were calculated as the total eggs divided by the number of females that survived through

the incubation. Females that died during incubation (< 1 %) were assumed to have been moribund at the beginning of the incubation and therefore to have laid no eggs. Egg-carbon production rates were then converted into total carbon ingestion rates by assuming a carbon-specific egg production efficiency of 33 % (Kiørboe et al. 1985, Peterson 1988, Båmstedt et al. 2000).

3. Results

Temperature and salinity profiles revealed that the water column was vertically well-mixed throughout the year. Figure IV-2 presents average temperature and salinity in the water column measured at the three stations in 2001. The seasonal variations of temperature show a winter minimum of about 5.5°C and a summer maximum of about 19.5°C. On average, the difference between stations 230 and 435 was about 1°C. Station 230 had an average salinity of 32 psu and seemed to be the more influenced by freshwater inputs, as shown by the amplitude of salinity variations. The average salinity average was 32.9 psu at station 330 and 33.6 psu at station 435.

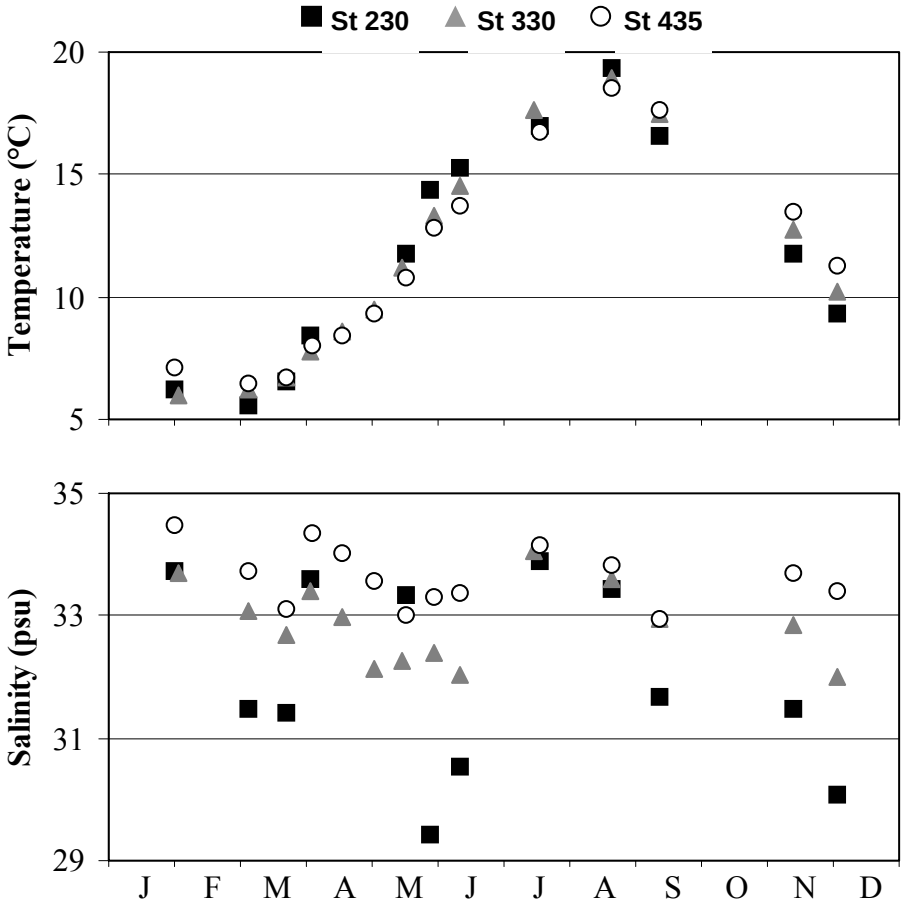

Figure IV-2: Temperature and salinity measured at the three sampling stations of the Belgian coastal zone during 2001.

3.1. Microplankton abundance and pigments biomarkers

Figure IV-3 shows the seasonal variation of diatom, *Phaeocystis globosa*, nanoflagellate (other than *P. globosa*), dinoflagellate and ciliate abundance at the three stations studied in 2001. Diatoms were present throughout the year, reaching maximum abundance in March (ca. 1700 cells ml^{-1}). At that time the genus *Chaetoceros*, largely composed of by *C. socialis*, became dominant, representing up to 90 % of the diatom community. In April and May *P. globosa* dominated diatoms and reached its maximum abundance around mid-April (more than 27,000 cells ml^{-1}). A second but rather modest *P. globosa* growth was observed in September (ca. 1,450 cells ml^{-1}). A bloom of nanoflagellates composed of Cryptophyceae, Prasinophyceae (*Pyramimonas* spp.) and other unidentified nanoplankton-sized flagellates occurred at the end of the *Phaeocystis* bloom in May and was particularly impressive at station 230 (ca. 2,300 cells ml^{-1}). Dinoflagellate and ciliate abundance increased at the wax and wane of the *P. globosa* bloom.

Representative fluorescence and absorbance chromatograms showing the elution pattern of chlorophyll and carotenoid pigments detected in water are presented in Fig. IV-4A,C, and the pigment concentration ranges are given in Table IV-1. Chlorophyll *a* concentrations integrated over the water column varied between 0.15 and 17.35 µg l^{-1} (Fig. IV-5). Increases in chl *a* concentrations were associated with increases in phytoplankton containing fucoxanthin (Spearman rank $r_s = 0.959$, $n = 40$, $p < 0.001$), and coincided with diatom (March and June) and *P. globosa* (April) blooms. Numerous carotenoid pigments were detected by HPLC. Our interest, however, was in those carotenoids that can be utilized as taxonomic markers of classes or groups of classes of phytoplankton. The major carotenoid, fucoxanthin, ranged in concentration from 0.08 to 6.57 µg l^{-1} and was significantly correlated with the abundance of the two dominant golden-brown algae ($r_s = 0.773$, $n = 32$, $p < 0.001$), diatoms and *P. globosa*. Chlorophyll c_3, which was highly correlated with *P. globosa* biomass, ($r_s = 0.751$, $n = 32$, $p < 0.001$) increased from concentrations < 0.10 µg l^{-1} in March up to 1.90 µg l^{-1} in April 2001, corresponding with the maximum abundance of *P. globosa*. Alloxanthin was commonly found in small amounts with highest values observed in May and June corresponding to an increase in cyptomonad abundance ($r_s = 0.597$, $n = 32$, $p < 0.001$). Both 19'-hexanoyloyfucoxanthin and peridinin were found as trace levels and were not correlated with any of the phytoplankton groups we examined. As no chlorophyll *b* standard was available we used chromatographic peak area to compare relative abundance of this pigment

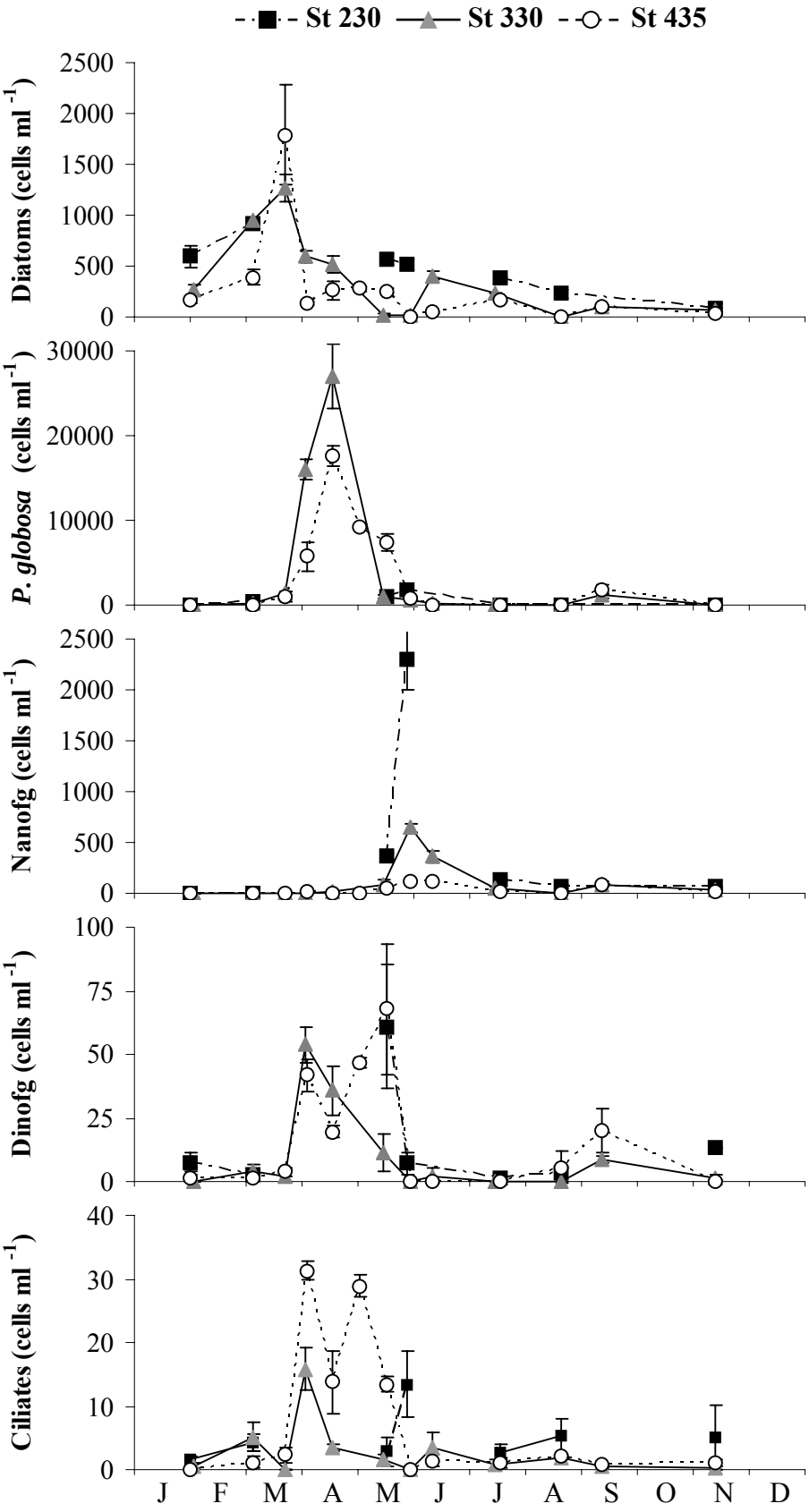

Figure IV-3: Abundance (mean ± SE) of diatoms, *Phaeocystis globosa*, nanoflagellates (other than *P. globosa*), dinoflagellates and ciliates at the three stations of the Belgian coastal zone during 2001. Note the vertical scales.