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Taxon-specific ingestion rates of natural phytoplankton by calanoid copepods in an estuarine environment (Pomeranian Bight, Baltic Sea) determined by cell counts and HPLC analyses of marker pigments

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ABSTRACT: This paper provides information on the application of marker pigment analyses for estimation of phytoplankton biomass as well as qualitative and quantitative estimates of food uptake by calanoid copepods *Acartia bifilosa*, Giesbrecht in early summer (27 June to 2 July 1994) in the Oder estuary (Pomeranian Bight, southern Baltic Sea). The marker pigment concentrations were converted into C-equivalents for comparison with data obtained by the classical Utermöhl method. Both data sets were generally in good agreement, although differences were found at the taxon-specific level, which could be explained largely by limitations of the Utermöhl method. The ingestion rates estimated from cell counts and marker pigment concentrations were within a comparable range (0.31 to 0.84 µgC ind.$^{-1}$ d^{-1} and 0.50 to 1.04 µgC ind.$^{-1}$ d^{-1} respectively). *A. bifilosa* showed a positive food selection for dino- and cryptophytes and an avoidance of cyanobacteria and chlorophytes. Diatoms were ingested according to their abundance. Marker pigment detection has been shown to be a useful tool for estimation of taxon-specific grazing activity in estuarine environments.

KEY WORDS: Pigment biomarkers · Chl *a*:marker pigment ratios · C:chl *a* ratios · Ingestion rates · Food selection · *Acartia bifilosa* · Pomeranian Bight · Baltic Sea

INTRODUCTION

Attempts to quantify the diet of copepods and to understand its significance in terms of secondary production have been discussed in the literature for many decades (reviewed in Kleppel et al. 1991, Kleppel 1993). A serious problem in estimating feeding activity is differentiating between methodological artifacts and natural variability, caused by factors such as species differences, environmental factors, and spatio-temporal scales (Morales & Harris 1990). Methods of estimating grazing activity have included gut fluorescence measurements to estimate food uptake by calanoid copepods *in situ* (e.g. Head & Harris 1987, Morales et al. 1993), or methods such as Utermöhl cell counting or electronic particle counting to estimate ingestion rates in bottle incubations (e.g. Omori & Ikeda 1976). All these techniques have their specific limitations. The major drawback of the gut fluorescence method is the lack of differentiation between different phytoplankton groups, because chlorophyll *a* (chl *a*) is a marker for phytoplankton in general (see review by Morales & Harris 1990). The Utermöhl method allows reliable quantification of single phytoplankton species, but is very time consuming. Additionally, high densities of phytoplankton particles, a wide size spectrum and a high proportion of detritus influence the accuracy of this method, especially in estuarine waters (Tackx et al. 1995). Electronic particle counters, which have been used for at least 15 yr, produce highly reliable

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data only when spherical unicellular algal cultures are used. Applications of this technique to natural phytoplankton communities have often been misleading because of the high variability in shape and size of natural phytoplankton populations. Furthermore, particle counters cannot distinguish phytoplankton particles from other particles of similar size, such as fecal pellets and detritus (Omori & Ikeda 1976).

Measurements of algal pigments other than chl *a* in animals' guts, especially for copepods, have been used for investigating food composition (Kleppel & Pieper 1984, Kleppel et al. 1988a, b, Nelson 1989, Swadling & Marcus 1994, Buffan-Duban et al. 1996), but this approach only allows qualitative estimation of grazing activity, because the stability of the different pigments during their passage through the gut is unknown in many cases (Kleppel et al. 1988a, b, 1991, Head 1992, Head & Harris 1992, 1994, 1996). By measuring the marker pigment composition in the incubation medium at the beginning and the end of a feeding experiment (Balance Method: Frost 1972, Omori & Ikeda 1976), it is possible to quantify taxon-specific grazing activity. This method has been used for microzooplankton in dilution experiments (Burkill et al. 1987, Strom & Welschmeyer 1991, McManus & Ederington-Cantrell 1992). To our knowledge only Head & Harris (1994) have used this technique to quantify uptake rates for different pigments by copepods.

In order to calculate carbon fluxes through the food web it is essential to convert the estimated pigment uptake into carbon (C) equivalents. Such a conversion from marker pigment concentration to C-equivalents can only be achieved by a 2-step calculation. Firstly, the marker pigment concentrations have to be converted into chl *a* equivalents, and these then further to C-equivalents. Conversion factors are available for the first step, but only for laboratory-cultivated phytoplankton species (Burkill et al. 1987). If these factors are used, it is an implicit assumption that different light conditions only influence the total pigment content without changing the ratio of chl *a* to the respective marker pigment (Vesk & Jeffrey 1977). However, in many cases, light conditions do alter pigment ratios in laboratory-cultured algae (Johnsen et al. 1992, Johnsen & Sakshaug 1993) as well as in the field (Gieskes et al. 1988, Brunet et al. 1993, Letelier et al. 1993, McManus 1995). Variable pigment ratios might be expected in estuarine environments because of variations in irradiance caused by mixing processes between river outflow and estuarine water (McManus 1995). Chl *a*:marker pigment ratios based on specific light regimes are therefore of limited value (McManus 1995). For the quantification of carbon fluxes in the field, habitat-specific chl *a*:marker pigment ratios are thus needed, as described in this study.

Recent technological developments have led to new methods for the quantification of biological interactions in natural environments. Before using these techniques in standardized experiments, however, there is a need to compare the results they give with results obtained using existing classical methods (Neveux et al. 1990). The aim of this study was to compare a classical method (Utermöhl method) with a newer technique (HPLC pigment analysis) to estimate qualitative and quantitative food uptake (or grazing rates) of calanoid copepods, specifically *Acartia bifilosa*, the dominant species during an investigation in the Oder estuary (Pomeranian Bight, southern Baltic Sea), in order to gain a better understanding of taxon-specific energy fluxes of phytoplankton to higher trophic levels in this environment. C-equivalents were chosen for comparision of the different methods tested because this study was a part of a joint research project in the Pomeranian Bight (TRUMP) and the investigation focused on the distribution, transport and modification of biogenic and anthropogenic inputs from the Oder river (Bodungen et al. 1995).

MATERIALS AND METHODS

Investigation area. The Pomeranian Bight is influenced by the outflows from the Oder, the Swina (Oder mouth), the Dwina and the Peene rivers. There are strong gradients in salinity, nutrients- and phytoplankton concentrations between the Swina (Oder mouth) and the open Baltic Sea (Fig. 1; Bodungen et al. 1995).

Water samples. Sampling for grazing experiments was undertaken at 6 stations (Fig. 1) from 27 June to 2 July 1994. Water samples from 2 to 3 m depth (the layer with the highest chl *a* concentration) were collected with a rosette water sampler equipped with 12 l bottles. The samples were passed through a 200 µm mesh to exclude larger grazers such as copepods. One subsample was used to fill eight 3 l polycarbonate bottles for the grazing experiments. For cell counts, 4 subsamples (250 ml) were taken and fixed with 1% Lugol. Another 4 subsamples (500 ml) for pigment analysis (HPLC) were filtered through Whatman GF/F glass-fiber filters (⌀ 25 mm) and stored in liquid nitrogen on board and at −80°C in the laboratory until further analyses 2 wk later. These operations were carried out under subdued light (<10 µmol m^{-2} s^{-1}).

The copepods used in the grazing experiments were collected at the same stations as the water samples using a 200 µm mesh ring net (⌀ 0.75 m), towed vertically between 10 m and the surface. Immediately after capture, the zooplankton were screened on to a 200 µm mesh and immersed in a bucket of GF/F filtered seawater which was equipped with a light trap

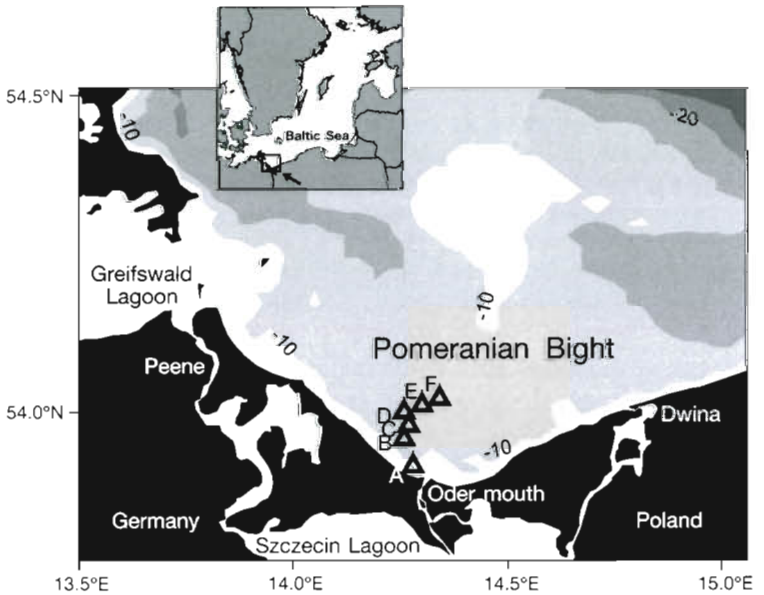

Fig. 1. Sampling stations (A to F) in the Oder estuary (Pomeranian Bight, southern Baltic Sea)

(for procedure see Behrendt & Krocker 1990). Healthy *Acartia bifilosa* were isolated under a binocular microscope and transferred into filtered seawater for 3 h to clear their guts.

Experiments/analytical procedure. Four bottles were inoculated with 50 to 60 adult *Acartia bifilosa*. The other 4 bottles, without copepods, were used as controls. The 8 incubation bottles were placed in a plexiglass box which was fixed to a drifting buoy for 24 h at the depth at which the incubation water had been collected. In this way, the contents were mixed by water movements. The temperature at this depth ranged between 17 and 18°C at the different stations. At the end of the experiment the water from the incubation bottles was passed through a 200 µm mesh to isolate the copepods from the phytoplankton. The copepods were transferred into filtered seawater to check their mortality. Subsamples of the incubation water were collected from each of the 8 bottles for both cell counts and pigment analyses as described above.

Phytoplankton cell concentrations for the major taxonomic groups were determined by inverted microscopy at ×100 and ×400 magnification after settling in a 10 ml counting chamber (Utermöhl 1931, 1958). Phytoplankton counts were converted into carbon equivalents via biovolume calculations using a volume to carbon conversion coefficient according to Strathmann (1967).

Phytoplankton marker pigments for the different phytoplankton groups in the Pomeranian Bight (Table 1) were analysed by HPLC using the method described by Kraay et al. (1992). This method allows a good separation of lutein and zeaxanthin. The system consisted of a Merck-Hitachi liquid chromatograph equipped with a L6200A gradient pump with system controller (interface module D-600), a photodiode array detector (L4500) and a F-1050 fluorescence spectrophotometer. Pigment detection was at 436 nm for all chlorophylls and carotenoids and 405 nm for phaeophytin *a* and phaeophorbide *a*. Pigments were identified by diode array spectroscopy during elution, and by transferring HPLC fractions to standard solvents and comparing their visible absorption spectra with reference standards according to Wright et al. (1991). A Kontron UVIKON 941 spectrophotometer was used for UV-visible spectroscopy of purified pigments.

The chemotaxonomic characteristics of the diagnostic pigments (Table 1) is given by Bustillos-Guzmán et al. (1995) for 1 to 5 and by Hooks et al. (1988) and Letelier et al. (1993) for 6. Fucoxanthin is a major accessory pigment in diatoms. It can also occur in prymnesio- and chrysophytes (Gieskes et al. 1988) but this had no effect in the present study because these algal classes were not detected in cell counts.

Grazing rates were calculated according to the method given by Frost (1972).

To calculate conversion factors for the estimation of chl *a* equivalents based on marker pigment concentrations, multiple regression analysis was performed, based on the measured chl *a* in the subsamples from the control bottles and the respective marker pigment concentrations (n = 48) according to e.g. Gieskes & Kraay (1983), Gieskes et al. (1988) and Barlow et al. (1993). The further transformation to C-equivalents was based on a C:chl *a* ratio of 50, which is commonly used for estuarine environments (Irigoien et al. 1993, Dagg 1995, McManus 1995, Sautour et al. 1996). To check the applicability of this value in this case, C:chl *a* ratios were also calculated based on the chl *a* concentrations measured by HPLC and the C-equivalents calculated from the microscopic counts (Table 2).

Table 1. Diagnostic pigments for the characterization of the different algal groups in the Pomeranian Bight

Phytoplankton group	Phytoplankton marker pigment
1. Cyanobacteria	Zeaxanthin
2. Diatoms	Fucoxanthin
3. Chlorophytes	Lutein
4. Dinophytes	Peridinin
5. Cryptophytes	Alloxanthin
6. Prasinophytes	Prasinoxanthin

Table 2. Chlorophyll *a* (chl *a*) measured by HPLC and C:chl *a* ratios estimated for the different stations in the Pomeranian Bight (calculated from microscopic cell counts)

Station	Chl *a* ($\mu g\ l^{-1}$) n = 12	C:chl *a* ratio n = 12
A	22.44 ± 0.93	45 ± 2
B	19.45 ± 0.55	50 ± 3
C	11.94 ± 0.53	48 ± 3
D	9.33 ± 0.42	56 ± 13
E	21.60 ± 1.00	48 ± 3
F	8.16 ± 0.08	32 ± 7

Food selection was quantified by the selectivity coefficient α (Manley et al. 1972, Chesson 1983). This index ranges from 0 to 1.0. A value of 0.5 indicates non-selective feeding, α values >0.5 indicate a preference for a phytoplankton group and $\alpha < 0.5$ a discrimination against a group. The calculations for α were based on the estimated taxon-specific phytoplankton biomass ($\mu gC\ l^{-1}$) in the incubation water at the beginning of the experiment and the calculated ingestion rates ($\mu gC\ ind.^{-1}\ d^{-1}$) measured by the Utermöhl method and HPLC analyses (n = 24, for all stations). The coefficient α calculated by cell counts and pigment analysis is termed α^c and α^p, respectively.

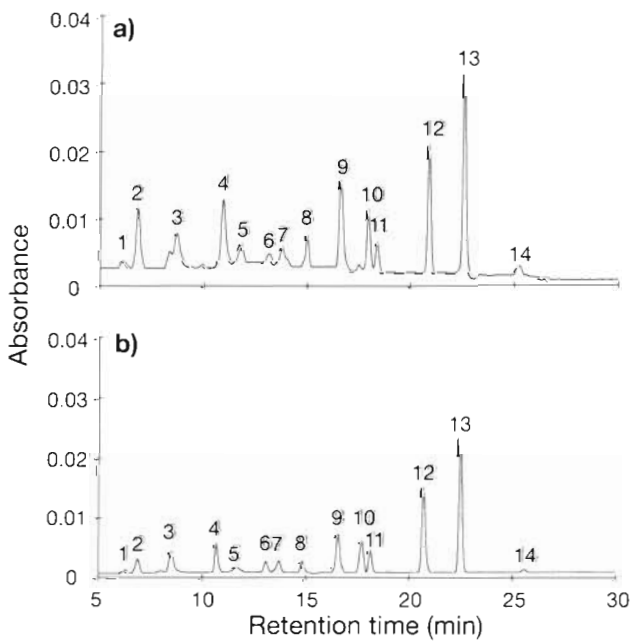

Fig. 2. Chromatograms (fixed wavelength 436 nm) with the typical pigment patterns in the incubation water (a) at the beginning (t_0) of the grazing experiments, and (b) after 24 h incubation (t_1). 1, chlorophyll c_1; 2, chlorophyll c_2; 3, peridinin; 4, fucoxanthin; 5, neoxanthin; 6, prasinoxanthin; 7, violaxanthin; 8, diadinoxanthin; 9, alloxanthin; 10, lutein; 11, zeaxanthin; 12, chlorophyll *b*; 13, chlorophyll *a*; and 14, β-carotene

RESULTS

Examples of absorbance chromatograms are presented in Fig. 2a, b, showing the elution pattern of chlorophyll and carotenoid pigments detected in the incubation water before (Fig. 2a) and after (Fig. 2b) the experiment. The individual marker pigments detected by HPLC analyses were consistent with the algal classes found by microscopical examination. The phytoplankton population in the Pomeranian Bight consisted of 6 algal classes (Fig. 3) with a wide size range in the group of cyanobacteria and chlorophytes (Table 3). The most dominant groups were chlorophytes, diatoms and cyanobacteria. Dinophytes, prasinophytes and cryptophytes only occurred in low numbers (Fig. 3).

As described in the 'Materials and methods', a 2-step calculation is needed to convert marker pigment concentrations into carbon equivalents. The following equation was derived to first estimate the contribution of single phytoplankton groups represented by the marker pigments to the total phytoplankton population:

$$[\text{chl } a] = 0.01 + 0.5[\text{zea.}] + 1.4[\text{fuco.}] + 4.9[\text{lutein}] + 2.8[\text{peri.}] + 2.3[\text{allo.}] + 0.2[\text{prasino.}] \quad (1)$$

The r^2 for this regression was 0.981 (significance level $p < 0.001$, n = 48). The constant 0.01 was the amount of remaining chl *a* when the other terms were zero. Therefore only a small part of the total chl *a* concentration could not be accounted for by any of the phytoplankton classes considered (1.9%).

Table 3. Size distribution of the dominant phytoplankton species within the 6 algal classes during the investigation period (27 June–2 July 1994) in the Pomeranian Bight

Phytoplankton group	Dominant phytoplankton species with average size
Cyanobacteria	*Oscillatoria* sp. (trichomes, 70 × 4 µm) *Merismopedia* sp. (colonies, 9 × 9 µm) *Microcystis* sp. (colonies, Ø 16 µm) *Woronichinia* sp. (colonies, Ø 15 µm)
Diatoms	*Thalassiosira levanderi* (Ø 10 µm) *Skeletonema costatum* (10 × 5 µm) Unknown centric diatoms (Ø 10 µm)
Chlorophytes	*Actinastrum hatzschii* (13 × 4 µm) *Monoraphidium* sp. (24 × 3, 100 × 2, 11 × 5 µm) *Coelastrum microporum* (Ø 8 µm) *Scenedesmus* sp. (10 × 5, 12 × 7 µm)
Dinophytes	*Katodinium rotundatum* (12 × 8 µm) *Prorocentrum balticum* (13 × 8 µm)
Cryptophytes	*Plagioselmis prolonga* (10 × 5 µm) *Teleaulax amphioxeia* (12 × 8)
Prasinophytes	*Pyramimonas* sp. (4 × 3, 6 × 5 µm)

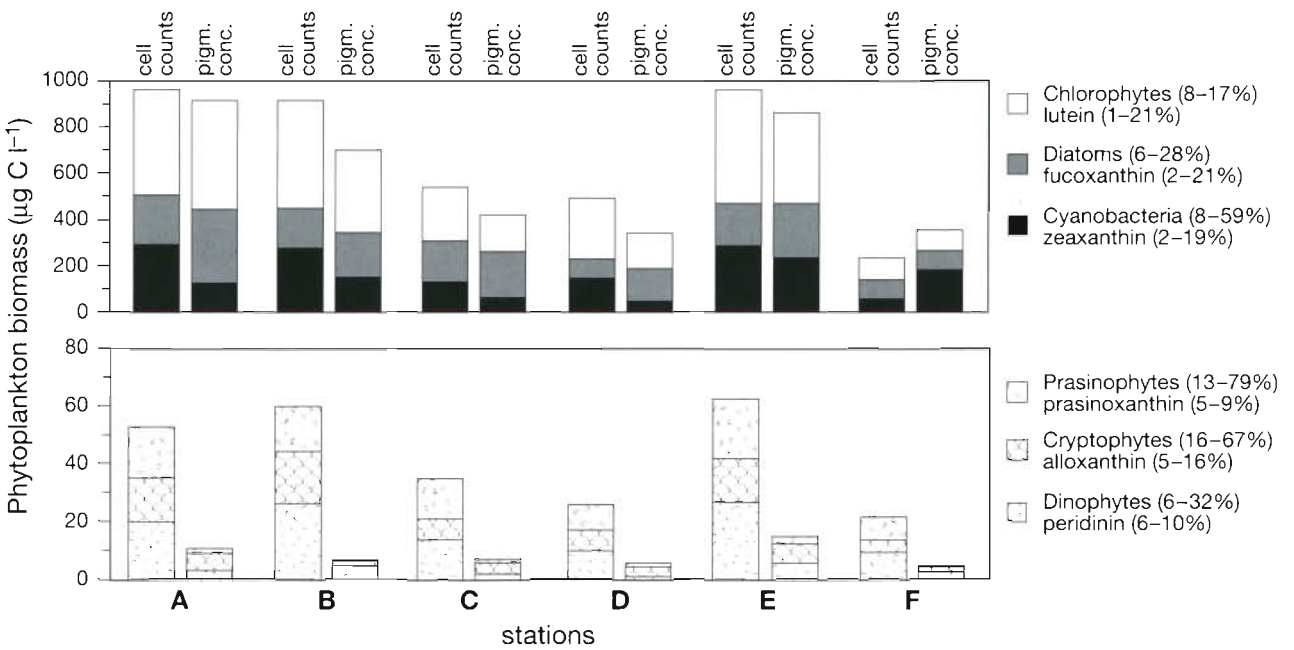

Fig. 3. Mean biomass values of the phytoplankton composition in the incubation water at Stns A–F at the beginning of the grazing experiments represented in carbon (C) equivalents, calculated from microscopic cell counts and marker pigment (pigm.) concentrations (conc.). The standard deviation for the cell counts of each algal group and the corresponding marker pigment is given in % for all stations tested (n = 24)

To check the reliability of the chosen C:chl *a* value of 50, habitat-specific C:chl *a* ratios were estimated based on carbon equivalents calculated from cell counts and on chl *a* concentrations measured by HPLC. The chl *a* concentrations ranged between 8.16 µg l^{-1} at Stn F and 22.44 µg l^{-1} at Stn A near the river mouth (Table 2). In general, chl *a* concentrations decreased with increasing distance from the river mouth. The calculated C:chl *a* ratios varied between 32 and 56 at the different stations analysed (Table 2) with a mean value of 46 ± 9 (n = 72). Thus, the value of 50, which was used for further calculations, was within the range of values found in the Pomeranian Bight.

The sum of phytoplankton carbon derived from cell counts (258 to 1026 µgC l^{-1}) or from marker pigment concentrations (363 to 926 µgC l^{-1}) were similar (Fig. 3). Differences between the 2 methods occurred at the taxon level, however. For dino-, crypto- and prasinophytes, estimates based on cell counts yielded systematically higher values than HPLC measurements (Fig. 3). With the exception of Stn F, cyanobacteria were also more abundant in the calculations based on cell counts. The variability within the different algal groups was generally higher when estimated by cell counts, compared with that derived from HPLC measurements.

To examine the practicability of marker pigments analysis as an analytical tool for grazing experiments, ingestion rates based on microscopic cell counts and marker pigment analyses were compared. The sum of the taxon-specific ingestion rates calculated by cell counts was lower (0.31 to 0.87 µgC ind.$^{-1}$ d^{-1}) compared with that derived from detected pigment concentrations (0.50 to 1.04 µgC ind.$^{-1}$ d^{-1}; Fig. 4). At the taxon-specific level, similar ingestion rates were found with both methods for diatoms, chlorophytes and cryptophytes. For cyanobacteria and prasinophytes ingestion rates calculated from cell counts (0.024 to 0.090 µgC ind.$^{-1}$ d^{-1} and 0.016 to 0.052 µgC ind.$^{-1}$ d^{-1}) were higher than those derived from pigment measurements (0.009 to 0.021 µgC ind.$^{-1}$ d^{-1} and 0.001 to 0.003 µgC ind.$^{-1}$ d^{-1}). By contrast, however, in the case of dinophytes, lower ingestion rates were obtained from cell counts than from marker pigment analyses.

Comparison between the abundances of the different phytoplankton groups in the incubation water (Fig. 3) and the calculated ingestion rates of *Acartia bifilosa* (Fig. 4) suggested that dino- and chryptophytes were major components of the diet. In contrast, cyanobacteria and chlorophytes, two of the dominant algae groups, only constituted a relatively small amount of the overall phytoplankton biomass ingested (Figs. 3 & 4).

For the quantification of the feeding behaviour of *Acartia bifilosa*, the selectivity coefficient α was determined (Fig. 5). The values α calculated with the data estimated by cell counts (α_c) and pigment concentra-

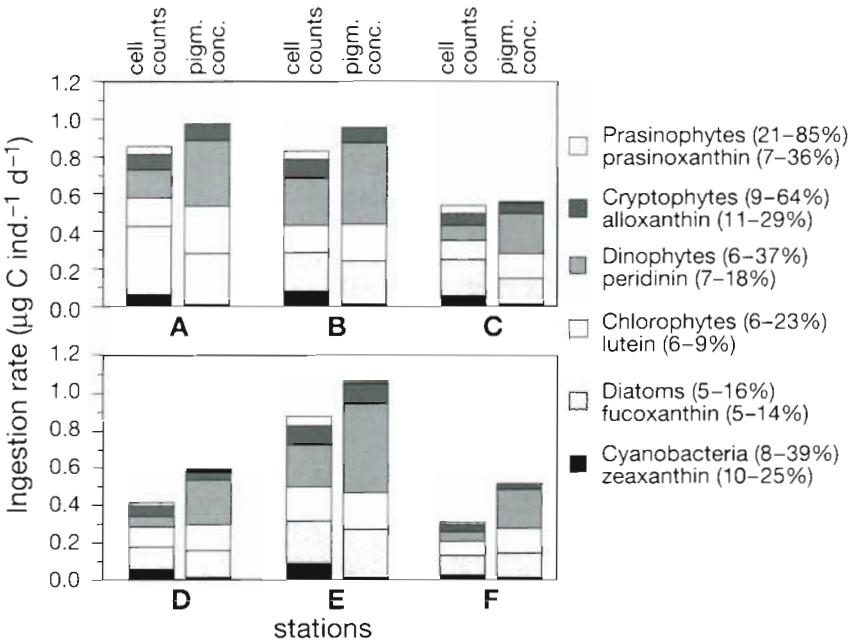

Fig. 4. *Acartia bifilosa*. Mean values of the ingestion rates at the different stations expressed in carbon (C) equivalents, calculated from microscopic cell counts and marker pigment (pigm.) concentrations (conc.). The standard deviation for the cell counts of each algal group and the corresponding marker pigment is given in % for all stations tested (n = 24)

tions (α_p) showed a similar pattern, except for the prasinophytes. Positive selection was found in the case for dino- (α_c = 0.90, α_p = 0.99) and cryptophytes (α_c = 0.89, α_p = 0.94), no selection for diatoms (α_c = 0.62, α_p = 0.42) and a discrimination against cyanobacteria (α_c = 0.24, α_p = 0.01) and chlorophytes (α_c = 0.25, α_p = 0.31). For prasinophytes, positive selection was only found based on cell counts (α_c = 0.74). The calculation of pigment analyses gave, in contrast, evidence for non-selection (α_p = 0.48).

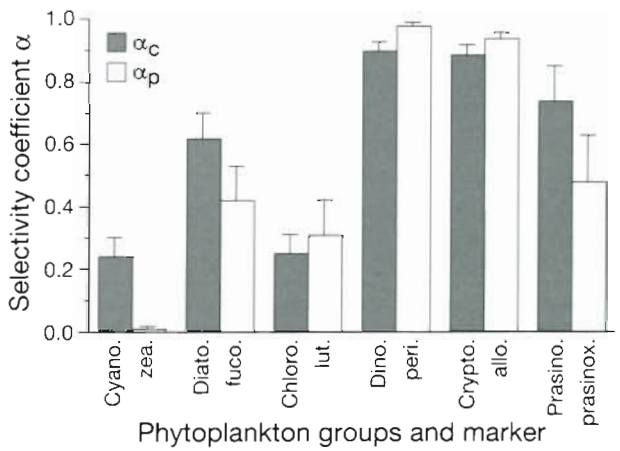

Fig. 5. *Acartia bifilosa*. Selectivity coefficient α calculated by cell counts (α_c) and pigment analysis (α_p) (x + SD, n = 24 for all stations) feeding on natural phytoplankton from the Oder estuary (Pomeranian Bight). $\alpha > 0.50$ indicates a positive selection for a phytoplankton group, and $\alpha < 0.5$ indicates avoidance of this group. (Cyano., Cyanobacteria; zea., zeaxanthin; Diato., Diatoms; fuco., fucoxanthin; Chloro., Chlorophytes; lut., lutein; Dino., Dinophytes; peri., peridinin; Crypto., Cryptophytes; allo., alloxanthin; Prasino., Prasinophytes; prasinox., prasinoxanthin)

DISCUSSION

Multiple regression analysis based on the marker pigment composition and chl *a* content is necessary for a reliable calculation of taxon-specific ingestion rates for specific habitats, because chl *a*:marker pigment ratios vary with changing light intensities in the field (Gieskes et al. 1988, Brunet et al. 1993, Letelier et al. 1993, McManus 1995, Waterhouse & Welschmeyer 1995). The use of chl *a*:marker pigment ratios based on specific light regimes (as in laboratory-cultured algae) are therefore of limited value (McManus 1995) and could result in over- or underestimations of calculated chl *a* equivalents. The estimated chl *a*:marker pigment ratios calculated in this study were, with the exception of prasinoxanthin, within the range of ratios found in the literature (Gieskes & Kraay 1983, Gieskes et al. 1988, Barlow et al. 1995, Bustillos-Guzmán et al. 1995). A cross-check with Utermöhl analyses should always be carried out, however, because some phytoplankton show irregularities in their pigment composition. For example, in the group of dino-, prasino- and prymnesiophytes, the marker pigments of the different groups can be replaced or supplemented by other pigments (Millie et al. 1993 and refs. therein). But such irregularities are only known for a relatively small number of species within these groups. Unfortunately so far we do not know the exact pigment composition for each species. Therefore there are still some limitations in the interpretation of HPLC analyses, which might explain the differences found between biomass and ingestion rates calculated by pigment concentrations and those derived from cell counts (Fig. 3). However, the good agreement between the chl *a*:marker pigment ratios

found in this study with ratios from the literature (see above), and the fact that 98% of the chl *a* could be attributed to the 6 detected marker pigments, suggests that HPLC analysis detected most of the relevant pigments. Thus, HPLC analysis adequately described the phytoplankton populations within the samples. Differences between taxon-specific phytoplankton biomass and ingestion rates derived from marker pigment concentrations and cell counts are probably due to problems in quantification of algal cells by the Utermöhl method. It is well known that methodological aspects and the skill of the persons counting can have a major effect on the accuracy of this method (Gieskes & Kraay 1983, Booth 1987, Millie et al. 1993). In estuarine waters, detritus and high variations in cell size further influence the reproducibility of the Utermöhl method (Tackx et al. 1995). In the Pomeranian Bight, chain-forming and colonial cyanobacteria may have been overestimated. This group is difficult to count as individuals due to the low visibility of their cell membranes within the trichomes, or because of the spherical nature of the colonies, which can only be roughly estimated by permanent focusing. Further difficulties in counting at the species level occur when, due to the fixation process, colonies fall apart, yielding single coccal cells, which can no longer be determined exactly as specific phytoplankton groups. Such problems occurred in the Pomeranian Bight in differentiating between cyanobacteria and chlorophytes. Cyanobacteria seem to have been overestimated (Figs. 3 & 4), whereas chlorophytes were underestimated by the Utermöhl method, when compared with pigment analyses. This is especially true for calculated ingestion rates. The overestimation of the prasinophytes by cell counts might be related to the difficulty of identifying them because of their small size and the fact that their morphology is similar to some chlorophytes.

C:chl *a* ratios are affected by nutrient and light conditions (Banse 1977, Laws et al. 1983), so that reliable ratios must be estimated for each specific environment. The C:chl *a* ratio of 50 given in the literature for estuarine environments (e.g. Bianchi et al. 1993, Irigoien et al. 1993, Dagg 1995, McManus 1995, Sautour et al. 1996) was confirmed by the results presented in this study.

Taxon-specific ingestion rates from field studies based on marker pigment measurements are scarce, and mostly related to microzooplankton (Burkill et al. 1987, Strom & Welschmeyer 1991, McManus & Ederington-Cantrell 1992). There has only been one study with copepods (Head & Harris 1994). The estimates of total carbon ingestion rates, which were in the range 0.5 to 1.04 µgC ind.$^{-1}$ d^{-1} (Fig. 4), were similar to values reported in literature. Ingestion rates of 0.5 to 1.3 µgC ind.$^{-1}$ d^{-1} have been reported for *Acartia bifilosa* in the Gironde estuary (France) at phytoplankton concentrations comparable with those in the Pomeranian Bight (Irigoien et al. 1993). In Fourleague Bay (Louisiana, USA), ingestion rates of 0.6 to 2.2 µgC ind.$^{-1}$ d^{-1} were reported for *A. tonsa* (Dagg 1995). A comparison of taxon-specific grazing rates with the available phytoplankton standing stock allows preferences for certain food taxa to be identified. The data presented here indicated that dino- and cryptophytes were the major components of the diet of *A. bifilosa*, although these phytoplankton groups were of only minor importance in the natural algal population (see Fig. 3).

Prior to the work of Paffenhöfer et al. (1982) using high-speed microcinematography, the mechanism of food selection of herbivorous calanoid copepods was thought to be simply mechanical, reflecting variable retention efficiency of the second maxillae for particles of different sizes (Frost 1977). Since then it has become obvious that selective feeding behaviour is more complex. It is influenced not only by the size of the algae and the phytoplankton concentration in the water column, but also by the nutritional value of the different algae and the composition of the algal population (Vanderploeg 1990, Kleppel 1993 and refs. cited). The data presented in this study suggest that it was not the size of the algae which was responsible for food selection. Amongst the algae which were not positively selected (diatoms, chlorophytes) many species were similar in size to algae which were selected (dino- and cryptophytes; Table 3). In habitats such as the Pomeranian Bight, where food is available in sufficient quantities, it has been suggested that food quality may be the deciding factor for food selection (Vanderploeg et al. 1988, Paffenhöfer et al. 1995). For dinoflagellates which were highly selected by *Acartia bifilosa*, it is known that volume-specific concentrations of protein, lipids and carbohydrates are higher than they are in diatoms under the same growth conditions (Hitchcock 1982). This agrees with studies in the nearshore waters off Los Angeles (USA), which showed that egg production of *A. tonsa* was related to the standing stock of dinoflagellates rather than to that of diatoms (Kleppel et al. 1991). In the Pomeranian Bight, egg production of *A. bifilosa* increased with increasing concentration of dinoflagellates (Schmidt unpubl.).

Cryptophytes, also positively selected by *Acartia bifilosa*, are known to be a valuable food for copepod cultures (Klein Breteler 1980). Although diatoms are thought to be an important food source for calanoid copepods (Bathmann et al. 1990), in this study they were only ingested by *A. bifilosa* according to their abundance. Recent studies have suggested that growth phase may affect the nutritional value of diatoms. In the stationary phase, increased mucus production may cause decreases in feeding rates of copepods (Malej &

Harris 1993). In feeding experiments in the laboratory, daily egg production and gross growth efficiencies of *A. tonsa* were lower when fed with the diatoms *Thalassiosira weissflogii* and *Ditylum brightwellii* compared with animals fed with the cryptophyte *Rhodomonas baltica*. Egg production ceased entirely within 4 d when the diet was the chlorophyte *Dunaliella tertiolecta* (Støttrup & Jensen 1990). In the Pomeranian Bight cyanobacteria were only ingested in low amounts. According to most studies, cyanobacteria are a poor food for zooplankton (e.g Lampert 1981, Ahlgren et al. 1990), because of difficulties resulting from the manageability of filaments, nutritional inadequacy and toxicity.

The degree of marker pigment and chl *a* destruction during digestion varies with ingestion rate (Head & Harris 1996) and depends in part on the previous feeding history of the copepods (Head 1992, Head & Harris 1992). The species composition of the algae and incubation conditions may also affect levels of pigment destruction (Head & Harris 1994, 1996). Calculation of grazing activity by pigment measurements using the balance method must take into account that in addition to the phytoplankton composition at the end of the experiments, the residual pigments remaining in faecal pellets will also be analysed and thus may influence the calculation of taxon-specific ingestions rates (Head & Harris 1994). To estimate the extent to which chl *a* and marker pigment concentration in faecal pellets might have affected calculations of grazing activity, faeces collected from *Acartia bifilosa* were analysed by HPLC (results not shown). In most of the faeces analysed, marker pigments and chl *a* could only be detected in low concentrations (<0.01 µg pigments per sample). Head (1992) reported that copepods which have fed at high levels of food *in situ* may have a greater potential ability to destroy ingested chl *a*. This seems also to be the case in the Pomeranian Bight because the chl *a* concentration ranged from 8 to 22 µg chl *a* during the investigation.

The marker pigment analysis and the classical Utermöhl method gave comparable estimates for the uptake of different algal classes, and summed carbon ingestion rates were within the range reported for *Acartia* sp. in estuarine waters (see above). The results show that the HPLC technique is a reliable method to estimate taxon-specific carbon flux between primary producers and *A. bifilosa* in the Pomeranian Bight. Further studies on grazing pressure and selective grazing behaviour of different zooplankton species analysed by HPLC in areas of different nutritional regimes and comparable laboratory experiments are needed to demonstrate the general usefulness of the method. The following considerations should be taken into account: (1) Determination of habitat-specific chl *a*:marker pigment ratios by multiple regression analysis, and (2) estimation of specific C:chl *a* ratios for the area of investigation.

C:chl *a* ratios can vary widely and it may be misleading to use values from the literature without cross validation in the investigation area.

Because HPLC analyses can be automated and are not subject to operator error (Millie et al. 1993), we recommend the use of marker pigment analyses as a standard method for determination of grazing activities in estuarine environments and perhaps also for the estimation of phytoplankton biomass in field samples. Utermöhl measurements are probably only needed for random controls of the actual species composition within phytoplankton. A more detailed knowledge of the grazing activity on natural phytoplankton might help provide new understanding of the success of zooplankton and, ultimately, repercussions on higher trophic levels.

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LITERATURE CITED

Ahlgren G, Lunstedt L, Brett M, Forsberg C (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J Plankton Res 12: 809–818

Banse K (1977) Determining the carbon-to chlorophyll ratio of natural phytoplankton. Mar Biol 41:199–212

Barlow RG, Mantoura RFC, Gough MA, Fileman TW (1993) Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep Sea Res II 40:459–477

Barlow RG, Mantoura RFC, Peinert RD, Miller AEJ, Fileman TW (1995) Distribution, sedimentation and fate of pigment biomarkers following thermal stratification in the western Alboran Sea. Mar Ecol Prog Ser 125:279–291

Bathmann UV, Noji TT, von Bodungen B (1990) Copepod grazing potential in late winter in the Norwegian Sea—a factor in the control of spring phytoplankton growth. Mar Ecol Prog Ser 60:225–233

Behrendt H, Krocker M (1990) A method for separation of zooplankton in high eutrophic water bodies. Limnologica 20:79–81

Bianchi TS, Findlay S, Dawson R (1993) Organic matter sources in the water column and sediments of the Hudson River Estuary: the use of plant pigments as tracers. Estuar Coast Shelf Sci 36:359–376

Bodungen B von, Graeve M, Kube J, Lass U, Meyer-Harms B, Mumm N, Nagel K, Pollehne F, Powilleit M, Reckermann M, Sattler C, Siegel H, Wodarg D (1995) Stoff-Flüsse am Grenzfluß-Transport- und Umsatz-Prozesse im Übergangsbereich zwischen Oderästuar und Pommerscher Bucht (TRUMP). Geowissenschaften 13:479–485

Booth BC (1987) The use of autofluorescence for analyzing oceanic phytoplankton communities. Bot Mar 30:101–108
Buffan-Dubau E, Wit de R, Castel J (1996) Feeding selectivity of the harpacticoid copepod *Canuella perplexa* in benthic muddy environments demonstrated by HPLC analyses of chlorin and carotenoid pigments. Mar Ecol Prog Ser 137: 71–82
Burkill PH, Mantoura RFC, Llewellyn CA, Owens NJP (1987) Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Mar Biol 93:581–590
Bustillos-Guzmán J, Claustre H, Marty JC (1995) Specific phytoplankton signatures and their relationship to hydrographic conditions in the coastal northwestern Mediterranean Sea. Mar Ecol Prog Ser 124:247–258
Brunet C, Brylinski JM, Lemoine Y (1993) *In situ* variations of the xantophylls diatoxanthin and diadinoxanthin: photoadaptation and relationships with a hydrodynamical system in the eastern English Channel. Mar Ecol Prog Ser 102:69–77
Chesson J (1983) The estimation and analysis of preference and its relationship to foraging models. Ecology 65: 1297–1304
Dagg MJ (1995) Ingestion of phytoplankton by micro- and mesozooplankton communities in a productive suptropical estuary. J Plankton Res 17:845–857
Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815
Frost BW (1977) Feeding behavior of *Calanus pacificus* in mixtures of food particles. Limnol Oceanogr 22:472–492
Gieskes WWC, Kraay GW (1983) Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Mar Biol 75:179–185
Gieskes WWC, Kraay GW, Nontji A, Setiapermana D, Sutomo (1988) Monsoonal alteration of mixed and a layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal fingerprints. Neth J Sea Res 22:123–137
Head EJH (1992) Gut pigment accumulation and destruction by arctic copepods *in vitro* and *in situ*. Mar Biol 112:583–592
Head EJH, Harris LR (1987) Copepod feeding patterns before and during a spring bloom in Bedford Basin, Nova Scotia. Mar Ecol Prog Ser 40:221–230
Head EJH, Harris LR (1992) Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar Ecol Prog Ser 86:229–238
Head EJH, Harris LR (1994) Feeding selectivity by copepods grazing on natural mixtures of phytoplankton determined by HPLC analysis of pigments. Mar Ecol Prog Ser 110: 75–83
Head EJH, Harris LR (1996) Chlorophyll destruction by *Calanus* spp. grazing on phytoplankton: kinetics, effect of ingestion rate and feeding history, and a mechanistic interpretation. Mar Ecol Prog Ser 135:223–235
Hitchcock GL (1982) A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates. J Plankton Res 4:363–377
Hooks CE, Bidigare RM, Keller D, Guillard RRL (1988) Coccoid eukaryotic marine ultraplankters with four different HPLC pigment signatures. J Phycol 24:571–580
Irigoien X, Castel J, Sautour B (1993) *In situ* grazing activity of planktonic copepods in the Gironde estuary. Cah Biol Mar 34:225–237
Johnsen G, Sakshaug E (1993) Bio-optical characteristics and photoadaptive responses in toxic and bloom-forming dinoflagellates *Gyrodinium aurelum*, *Gymnodinium galatheanum*, and two strains of *Prorocentrum minimum*. J Phycol 29:627–642
Johnsen G, Sakshaug E, Vernet M (1992) Pigment composition, spectral characterisation and photosynthetic parameters in *Chrysochromolina polylepsis*. Mar Ecol Prog Ser 83:241–249
Klein Breteler WCM (1980) Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. Mar Ecol Prog Ser 2:229–233
Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183–195
Kleppel GS, Franzel D, Pieper RE, Holliday DV (1988a) Natural diets of zooplankton off southern California. Mar Ecol Prog Ser 49:231–241
Kleppel GS, Holliday DV, Pieper RE (1991) Trophic interactions between copepods and microplankton: a question about the role of diatoms. Limnol Oceanogr 36:172–178
Kleppel GS, Pieper RE (1984) Phytoplankton pigments in the gut contents of planktonic copepods from coastal waters off southern California. Mar Biol 78:193–198
Kleppel GS, Pieper RE, Trager G (1988b) Variability in the gut contents of individuel *Acartia tonsa* from waters off Southern California. Mar Biol 97:185–190
Kraay GW, Zapata M, Veldhius MJ (1992) Separation of chlorophylls c_1, c_2 and c_3 of marine phytoplankton by reversed-phase-C18-high-performance liquid chromatography. J Phycol 28:708–712
Lampert W (1981) Inhibitory and toxic effects of blue-green algae on *Daphnia*. Int Rev Ges Hydrobiol 66:285–298
Laws EA, Karl DM, Redalje DG, Jurick RS, Winn CD (1983) Variability in ratios of phytoplankton carbon and RNA to ATP and chlorophyll a in batch and continuous cultures. J Phycol 19:439–445
Letelier RM, Bidigare RR, Hebel DV, Ondrusek M (1993) Temporal variability of phytoplankton community structure based on pigment analysis. Limnol Oceanogr 38: 1420–1437
Malej A, Harris RP (1993) Inhibition of copepod grazing by diatom exudates: a factor in the development of mucus aggregates? Mar Ecol Prog Ser 96:33–42
Manley BFJ, Miller P, Cook LM (1972) Analysis of a selective predation experiment. Am Nat 106:719–736
McManus GB (1995) Phytoplankton abundance and pigment changes during simulated *in situ* dilution experiments in estuarine waters: possible artifacts caused by algal light adaptation. J Plankton Res 17:1705–1716
McManus GB, Ederington-Cantrell MC (1992) Phytoplankton pigments and growth rates, and microplankton grazing in a large temperate estuary. Mar Ecol Prog Ser 87:77–85
Millie DF, Pearl HW, Hurley JP (1993) Microalgae pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications. Can J Fish Aquat Sci 50:2513–2527
Morales CE, Harris RP (1990) A review of gut fluorescence method for estimating infestion rates of planktonic herbivores. ICES CM 1990/L:26
Morales CE, Harris RP, Head RN, Tranter PRG (1993) Copepod grazing in the oceanic northeast Atlantic during a 6 week drifting station: the contribution of size classes and vertical migrants. J Plankton Res 15:185–211
Nelson JR (1989) Phytoplankton pigments in macrozooplankton faeces: variability in carotenoid alterations. Mar Ecol Prog Ser 52:129–144
Neveux J, Delmas D, Romano JC, Algarra P, Ignatiades L, Herbland A, Morand P, Neori A, Bonin D, Barbe J, Surenik A (1990) Comparison of chlorophyll and phaeopigment determinations by spektrophotometric, flurometric, spec-

trofluorometric and HPLC methods. Mar Microb Food Webs 4:217–238
Omori M, Ikeda T (1976) Feeding. In: Omori M, Ikeda T (eds) Methods in marine zooplankton ecology. John Wiley and Sons, New York, p 134–172
Paffenhöfer GA, Bundy MH, Lewis KD, Metz C (1995) Rates of ingestion and their variability between individual calanoid copepods: direct observation. J Plankton Res 17: 1573–1583
Paffenhöfer GA, Strickler JR, Alcaraz M (1982) Suspension-feeding by herbivorous calanoid copepods: a cinematographic study. Mar Biol 67:193–199
Sautour B, Artigas F, Herbland A, Laborde P (1996) Zooplankton grazing impact in the plume of dilution of the Gironde estuary (France) prior to the spring bloom. J Plankton Res 18:835–853
Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 141:87–105
Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12:411–418
Strom SL, Welschmeyer NA (1991) Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol Oceanogr 36: 50–63
Swadling KM, Marcus NM (1994) Selectivity in the natural diets of *Acartia tonsa* Dana (Copepoda: Calanoida): comparison of juveniles and adults. J Exp Biol Ecol 181:91–103
Tackx MLM, Zhu L, De Coster W, Billones R, Daro MH (1995) Measuring selectivity of feeding by estuarine copepods using image analysis combined with microscopic and Coulter counting. ICES J Mar Sci 52:419–425
Utermöhl H (1931) Über das umgekehrte Mikroskop. Int Ver Theor Angew Limnol 5:567–569
Utermöhl H (1958) Zur Vervollkommnung der qualitativen Phytoplankton-Methodik. Mitt Int Theor Angew Limnol 9:1–38
Vanderploeg HA (1990) Feeding mechanisms and particle selection in suspension-feeding zooplankton. In: Wotton RS (ed) The biology of particles in aquatic systems. CRC Press, Boca Raton, p 191–197
Vanderploeg HA, Paffenhöfer GA, Liebig JR (1988) *Diaptomus* vs. net phytoplankton: effects of algal size and morphology on selectivity of a behaviorally flexible, omnivorous copepod. Bull Mar Sci 43:377–394
Vesk M, Jeffrey SW (1977) Effect of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes. J Phycol 13: 280–288
Waterhouse TT, Welschmeyer NA (1995) Taxon specific analysis of microzooplankton growth rates. Limnol Oceanogr 40:827–834
Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjørnland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar Ecol Prog Ser 77: 183–196

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