Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 45, Issue 2, June 2016
pages (216-229)

ISSN 1730-413X
eISSN 1897-3191

Growth rates of common pelagic ciliates in a highly eutrophic lake measured with a modified dilution method

by

Krzysztof Rychert

DOI: **10.1515/ohs-2016-0020**
Category: **Original research paper**
Received: **July 4, 2015**
Accepted: **October 9, 2015**

Institute of Biology and Environmental Protection, Pomeranian University in Słupsk, ul. Arciszewskiego 22b, 76-200 Słupsk, Poland

Abstract

The growth rates of ciliates estimated under natural conditions with the widely used size fractionation method are much lower than those observed in cultures. However, recent studies performed with a modified dilution method demonstrated that the size fractionation method underestimates the ciliate growth, because it does not remove predators of the same size as the organisms studied. Thus, it is still unresolved whether ciliates are food-limited in different systems and whether their growth rates are indeed lower than those in cultures. This study was conducted in highly eutrophic Lake Gardno using a modified dilution method. Each time, two dilution experiments were performed (around noon and around midnight). Four small, common ciliates from the genera *Rimostrombidium*, *Tintinnidium*, *Cyclidium*, and *Urotricha* were studied. The first three ciliates demonstrated very high mean diel growth rates exceeding 0.1 h^{-1}, which corresponded well to the highest values reported in the literature for the ciliate growth in cultures at similar temperatures. *Tintinnidium* sp. demonstrated a diel growth rhythm. *Urotricha* sp. was sensitive to the experimental procedure, and measurements of its growth were unsuccessful. Concentrations of food particles were analyzed to check whether organisms studied were food satiated.

Key words: growth, ciliate, dilution technique, allometric equation, food limitation, diel rhythm, Lake Gardno, eutrophic

* Corresponding author: *krychert@wp.pl*

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at oandhs.ocean.ug.edu.pl

Introduction

Protists and prokaryotes are crucial organisms involved in the cycling of matter and the biological carbon pump (Stocker 2012; Caron & Hutchins 2013; Mitra et al. 2014). To estimate their role in matter turnover, not only their biomass but also their growth rates have to be measured (Müller 1989; Leakey et al. 1992; Cleven & Königs 2007; Lai et al. 2014). There are direct, reliable methods to measure algal (that of autotrophic protists) and bacterial production under natural conditions, but assessment of protozoan production (that of heterotrophic protists) is much more difficult (Rychert 2013).

Growth rates of heterotrophic protists, which are necessary for production estimates, have long been estimated by the size fractionation method in which the organisms studied are separated from larger grazers (Verity 1986; Carrick et al. 1992; McManus 1993; Leakey et al. 1994; Nielsen & Kiørboe 1994; Macek et al. 1996; Wallberg et al. 1999; Carrias et al. 2001; Carrick 2005; Seuthe et al. 2011). In the case of ciliates, growth rates measured with this method are typically lower than 50% of those estimated from allometric equations (Leakey et al. 1994; Macek et al. 1996) that describe the relationship between cell volume, ambient temperature, and the growth rate and were formulated based on the growth of ciliates in cultures (Montagnes et al. 1988; Müller & Geller 1993). Lower rates observed during field works in size fractionation experiments are explained by food limitation of ciliate growth, but also by underestimating growth rates due to grazing between heterotrophic protists, because size fractionation does not remove predators of the same size as the organisms studied (Müller 1989; Leakey et al. 1992; Leakey et al. 1994; Macek et al. 1996; Wallberg et al. 1999; Carrias et al. 2001). Recently, Rychert (2013) and Franzé & Modigh (2013) have demonstrated that grazing among heterotrophic protists does indeed cause underestimated ciliate growth rates measured with the size fractionated method. The second problem is that ciliate growth is usually measured during a 24-hour incubation period since this includes any possible diel growth rhythms (Carrick et al. 1992; Jakobsen & Strom 2004; McManus & Santoferrara 2013) and is long enough to detect ciliate growth with the size fractionation method. However, such long incubation periods lead to the deceleration of biological processes (Carrick et al. 1992; McManus 1993; Rychert 2013), and different containment effects such as ciliate grazing on bacterial biofilm that develops on internal bottle surfaces (e.g. Macek et al. 1996). In conclusion, more field research on ciliate growth rates are required and they should be performed with methods better than size fractionation.

A method that completely removes the grazing pressure exerted on ciliates and allows a short incubation period is the modified dilution method (Rychert 2013; see also Franzé & Modigh 2013). It consists of the whole-community manipulation by creating a gradient of dilutions of whole water with a 10-μm filtrate. Through the dilution, grazing pressure decreases toward zero, and the apparent growth rate approximates the specific growth rate. Because of the filtrate used, the method allows to study the specific growth rates of protists larger than 10 μm (e.g. ciliates) that feed on prey smaller than 10 μm (nanoflagellates, small algae, or bacteria). Modifications of the dilution method (Landry & Hassett 1982) were previously applied to measure the growth rates of pelagic heterotrophic nanoflagellates (Landry et al. 1984; Berglund et al. 2005; Dupuy et al. 2007) and interstitial ciliates (Cleven & Königs 2007).

In this study, the measurements of the growth rates of common pelagic ciliates were performed with the modified dilution method (Rychert 2013) and growth rates were compared with literature data and also with rates estimated from an allometric equation formulated by Müller & Geller (1993). Experimental incubation periods were shortened to approximately 4 hours to minimize artifacts. Separate measurements performed around noon and midnight allowed the observation of diel rhythms in ciliate growth. It is still unresolved whether ciliates are food-limited in different systems and whether their growth rates are indeed lower than those in cultures. Food limitation is difficult to study, because resources are typically distributed in patches and ciliates can exploit them and be satiated even when the mean abundance of food particles calculated for larger volumes is below the saturation level (Paffenhöfer et al. 2007). In this study, the mean concentrations of food resources of the organisms in question were analyzed and discussed in order to check whether high growth rates were accompanied by saturation levels of food resources or whether the organisms exploited unevenly distributed food and were satiated despite mean concentrations of food resources that were below the saturation level. It was hypothesized that the ciliate growth in the highly eutrophic lake was as high as in cultures.

Materials and Methods

The studies were performed in large (24.7 km^2), shallow (mean depth: 1.2 m), brackish (mean salinity: 0.6–0.7 PSU, Trojanowski & Antonowicz 2011, Rychert et al. 2012, Wielgat-Rychert et al. 2015) Lake Gardno (54°39'N, 17°07'E), at an offshore station in the southern part of the lake representing an open water area (Ficek & Wielgat-Rychert 2009). The lake is highly eutrophic with high average chlorophyll concentrations in the growing season (87 µg l^{-1}) and high annual integrals of primary production at 402–471 g C m^{-2} (Wielgat-Rychert et al. 2010). The mean annual ciliate biomass is 107–115 µg C l^{-1} (Rychert et al. 2012), which corresponds to about 100 cells ml^{-1}. In this study, the experiments were carried out in September 2011, June 2012, and June 2013. Each time, two experiments were performed (around noon and around midnight) for a total of six experiments (Table 1). Water for the experiments was taken from the well-oxygenated subsurface layer, and temperature, salinity, bacterial and flagellate abundances, and chlorophyll *a* concentration measurements were performed. Bacterial abundance was studied under an epifluorescence microscope after fixation with formalin (final concentration 1%) and staining with acridine orange (Hobbie et al. 1977). Flagellates were also analyzed under an epifluorescence microscope after fixation with glutaraldehyde (final concentration 0.5%) and staining with primulin (Caron 1983). Since flagellates were analyzed as a potential food resource, both autotrophic (ANF) and heterotrophic flagellates (HNF) were counted. Chlorophyll *a* concentration was measured according to Jeffrey and Humphrey (1975) without correction for phaeopigments. On each occasion, the second experiment was performed about 12 h after the first. Despite the short period of time elapsed between the two experiments, some differences in biological parameters (chlorophyll *a* concentration, bacterial abundance, etc.) were observed (Table 1). Such changes are typical for Lake Gardno, because bottom sediments are frequently resuspended due to its shallow depth and wind-induced mixing (Wielgat-Rychert et al. 2015).

Dilution experiments

Before each experiment, all the equipment was acid-washed and thoroughly rinsed with deionized water. Lake water for the experiments was pre-screened through 100-µm mesh nylon gauze to remove larger zooplankton that could not be distributed representatively in the treatments. This water was treated as whole water and was carefully mixed. To produce the 10-µm filtrate, part of the water was sequentially gravity-filtered through 25-µm mesh gauze, and then twice through 10-µm mesh gauze. To prevent clogging, the first filtration with 10-µm mesh gauze was carried out through three parallel filtration sets. Next, filtrates were combined and filtered through the fourth filtration set. Samples were taken from the 10-µm filtrate to assess the impact of filtration on bacterial abundance, flagellate abundance, and chlorophyll concentration. Subsequently, whole water and 10-µm filtrate were used to prepare the dilutions, in which fractions of whole water were 20% (in triplicate), 40%, 60%, 80%, and 100% (undiluted whole water, in triplicate). All dilutions were prepared in 120-ml bottles (9 in total), and each bottle was gently mixed by rotating it 50 times. Two bottles, one each for 20% and 100% dilutions, were used to assess changes

Table 1

Environmental conditions and food resources during experiments carried out in highly eutrophic Lake Gardno. Mean values for the incubation of whole water and 20% dilution were calculated for bacterial and flagellate abundances (see Materials and Methods). In the case of chlorophyll *a* concentration, values for whole water and 10-µm filtrates are provided, because only such small algae (up to 10 µm) represented the available food resource for the ciliates studied.

Experiment		Temp. (°C)	Salinity (PSU)	Ciliates (cells ml^{-1})	Chlorophyll *a* (µg l^{-1})		Bacteria (10^6 cells ml^{-1})		Nanoflagellates (10^3 cells ml^{-1})	
					whole water	10-µm filtrate	whole water	20% dilution	whole water	20% dilution
21–22/09/2011	midnight	15.6	0.59	67.3	60.9	7.03	7.04	5.22	18.7	11.2
22/09/2011	noon	15.4	0.67	42.2	61.9	6.28	5.89	3.26	19.3	16.9
13/06/2012	noon	20.0	0.92	118	50.3	9.17	8.76	7.87	20.3	14.6
13–14/06/2012	midnight	21.1	0.84	119	33.8	6.89	9.76	9.14	16.0	12.8
28–29/06/2013	midnight	18.4	0.17	307	45.3	7.58	16.4	14.9	18.1	15.5
29/06/2013	noon	18.2	0.18	423	46.8	8.69	12.7	10.7	16.6	15.4

in bacterial and flagellate abundances as well as chlorophyll *a* concentration during incubation. Next, initial samples were collected for ciliate counts (7 samples), bacterial and flagellate counts (one each for 20% and 100% dilutions), and water was sampled for chlorophyll *a* concentration measurements. Experimental incubation was conducted for 3.5–4.8 h under natural conditions (temperature, wave motion, irradiance), using an anchored experimental set-up. The exact duration time was used to calculate growth rates. After incubation, final samples were taken for the determination of ciliate, bacterial and flagellate abundances, and chlorophyll *a* concentration measurements (similarly to the initial samples). Finally, I took an additional sample from the lake to compare the ciliate dynamics in the whole experimental water with that in the environment.

The ciliates were analyzed in samples fixed with acid Lugol's solution (final concentration 0.5%). The samples were stored in the dark at 4°C until they were analyzed using the Utermöhl (1958) method. The observations were conducted using an Olympus CKX41 inverted microscope. The ciliates were identified according to Foissner & Berger (1996). It was crucial to analyze the entire bottom of the Utermöhl chamber and not half of it to minimize the error caused by the uneven distribution of specimens on the bottom. Samples were dense with algae and detritus, so small volumes of water were taken for analyses: 3 ml or 10 ml for the more diluted treatments. If necessary, additional counts were performed to gather a sufficient number of specimens. In each experiment, the entire ciliate community was analyzed in one initial sample collected from whole water (see abundance values in Table 1). In the remaining samples, only two ciliate species and their growth rates were analyzed. The ciliate species were selected according to (i) high abundance, which allowed for an accurate assessment of changes in the abundance during incubation, and (ii) distinct morphology, which facilitated the identification of all specimens present in samples fixed with Lugol's solution. Because fixation with Lugol's solution obscures taxonomic features, the ciliates were identified to the genus level. During incubation, no evident changes in ciliate cell volumes were observed. For each dilution, the apparent growth rate (k, h^{-1}) was calculated assuming the exponential growth during incubation:

$$k = \frac{\ln\left(\frac{N_1}{N_0}\right)}{t} \quad (1)$$

where N_1 was the final abundance (cells ml^{-1}), N_0 was the initial abundance (cells ml^{-1}), and t was the duration of incubation (h).

The experiments were analyzed according to Landry & Hassett (1982). The encounter rates between predators and prey were gradually reduced along the dilution gradient from undiluted to the most diluted water. Thus, the apparent growth rate (k, h^{-1}) in each dilution depended on the specific growth rate (μ, h^{-1}), i.e. the intrinsic rate of increase, grazing pressure (g), and the fraction of whole water in the dilution (D, %):

$$k = \mu - \left(\frac{g \times D}{100\%}\right) \quad (2)$$

Plotting the apparent growth rates against fractions of whole water in dilutions enabled the estimation of specific growth rates (μ, h^{-1}) for the theoretically complete relaxation of grazing pressure (D = 0%, y-intercept, Fig. 1). A single measurement comprised 7 experimental treatments and the analysis of 15 samples (7 initial and 7 final samples, and also one additional environmental sample); thus, the growth rate estimates were statistically significant. Growth rates were measured during short incubations (3.5–4.8 h), therefore they were expressed per hour.

Potential ciliate growth rates

Measurements of ciliate growth rates were compared with potential (maximum) growth rates estimated from an allometric equation formulated on the basis of ciliate growth in cultures. The model published by Müller & Geller (1993) was chosen because it is based on the largest data set and is considered to be the best (Macek et al. 1996, Montagnes 1996). In this model, potential specific growth rates (μ, d^{-1}) were estimated from the mean cell volume of the organism studied (V, μm^3) and the ambient temperature (T, °C):

$$\ln \mu = 1.52 \times \ln T - 0.27 \times \ln V - 1.44 \quad (3)$$

To calculate the volume of organisms studied, they were measured in samples of the whole water collected before incubation. Measurements were carried out with an ocular micrometer or image analysis software. The volume (V, μm^3) of

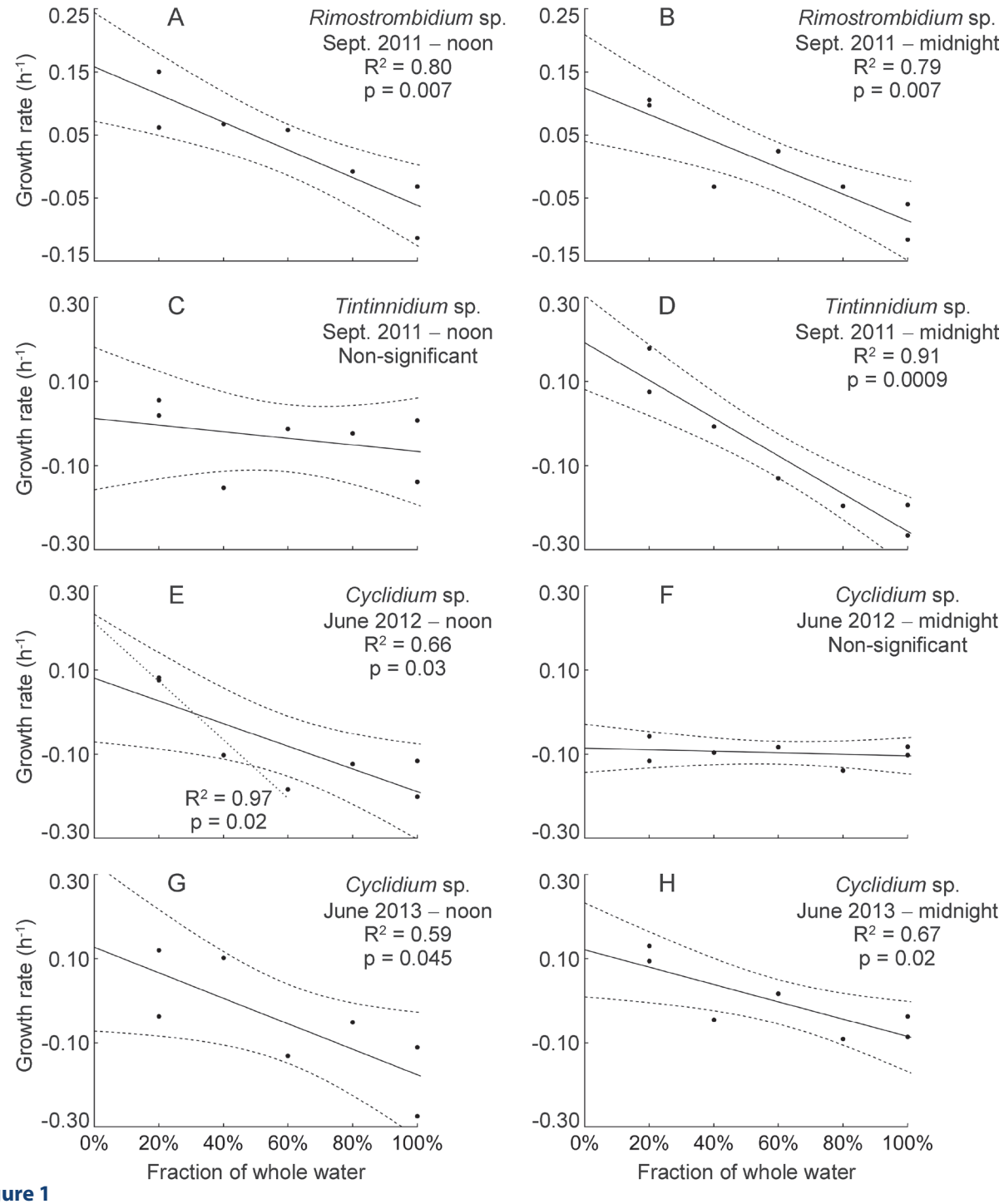

Figure 1

Growth responses of ciliates to gradual dilution observed during approx. 4 hours of incubation performed around noon and midnight on three occasions in Lake Gardno. Through dilution, grazing pressure decreased toward zero and the apparent growth rate approximates the specific growth rate (y-intercept). The date of the experiments and statistical significances are depicted on the graphs. The dashed line represents the 95% confidence interval. In the experiment with Cyclidium sp. performed around noon in June 2012 (E), the grazing pressure was relaxed only in more diluted treatments, thus the additional grazing curve was plotted for 20–60% dilutions (dotted line) that demonstrated higher statistical significance.

Rimostrombidium sp., *Cyclidium* sp., and *Urotricha* sp. were calculated from mean values of their length (L, µm) and width (W, µm) using standard formulae for the volume of a rotational ellipsoid:

$$V = \frac{4}{3} \times \pi \times \left(\frac{W}{2}\right)^2 \times \left(\frac{L}{2}\right) \quad (4)$$

The volume of ciliates can shrink after fixation with Lugol's solution. This shrinkage is species-specific and also depends on the physiological state of the cells (Choi & Stoecker 1989; Wiackowski et al. 1994). Thus, no correction was applied to avoid introducing additional error. The potential specific growth rates computed (µ, d^{-1}) were divided by 24 to calculate values per hour. Growth rate estimation for tintinnid ciliates (*Tintinnidium* sp.) was more complicated, because tintinnid loricae are built by protoplasts and the energy allocated for lorica construction has to be taken into account (Rychert 2011; Agatha et al. 2013). First, the carbon content of a tintinnid ciliate (CC, pgC), both lorica and protoplast, was calculated from the volume of lorica using the coefficient 0.053 pgC μm^{-3} (Verity & Langdon 1984; Seuthe et al. 2011). The volume of lorica (V, μm^3) was calculated from the mean width (W, µm) and length (L, µm) of lorica. Lorica shape was described as a cylinder and hemisphere:

$$V = \pi \times \left(\frac{W}{2}\right)^2 \times \left(L - \frac{W}{2}\right) + \frac{2}{3} \times \pi \times \left(\frac{W}{2}\right)^3 \quad (5)$$

Next, the carbon content was calculated back to the volume using the coefficient 0.11 pgC μm^{-3} (Turley et al. 1986) to calculate the surrogate cell volume, i.e. volume of hypothetical protoplast containing the organic carbon allocated for production of both protoplast and lorica. This surrogate cell volume was used to calculate the potential growth rate according to the formula by Müller & Geller (1993), similarly as with aloricate ciliates.

Additionally, the tintinnid ciliate growth (µ, d^{-1}) was calculated based on the lorica oral diameter (LOD, µm) according to the formula by Dolan (2010), which was further developed by Montagnes (2013):

$$\mu = -0.0082 \times LOD + 1.4 \quad (6)$$

The estimated growth rates (d^{-1}) were recalculated per hour.

Assessment of food resources

During all six experiments, I assessed the impact of dilution on food resources of the organisms studied in the whole water and in 20% dilution. Three parameters were studied: bacterial abundance, flagellate (ANF and HNF, size: 2–10 µm) abundance, and chlorophyll *a* concentration. Their changes during incubation were small to moderate, so the mean values (N) were calculated from initial (N_0) and final (N_1) values according to Frost (1972):

$$N = \frac{(N_1 - N_0)}{\ln \frac{N_1}{N_0}} \quad (7)$$

The quantity of food resources was analyzed in each experiment separately according to the food demands of the organism in question. When necessary, they were recalculated into carbon units. The nutritional value of algae was calculated from chlorophyll *a* concentration (Table 1) assuming the C:Chl *a* conversion factor of 40.7 (Montagnes et al. 1994). The bacterial biomass was calculated from the abundance (Table 1) using a coefficient of 17 fgC $bacterium^{-1}$ (Cleven & Weisse 2001), which is a mean value among those reported in the literature. The biomass of nanoflagellates was also calculated from their abundance (Table 1) assuming a conservative mean flagellate size (3 µm) and a conversion factor of 0.22 pgC μm^{-3} (Børsheim & Bratbak 1987).

Results

Four common ciliates were studied. In September 2011, I measured the growth rates of *Rimostrombidium* sp. (mean volume after fixation: 936 μm^3) and *Tintinnidium* sp. (mean lorica oral diameter: 12.5 µm, mean lorica length: 38.0 µm). In June 2012, *Cyclidium* sp. (mean volume after fixation: 586 μm^3) and *Urotricha* sp. (mean volume after fixation: 830 μm^3) were studied. On the third occasion (June 2013), I studied *Cyclidium* sp. (mean volume after fixation: 883 μm^3) and *Urotricha* sp. (mean volume after fixation: 1505 μm^3). The abundance of each organism studied was high and was typically 20–30 cells ml^{-1} in whole water (maximum abundance: 70 cells ml^{-1}). On each occasion, two experiments were performed, one around noon and another one around midnight (6 experiments altogether).

As already mentioned, the growth rates of *Rimostrombidium* sp. were studied in September

2011. The two experiments conducted around noon and midnight demonstrated high specific growth rates of 0.16 h^{-1} and 0.12 h^{-1}, respectively (Fig. 1A–B). On the same occasion, the specific growth of *Tintinnidium* sp. was observed, and a statistically significant, exceptionally high growth rate (0.19 h^{-1}) was observed in only one experiment performed around midnight (Fig. 1D). The specific growth rate was close to zero around noon (Fig. 1C), which indicated that *Tintinnidium* sp. growth was subjected to diel rhythms with cell divisions occurring at night. The growth of *Cyclidium* sp. was studied twice – in June 2012 and in June 2013. In June 2013, specific growth rates measured around noon (0.13 h^{-1}) and midnight (0.12 h^{-1}) were similar (Fig. 1G–H) indicating no diel rhythm in cell division. In June 2012, the specific growth rate was lower around noon and was 0.08 h^{-1}. At night (June 2012), a negative growth rate of *Cyclidium* sp. was observed. The same negative apparent growth rate in all dilutions (flat line, mean value for all dilutions: –0.10 h^{-1}, Fig. 1F) indicated that the dilution procedure failed to relax the grazing pressure. The last organism studied, i.e. *Urotricha* sp., was studied twice – in June 2012 and in June 2013. *Urotricha* sp. was sensitive to the experimental procedure, and its abundance during incubation changed dramatically and randomly, thus the growth rate measurements were unsuccessful (not presented).

Discussion

Ciliate growth rates

Rimostrombidium sp. grew at rates of 0.16 h^{-1} at 15.4°C and 0.12 h^{-1} at 15.6°C (Fig. 1A–B, Table 1), which corresponded to the highest estimates of the maximum growth rates reported in the literature for small, satiated oligotrich ciliates, e.g. 0.15 h^{-1} at 15°C (Rivier et al. 1985) or 0.11 h^{-1} at 20°C (Ohman & Snyder 1991). The field research conducted in hypereutrophic waters by Lavrentyev et al. (2004) also revealed similar growth rates of *Rimostrombidium humile*: 0.11 h^{-1}; however, measurements were taken at a higher temperature (22–25°C).

Tintinnidium sp. demonstrated an exceptionally high growth rate of 0.19 h^{-1} around midnight at 15.6°C (Table 1), whereas around noon the specific growth rate was close to zero (Fig. 1C–D). This indicated that *Tintinnidium* sp. demonstrated a diel rhythm in growth with cell divisions occurring during the night. Some researchers studying the growth of tintinnids reported this cycle (Campbell 1926; Biernacka 1952), while others did not (e.g. Heinbokel & Coats 1986). Assuming that estimates for the night were 0.19 h^{-1} and for the day – zero (no growth), the mean diel growth rate would be 0.10 h^{-1}. This value is twice as high as the maximum growth rate reported for *Tintinnidium fluviatile* (0.05 h^{-1}, Verity 1986) and several small tintinnid ciliates studied in cultures (0.05–0.06 h^{-1}, 18°C, Heinbokel 1978). A similar low value of 0.05 h^{-1} was also calculated using a formula relating the growth rate to the lorica oral diameter (Dolan 2010; Montagnes 2013, see Materials and Methods). On the other hand, Lavrentyev et al. (2004) reported an even higher growth rate of *Tintinnidium fluviatile*: 0.12 h^{-1} during the field research at higher temperatures of 22–25°C.

Cyclidium sp. grew very fast in June 2013 (0.12–0.13 h^{-1} at 18.2–18.4°C, Fig. 1G–H, Table 1) and around noon in June 2012 (0.08 h^{-1} at 20.0°C). However, in the latter experiment, *Cyclidium* sp. seemed to be relieved from grazing pressure only in the most diluted water (Fig. 1E), consequently the specific growth rate was underestimated. Assuming that the so-called "saturated feeding effect", in which grazing pressure is very strong and relaxes only in more diluted water (Gallegos 1989; Calbet & Saiz 2013), additional growth rate calculations were carried out only for the most diluted treatments (20–60%). This growth estimate (0.21 h^{-1}) was higher, as was the statistical significance (Fig. 1E). However, a 2.5 times higher growth rate than in the 20% dilution (Fig. 1E) is very unlikely, and it seems to be overestimated. Thus, the actual specific growth rate was somewhere between the low, underestimated value (0.08 h^{-1}) and the high, overestimated value (0.21 h^{-1}). Generally, the estimates of the growth rates of *Cyclidium* sp. are similar to the maximum estimate for *Cyclidium* sp. of a similar size (887 μm^3) at 0.16 h^{-1} at 20°C (Boenigk & Novarino 2004) and higher than estimates of the maximum growth rate reported in the literature for *Cyclidium glaucoma*, e.g. 0.079 h^{-1} at 20°C (Taylor 1978) or 0.10 h^{-1} at 20°C (Finlay 1977).

In conclusion, the growth rates measured in this study corresponded well with the maximum values reported in the literature at similar temperatures or were even higher. Therefore, the hypothesis posed in the Introduction was confirmed. As mentioned in the Introduction, ciliate growth could be approximated with the allometric equation by Müller & Geller (1993). However, it does not necessarily estimate the maximum growth rates of particular species, but approximate the "general ciliate growth" and

are especially useful for estimating the growth rates of multi-species ciliate communities (Montagnes 1996). To perform a general comparison (Fig. 2), the mean diel growth rates from measurements were compared with growth rates estimated with the allometric formula according to Müller & Geller (1993). This comparison also indicated that the growth rates measured are at the same level as rates expected from the growth observed in cultures.

Unsuccessful experiments with *Urotricha* sp. were unexpected, because organisms from this genus were introduced into cultures and were thoroughly studied under laboratory conditions (Weisse et al. 2001; Weisse et al. 2002). Thus they could be considered as robust. I cannot offer any explanation why *Urotricha* sp. was sensitive to the experimental procedure. Studies were performed in the highly eutrophic and brackish lake with unstable environmental conditions (Wielgat-Rychert et al. 2015). Therefore, it is possible that the sensitivity of *Urotricha* sp. was induced by an unidentified, perhaps abiotic, factor.

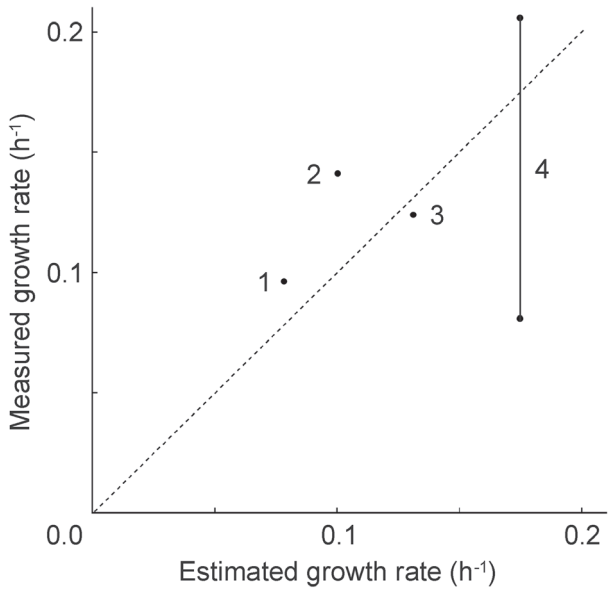

Figure 2

Mean diel growth rates versus growth rates estimated with the allometric equation according to Müller & Geller (1993): (1) *Tintinnidium* sp., (2) *Rimostrombidium* sp., (3) *Cyclidium* sp. in June 2013, (4) *Cyclidium* sp. in June 2012 (noon only, range of growth rates estimated with two methods of analysis (Fig. 1E). The dashed diagonal represents a 1:1 relationship.

Applicability of the dilution method

The dilution method is based on the two main assumptions: (i) the growth of the organisms studied is not food-limited in subsequent dilutions and (ii) grazing pressure depends linearly on the dilution factor, which is proportional to the concentration predators (Landry & Hassett 1982; Calbet & Saiz 2013). The first assumption holds if the experimental procedure does not cause a reduction in the food resources in dilutions or, alternatively, organisms are food-satiated, both in the whole water and in the dilutions, despite the reduced food resources in dilutions. Filtration excluded bacteria attached to detritus particles. In the 20% dilution, bacterial abundance was 55–94% (mean: 81%, Table 1) of that observed in whole water. However, neither attached bacteria nor detritus particles larger than 10 µm are edible for the organisms studied. Similarly, the abundance of flagellates was also reduced. Their abundance in 20% dilution was 60–93% (mean: 80%, Table 1) of that observed in whole water. Filtration had a pronounced effect on chlorophyll *a* concentration: the 10-µm filtrate contained only 10–20% (mean: 16%, Table 1) of the chlorophyll *a* concentration recorded in whole water. However, filtration excluded large, abundant colonial green algae, like *Scenedesmus quadricauda* and *Pediastrum* spp., colonial diatoms (genera: *Asterionella*, *Nitzschia*, and *Fragilaria*), and other large algae, which are not food resources for the organisms studied. Therefore, only the chlorophyll *a* concentration present in the 10-um-filtrate was treated as a food resource available to the organisms studied. The food resources were analyzed according to the food demands of the organisms in question in each experiment. However, food resources were assessed on the basis of three basic parameters (chlorophyll concentration, abundance of nanoflagellates, and abundance of bacteria; Table 1), consequently I estimated the potential food and not the most optimal prey grazed preferentially by organisms studied.

Rimostrombidium sp. and *Tintinnidium* sp. were studied in September 2011 (Table 1). On this occasion, the biomass of algae smaller than 10 µm was 286 µg C l^{-1} (night) and 256 µg C l^{-1} (day). The biomass of flagellates in the 20% dilution was 35 µg C l^{-1} (night) and 53 µg C l^{-1} (day), and the bacterial biomass was 89 µg C l^{-1} (night) and 55 µg C l^{-1} (day). Coccoid cyanobacteria were not studied separately, but their biomass was included in the group of small algae because they also contain chlorophyll *a*. These values were high, even though the biomass

contributed by algae (smaller than 10 µm) and nanoflagellates partially overlapped because both autotrophic and heterotrophic nanoflagellates were counted. *Rimostrombidium* sp. feeds primarily on small algae (including coccoid cyanobacteria), but it can also ingest bacteria (Cleven 2004; Sonntag et al. 2006; Peštová et al. 2008). When only algae were considered, the food resources considerably exceeded the satiating food level for oligotrichs and aloricate choreotrichs at 100 µg C l^{-1} (Montagnes 1996) or 150 µg C l^{-1} (Gismervik 2005). This was also true if only part of the small algae (e.g. only cyanobacteria) was available as food for *Rimostrombidium* sp., which is likely because the organism studied was small (mean diameter of fixed protoplasts was about 12 µm). In addition, *Rimostrombidium* sp. could also feed on bacteria. The second organism studied in September 2011 was *Tintinnidium* sp. Tintinnids can ingest particles up to one third of their lorica oral diameter, but they prefer food particles sized about one fifth of the lorica oral diameter (reviewed by Montagnes 2013). In this study, the lorica oral diameter of *Tintinnidium* sp. was 12.5±1.4 µm (mean±SD), thus its food resource were nanoflagellates (the mean diameter of which was about 3 µm) and the smallest algae. According to Heinbokel (1978), Verity (1986), and Montagnes (2013), tintinnids require 50–80 µg C l^{-1} for the saturated growth. Thus, the food demands of *Tintinnidium* sp. were satisfied by nanoflagellates and the small share of algae smaller than 10 µm. In conclusion, both *Rimostrombidium* sp. and *Tintinnidium* sp. were food-satiated, both in the 20% dilution and in the whole water, where food resources were more abundant. The excess of available food allowed them to achieve very high growth rates.

Cyclidium sp. was studied twice, in June 2012 and in June 2013. According to Fenchel (1986), Dolan & Coats (1991), Posch et al. (2001), Jezbera et al. (2005), and Bautista-Reyes & Macek (2012), organisms from the genus *Cyclidium* feed on bacteria and cyanobacteria. According to the functional response of *Cyclidium glaucoma*, bacterial abundance over 3.0 × 10^7 cells ml^{-1} (Posch et al. 2001) or 8.6 × 10^7 cells ml^{-1} (Taylor 1978; Fenchel 1980) saturates its food demands. During the experiments performed in June 2012, bacterial abundances in the most diluted treatment (20%) were 0.79 × 10^7 cells ml^{-1} (day) and 0.91 × 10^7 cells ml^{-1} (night, Table 1), thus below the saturated food level. The bacterial abundance in whole water was higher by 6–10% (depending on experiment, Table 1), but it was still below the satiation level. It is difficult to verify whether *Cyclidium* sp. was food-limited on that occasion or whether it was satiated due to the presence of coccoid cyanobacteria (additional food resource that was not counted, however, their abundance was few times lower than that of bacteria) or due to the exploitation of food patches, because the experiment performed around midnight was unsuccessful and that performed around noon resulted only in a rough estimation of the growth rate (0.08–0.21 h^{-1}, Fig. 1E). It should be admitted that data on coccoid cyanobacteria would not be conclusive, because not all species or even ecotypes from the genus *Cyclidium* feed efficiently on the former (Šimek et al. 1995; Peštová et al. 2008). During the experiments performed in June 2013, bacterial abundances were higher and exceeded 10^7 cells ml^{-1}, both in the 20% dilution and in the whole water (1.07–1.64 × 10^7 cells ml^{-1}, Table 1). However, they were slightly lower than the satiation level (3.0 × 10^7 cells ml^{-1}, Posch et al. 2001). In June 2013, the experimental procedure caused a decrease in the bacterial abundance between the whole water and the 20% dilution (9–16%, Table 1). Theoretically, *Cyclidium* sp. growth could be limited in the whole water and in the dilutions (more likely). However, the high growth rates observed on this occasion (discussed above) preclude this possibility. *Cyclidium* sp. could be satiated due to the presence of coccoid cyanobacteria (not counted) and could exploit food patches and maintain the maximum growth rates despite the fact that the mean abundance of food particles calculated for the larger volume was not satiating. Unfortunately, coccoid cyanobacteria were not counted separately. It is also possible that the organism studied requires less food for saturated growth. The *Cyclidium* sp. studied by Boenigk & Novarino (2004) grew very fast (0.16 h^{-1}) when cultured with a nominally food-limited abundance of bacteria (2 × 10^7 cells ml^{-1}).

The second assumption of the dilution method is that grazing pressure depends linearly on the dilution factor. Predatory ciliates, which could prey on the organisms studied, were observed during all the experiments. Depending on the occasion, their abundance in whole water ranged from 2.7 to 13.0 cells ml^{-1}. During all the experiments, I observed many specimens from the genera *Askenasia* and *Monodinium*. Ciliates from the genera *Lacrymaria*, *Didinium*, and *Mesodinium* were observed on two of the three dates. *Litonotus* sp. and others were less frequent. These ciliates are predatory (Gast 1985; Pratt & Cairns 1985; Dolan & Coats 1991; Nielsen & Kiørboe 1994; Mironova et al. 2012). Dilution of predatory ciliates in subsequent dilutions was accompanied by a linear increase in the apparent growth rates of the organisms studied (Fig. 1).

This was directly demonstrated in the experiments with *Rimostrombidium* sp. (Fig. 1A–B), *Cyclidium* sp. (June 2013, Fig. 1G–H), and in one experiment with *Tintinnidium* sp. (Fig. 1D). As described above, in the experiment performed with *Cyclidium* sp. around noon in June 2012 (Fig. 1E), the relaxation of grazing pressure was incomplete, which resulted in the underestimated growth rate, whereas in the corresponding experiment performed around midnight (Fig. 1F), the relaxation of grazing pressure was unsuccessful. Higher grazing pressure at night could be explained by the abundance of predatory ciliates in both experiments. In the experiment performed around noon, their abundance was lower (3.7 cells ml^{-1} in whole water) than around midnight (7.0 cells ml^{-1} in whole water). Unreleased grazing pressure around midnight suggested that the abundance of predatory ciliates in the 20% dilution was still high enough to exert top-down control on *Cyclidium* sp. In conclusion, the second assumption of the dilution method was positively verified in the experiments that were carried out in September 2011 and in June 2013. This assumption was violated in June 2012 and on that occasion the growth rate was analyzed with the additional method assuming the so-called "saturated feeding effect" (see Results and Fig. 1E) and is reported as a range.

As illustrated in Fig. 1, the apparent growth rates observed in whole water (dilution 100%) were typically negative. One could expect values close to zero, that is, balanced growth and mortality rates. At the end of each experiment, an additional environmental sample was taken to compare changes in the whole experimental water with that at the study site. In 6 out of 8 cases, apparent growth rates in whole water in bottles were lower than in the environment; however, the difference was not statistically significant (Wilcoxon's signed rank test). Theoretically, negative growth rates in less diluted water could be caused by trophic cascade effects, because whole water was pre-screened through 100-µm mesh nylon gauze and during incubation metazooplankton smaller than 100 µm was released from grazing pressure and could graze more strongly on ciliates (e.g. Klaas et al. 2008; First et al. 2009; Calbet & Saiz 2013). This would result in the overestimated slope of the grazing curve and grazing rates. However, it is of lesser importance in this study, because only growth rates and not grazing rates were taken into account. In most cases, the specific growth rates estimated (Fig. 1, y-intercept) were comparable to apparent growth rates observed in the 20% dilution.

The dilution method relaxes grazing pressure, but does not remove other mortality agents such as (i) viral lysis (McManus 1993), (ii) toxins excreted by other protists (Verity 1986, Leakey et al. 1994), or (iii) parasites (Verity 1986; Coats & Bachvaroff 2013; Coats et al. 2014). However, these mortality agents seem to be far less important than grazing, because successful elimination of grazing pressure generally results in very high growth rates close to the intrinsic rate of increase (e.g. Nielsen & Kiørboe 1994; Franzè & Lavrentyev 2014; this study).

Conclusions

The number of the studied ciliate species was rather low, but their statistically significant growth rates measured under natural conditions in the highly eutrophic lake corresponded well with the rates observed in cultures. Similarly, very high ciliate growth rates in eutrophic waters are also reported by Lavrentyev et al. (2004). Thus, the growth of ciliates in eutrophic waters is as fast as in the cultures and the hypothesis put forward in the Introduction was confirmed. It should be emphasized that the isolated measurements indicate that even in less nutrient-enriched systems some ciliates can grow with rates similar to or even higher than those estimated with allometric equations, e.g. in oligo-mesotrophic Lake Pavin (Carrias et al. 2001), oligo-mesotrophic Lake Michigan (Carrick 2005), meso-eutrophic Lake Constance (Müller 1989), and in sea waters (Nielsen & Kiørboe 1994; Rychert 2013; Franzè & Lavrentyev 2014). Confirmation of the hypothesis implies that higher ciliate growth rates should be applied in ecological models (e.g. Buitenhuis et al. 2010).

The mean concentrations of food particles available for *Rimostrombidium* sp. and *Tintinnidium* sp. indicated that both organisms were food-satiated. The third ciliate studied, *Cyclidium* sp., was not demonstrated to be satiated; however, in the successful experiment performed in June 2013 it grew at rates close to the maximum rates reported in the literature and those estimated with the allometric equation (Fig. 2). As mentioned above, this could be explained by the fact that ciliates can exploit food patches (Paffenhöfer et al. 2007), which means they could be well-fed despite mean concentrations of food resources that are not satiating. In conclusion, it is generally difficult to demonstrate whether any ciliate is or is not food-satiated under natural conditions. This is because of (i) the patchy distribution of food (Paffenhöfer et al. 2007) and (ii) ciliate food preferences resulting in prey selection (e.g. Wiackowski et al. 2001;

Montagnes et al. 2008; Bautista-Reyes & Macek 2012). Thus, direct measurements of ciliate growth rates cannot be replaced with general analysis of ciliate food resources.

The dilution method was confirmed to be useful for studying growth rates of pelagic ciliates during short incubations. Short incubation periods, lasting only a few hours, minimized artifacts and enabled the detection of diel rhythms in growth rates (e.g. *Tintinnidium* sp.). Unfortunately, the method is unable to remove grazing pressure if there is a very high abundance of predators (experiments performed in June 2012). Some ciliates were sensitive to the experimental procedure (*Urotricha* sp.), which is also reported for the size fractionation method (Carrick et al. 1992; Carrias et al. 2001). In conclusion, the dilution method is much better than the size fractionation method, which frequently fails (e.g. Carrias et al. 2001).

Acknowledgments

The study was supported by the Polish Ministry of Science and Higher Education through the statutory activities of the Pomeranian University in Słupsk (Poland), project number 11.6.14. The sponsor was not involved in the study design and execution.

References

Agatha, S., Laval-Peuto, M. & Simon, P. (2013). The tintinnid lorica. In J.R. Dolan, D.J.S. Montagnes, S. Agatha, D.W. Coats & D.K. Stoecker (Eds.), *The biology and ecology of tintinnid ciliates. Models for marine plankton* (pp. 17-41). Chichester: Wiley-Blackwell.

Bautista-Reyes, F. & Macek, M. (2012). Ciliate food vacuole content and bacterial community composition in the warm-monomictic crater Lake Alchichica, México. *FEMS Microbiol. Ecol.* 79: 85-97. DOI: 10.1111/j.1574-6941.2011.01200.x.

Berglund, J., Samuelsson, K., Kull, T., Müren, U. & Andersson, A. (2005). Relative strength of resource and predation limitation of heterotrophic nanoflagellates in a low-productive sea area. *J. Plankton Res.* 27: 923-935. DOI: 10.1093/plankt/fbi067.

Biernacka, I. (1952). Studies on the reproduction of some species of the genus *Tintinnopsis* Stein. *Ann. Univ. Mariae Curie-Sklodowska Sect. C* 6: 211-247. (In Polish with English abstract).

Boenigk, J. & Novarino, G. (2004). Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates. *Aquat. Microb. Ecol.* 34: 181-192.

Børsheim, K.Y. & Bratbak, G. (1987). Cell volume to carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Prog. Ser.* 36: 171-175.

Buitenhuis, E.T., Rivkin, R.B., Sailley, S. & Le Quéré, C. (2010). Biogeochemical fluxes through microzooplankton. *Global Biogeochem. Cy.* 24: GB4015. DOI: 10.1029/2009GB003601.

Calbet, A. & Saiz, E. (2013). Effects of trophic cascades in dilution grazing experiments: from artificial saturated feeding responses to positive slopes. *J. Plankton Res.* 35: 1183-1191. DOI: 10.1093/plankt/fbt067.

Campbell, A.S. (1926). The cytology of *Tintinnopsis nucula* (Fol) Laackmann with an account of its neuromotor apparatus, division, and a new intranuclear parasite. *Univ. Calif. Publs. Zool.* 29: 179-236.

Caron, D.A. (1983). Technique for enumeration of heterotrophic and phototrophic anoplankton, using epifluorescence microscopy, and comparison with other procedures. *Appl. Environ. Microbiol.* 46: 491-498.

Caron, D.A. & Hutchins, D.A. (2013). The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J. Plankton Res.* 35: 235-252. DOI: 10.1093/plankt/fbs091.

Carrias, J.-F., Thouvenot, A., Amblard, C. & Sime-Ngando, T. (2001). Dynamics and growth estimates of planktonic protists during early spring in Lake Pavin, France. *Aquat. Microb. Ecol.* 24: 163-174.

Carrick, H. (2005). An under-appreciated component of biodiversity in plankton communities: the role of protozoa in Lake Michigan (a case study). *Hydrobiologia* 551: 17-32. DOI: 10.1007/s10750-005-4447-0.

Carrick, H.J., Fahnenstiel, G.L. & Taylor, W.D. (1992). Growth and production of planktonic protozoa in Lake Michigan: In situ versus in vitro comparisons and importance to food web dynamics. *Limnol. Oceanogr.* 37: 1221-1235.

Choi, J.W. & Stoecker, D.K. (1989). Effects of fixation on cell volume of marine planktonic protozoa. *Appl. Environ. Microbiol.* 55: 1761-1765.

Cleven, E.-J. (2004). Pelagic ciliates in a large mesotrophic lake: seasonal succession and taxon-specific bacterivory in Lake Constance. *Internat. Rev. Hydrobiol.* 89: 289-304. DOI: 10.1002/iroh.200310701.

Cleven, E.-J. & Königs, S. (2007). Growth of interstitial ciliates in association with ciliate bacterivory in a sandy hyporheic zone. *Aquat. Microb. Ecol.* 47: 177-189.

Cleven, E.-J. & Weisse, T. (2001). Seasonal succession and taxon-specific bacterial grazing rates of heterotrophic nanoflagellates in Lake Constance. *Aquat. Microb. Ecol.* 23: 147-161.

Coats, D.W. & Bachvaroff, T.R. (2013). Parasites of tintinnids. In J.R. Dolan, D.J.S. Montagnes, S. Agatha, D.W. Coats & D.K. Stoecker (Eds.), *The biology and ecology of tintinnid ciliates. Models for marine plankton* (pp. 145-170). Chichester: Wiley-Blackwell.

Coats, D.W., Kim, Y.O., Choi, J.M. & Lee, E.S. (2014).

Observations on dinoflagellate parasites of aloricate ciliates in Korean coastal waters. *Aquat. Microb. Ecol.* 72: 89-97. DOI: 10.3354/ame01687.

Dolan, J.R. (2010). Morphology and ecology in tintinnid ciliates of the marine plankton: correlates of lorica dimensions. *Acta Protozool.* 49: 235-244.

Dolan, J.R. & Coats, D.W. (1991). A study of feeding in predacious ciliates using prey ciliates labeled with fluorescent microspheres. *J. Plankton Res.* 13: 609-627.

Dupuy, C., Ryckaert, M., Le Gall, S. & Hartmann, H.J. (2007). Seasonal variations in planktonic community structure and production in an Atlantic coastal pond: the importance of nanoflagellates. *Microb. Ecol.* 53: 537-548. DOI: 10.1007/s00248-006-9087-z.

Fenchel, T. (1980). Suspension feeding in ciliated protozoa: feeding rates and their ecological significance. *Microb. Ecol.* 6: 13-25.

Fenchel, T. (1986). Protozoan filter feeding. *Progr. Protistol.* 1: 65-113.

Ficek, D. & Wielgat-Rychert, M. (2009). Spatial distribution and seasonal variation in chlorophyll concentration s in the coastal Lake Gardno (Poland). *Oceanol. Hydrobiol. Stud.* 38: 3-15. DOI: 10.2478/v10009-009-0002-z.

Finlay, B.J. (1977). The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. *Oecologia (Berl.)* 30: 75-81.

First, M.R., Miller, H.L., Lavrentyev, P.J., Pinckney, J.L. & Burd, A.B. (2009). Effects of microzooplankton growth and trophic interactions on herbivory in coastal and offshore environments. *Aquat. Microb. Ecol.* 54: 255-267. DOI: 10.3354/ame01271.

Foissner, W. & Berger, H. (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicatiors in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biol.* 35: 375-482.

Franzè, G. & Lavrentyev, P.J. (2014). Microzooplankton growth rates examined across a temperature gradient in the Barents Sea. *PLoS ONE* 9: e86429. DOI: 10.1371/journal.pone.0086429.

Franzé, G. & Modigh, M. (2013). Experimental evidence for internal predation in microzooplankton communities. *Mar. Biol.* 160: 3103-3112. DOI: 10.1007/s00227-013-2298-1.

Frost, B.W. (1972). Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* 17: 805-815.

Gallegos, C.L. (1989). Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. *Mar. Ecol. Prog. Ser.* 57: 23-33.

Gast, V. (1985). Bacteria as a food source for microzooplankton in the Schlei Fjord and Baltic Sea with special reference to ciliates. *Mar. Ecol. Prog. Ser.* 22: 107-120.

Gismervik, I. (2005). Numerical and functional responses of choreo- and oligotrich planktonic ciliates. *Aquat. Microb. Ecol.* 40: 163-173.

Heinbokel, J.F. (1978). Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. *Mar. Biol.* 47: 177-189.

Heinbokel, J.F. & Coats, D.W. (1986). Patterns of tintinnine abundance and reproduction near the edge of seasonal pack-ice in the Weddell Sea, November 1983. *Mar. Ecol. Prog. Ser.* 33: 71-80.

Hobbie, J.E., Daley, R.J. & Jasper, S. (1977). Use of nucleopore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.* 33: 1225-1228.

Jakobsen, H.H. & Strom, S.L. (2004). Circadian cycles in growth and feeding rates of heterotrophic protist plankton. *Limnol. Oceanogr.* 49: 1915-1922.

Jeffrey, S.W. & Humphrey, G.F. (1975). New spectrophotometric equation for determining chlorophyll a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pfl.* 167: 191-194.

Jezbera, J., Horňák, K. & Šimek, K. (2005). Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridisation. *FEMS Microbiol. Ecol.* 52: 351-363. DOI: 10.1016/j.femsec.2004.12.001.

Klaas, C., Verity, P.G. & Schultes, S. (2008). Determination of copepod grazing on natural plankton communities: correcting for trophic cascade effects. *Mar. Ecol. Prog. Ser.* 357: 195-206. DOI: 10.3354/meps07262.

Lai, C.-C., Fu, Y.-W., Liu, H.-B., Kuo, H.-Y., Wang, K.-W. et al. (2014). Distinct bacterial-production–DOC–primary-production relationships and implications for biogenic C cycling in the South China Sea shelf. *Biogeosciences* 11: 147-156. DOI: 10.5194/bg-11-147-2014.

Landry, M.R. & Hassett, R.P. (1982). Estimating the grazing impact of marine micro-zooplankton. *Mar. Biol.* 67: 283-288.

Landry, M.R., Haas, L.W. & Fagerness, V.L. (1984). Dynamics of microbial plankton communities: Experiments in Kanoehe, Hawaii. *Mar. Ecol. Prog. Ser.* 16: 127-133.

Lavrentyev, P.J., McCarthy, M.J., Klarer, D.M., Jochem, F. & Gardner, W.S. (2004). Estuarine microbial food web patterns in a Lake Erie coastal wetland. *Aquat. Ecol.* 48: 567-577. DOI: 10.1007/s00248-004-0250-0.

Leakey, R.J.G., Burkill, P.H. & Sleigh, M.A. (1992). Planktonic ciliates in Southampton Water: abundance, biomass, production, and role in pelagic carbon flow. *Mar. Biol.* 114: 67-83.

Leakey, R.J.G., Burkill, P.H. & Sleigh, M.A. (1994). Ciliate growth rates from Plymouth Sound: comparison of direct and indirect estimates. *J. Mar. Biol. Assoc. UK* 74: 849-861.

Macek, M., Šimek, K., Pernthaler, J., Vyhnálek, V. & Psenner, R. (1996). Growth rates of dominant planktonic ciliates in two freshwater bodies of different trophic degree. *J. Plankton Res.* 18: 463-481.

McManus, G.B. (1993). Growth rates of natural populations

of heterotrophic nanoplankton. In P.F. Kemp, B.F. Sherr, E.B. Sherr, J.J. Cole (Eds.), *Handbook of methods in aquatic microbial ecology* (pp. 557-562). Boca Raton: Levis Publishing.

McManus, G.B. & Santoferrara, L.F. (2013). Tintinnids in microzooplankton communities. In J.R. Dolan, D.J.S. Montagnes, S. Agatha, D.W. Coats & D.K. Stoecker (Eds.), *The biology and ecology of tintinnid ciliates. Models for marine plankton* (pp. 198-213). Chichester: Wiley-Blackwell.

Mironova, E., Telesh, I. & Skarlato, S. (2012). Diversity and seasonality in structure of ciliate communities in the Neva Estuary (Baltic Sea). *J. Plankton Res.* 34: 208-220. DOI: 10.1093/plankt/fbr095.

Mitra, A., Flynn, K.J., Burkholder, J.M., Berge, T., Calbet, A. et al. (2014). The role of mixotrophic protists in the biological carbon pump. *Biogeosciences* 11: 995-1005. DOI: 10.5194/bg-11-995-2014.

Montagnes, D.J.S. (1996). Growth responses of planktonic ciliates in the genera *Strobilidium* and *Strombidium*. *Mar. Ecol. Prog. Ser.* 130: 241-254.

Montagnes, D.J.S. (2013). Ecophysiology and behavior of tintinnids. In J.R. Dolan, D.J.S. Montagnes, S. Agatha, D.W. Coats & D.K. Stoecker (Eds.), *The biology and ecology of tintinnid ciliates. Models for marine plankton* (pp. 85–121). Chichester: Wiley-Blackwell.

Montagnes, D.J.S., Barbosa, A.B., Boenigk, J., Davidson, K., Jürgens, K. et al. (2008). Selective feeding behaviour of key free-living protists: avenues for continued study. *Aquat. Microb. Ecol.* 53: 83-98. DOI: 10.3354/ame01229.

Montagnes, D.J.S., Lynn, D.H., Roff, J.C. & Taylor, W.D. (1988). The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. *Mar. Biol.* 99: 21-30.

Montagnes, D.J.S., Berges, J.A., Harrison, P.J. & Taylor, F.J.R. (1994). Estimating carbon, nitrogen, protein, and chlorophyll *a* from volume in marine phytoplankton. *Limnol. Oceanogr.* 39: 1044-1060.

Müller, H. (1989). The relative importance of different ciliate taxa in the pelagic food web of Lake Constance. *Microb. Ecol.* 18: 261-273.

Müller, H. & Geller, W. (1993). Maximum growth rates of aquatic ciliated protozoa: the dependence on body size and temperature reconsidered. *Arch. Hydrobiol.* 126: 315-327.

Nielsen, T.G. & Kiørboe, T. (1994). Regulation of zooplankton biomass and production in a temperate coastal ecosystem. 2. Ciliates. *Limnol. Oceanogr.* 39: 508-519.

Ohman, M.D. & Snyder, R.A. (1991). Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. *Limnol. Oceanogr.* 36: 922-935.

Paffenhöfer, G.A., Sherr, B.F. & Sherr, E.B. (2007). From small scales to the big picture: persistence mechanisms of planktonic grazers in the oligotrophic ocean. *Mar. Ecol.* 28: 243-253. DOI: 10.1111/j.1439-0485.2007.00162.x.

Peštová, D., Macek, M. & Martínez-Pérez, M.E. (2008) Ciliates and their picophytoplankton-feeding activity in a high-altitude warm-monomictic saline lake. *Eur. J. Protistol.* 44: 13-25. DOI: 10.1016/j.ejop.2007.04.004.

Posch, T., Jezbera, J., Vrba, J., Šimek, K., Pernthaler, J. et al. (2001). Size selective feeding in *Cyclidium glaucoma* (Ciliophora, Scuticociliatida) and its effects on bacterial community structure: a study from a continuous cultivation system. *Microb. Ecol.* 42: 217-227. DOI: 10.1007/s002480000114.

Pratt, J.R. & Cairns, J.Jr. (1985). Functional groups in the protozoa: roles in differing ecosystems. *J. Protozool.* 32: 415-423.

Rivier, A., Brownlee, D.C., Sheldon, R.W. & Rassoulzadegan, F. (1985). Growth of microzooplankton: a comparative study of bactivorous zooflagellates and ciliates. *Mar. Microb. Food Webs* 1: 51-60.

Rychert, K. (2011). Dependence between volumes of protoplast and lorica in Lugol-fixed tintinnid ciliates. *Protist* 162: 249-252. DOI: 10.1016/j.protis.2010.05.004.

Rychert, K. (2013). A modified dilution method reveals higher protozoan growth rates than the size fractionation method. *Eur. J. Protistol.* 49: 249-254. DOI: 10.1016/j.ejop.2012.08.003.

Rychert, K., Wielgat-Rychert, M., Szczurowska, D., Myszka, M., Bochyńska, M. et al. (2012). The importance of ciliates as a trophic link in shallow, brackish and eutrophic lakes. *Pol. J. Ecol.* 60: 767-776.

Seuthe, L., Iversen, K.R. & Narcy, F. (2011). Microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): II. Ciliates and dinoflagellates. *Polar Biol.* 34: 751-766. DOI: 10.1007/s00300-010-0930-9.

Šimek, K., Bobková, J., Macek, M., Nedoma, J. & Psenner, R. (1995). Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. *Limnol. Oceanogr.* 40: 1077-1090.

Sonntag, B., Posch, T., Klammer, S., Teubner, K. & Psenner, R. (2006). Phagotrophic ciliates and flagellates in an oligotrophic, deep, alpine lake: contrasting variability with seasons and depths. *Aquat. Microb. Ecol.* 43: 193-207.

Stocker, R. (2012). Marine microbes see a sea of gradients. *Science* 338: 628-633. DOI: 10.1126/science.1208929.

Taylor, W.D. (1978). Maximum growth rate, size and commonness in a community of bactivorous ciliates. *Oecologia (Berl.)* 36: 263-272.

Trojanowski, J. & Antonowicz, J. (2011). Heavy metals in surface microlayer in water of Lake Gardno. *Arch. Environ. Prot.* 37: 75-88.

Turley, C.M., Newell, R.C. & Robins, D.B. (1986). Survival strategies of two small marine ciliates and their role in regulating bacterial community structure under experimental conditions. *Mar. Ecol. Prog. Ser.* 33: 59-70.

Utermöhl, H. (1958). Improving quantitative methods for phytoplankton analyses. *Mitt. Int. Ver. Limnol.* 9: 1-38. (In

German).

Verity, P.G. (1986). Growth rates of natural tintinnid populations in Narragansett Bay. *Mar. Ecol. Prog. Ser.* 29: 117-126.

Verity, P.G. & Langdon, C. (1984). Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. *J. Plankton Res.* 6: 859-867.

Wallberg, P., Jonsson P.R. & Johnstone, R. (1999). Abundance, biomass and growth rates of pelagic microorganisms in a tropical coastal ecosystem. *Aquat. Microb. Ecol.* 18: 175-185.

Weisse, T., Karstens, N., Meyer, V.C.L., Janke, L., Lettner, S. et al. (2001). Niche separation in common prostome freshwater ciliates: the effect of food and temperature. *Aquat. Microb. Ecol.* 26: 167-179.

Weisse, T., Stadler, P., Lindström, E.S., Kimmance, S.A. & Montagnes, D.J.S. (2002). Interactive effect of temperature and food concentration on growth rate: a test case using the small freshwater ciliate *Urotricha farcta*. *Limnol. Oceanogr.* 47: 1447-1455.

Wiackowski, K., Doniec, A. & Fyda, J. (1994). An empirical study of the effect of fixation on ciliate cell volume. *Mar. Microb. Food Webs* 8: 59-69.

Wiackowski, K., Ventelä, A.-M., Moilanen, M., Saarikari, V., Vuorio, K. et al. (2001). What factors control planktonic ciliates during summer in a highly eutrophic lake? *Hydrobiologia* 443: 43-57. DOI: 10.1023/A:1017592019513.

Wielgat-Rychert, M., Rychert, K. & Ficek, D. (2010). Factors controlling pelagic production and respiration in a shallow polymictic lake. *Pol. J. Ecol.* 58: 379-385.

Wielgat-Rychert, M., Jarosiewicz, A., Ficek, D., Pawlik, M. & Rychert, K. (2015). Nutrient fluxes and their impact on the phytoplankton in a shallow coastal lake. *Pol. J. Environ. Stud.* 24: 751-759. DOI: 1015244/pjoes/30925.