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Annual production to biomass (P/B) ratios of pelagic ciliates in different temperate waters

by

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Abstract

In aquatic environments, ciliates integrate the energy flowing through bacteria, smaller protozoa, and the prevailing fraction of algae and are further grazed upon by metazooplankton. Ciliates are incorporated into budgets and models describing ecosystem functioning. However, data for the parameterization and validation of models remain insufficient. In this study, annual production to biomass (P/B) ratios of pelagic ciliates were estimated in four lakes of different trophic status and at two sites located in the coastal zone of the Baltic Sea. All study sites were located in the temperate zone. The calculations were based on bulk data reflecting seasonal changes in ciliate production, which was estimated using an allometric equation. The annual ciliate P/B ratio for surface waters was 308 ± 81 yr^{-1} (mean ± standard deviation). There was no statistically significant relationship between the mean annual ciliate biomass in particular water bodies and the annual P/B ratio. In the near-bottom waters, the annual P/B ratio was 78 ± 39 yr^{-1}. Because of the possible food limitation of ciliate growth and reconstructions of the community due to changes in environmental conditions, the ciliate P/B ratio applied in models of temperate waters should range from 50% to 100% of the P/B ratios estimated in this study.

Key words: protozoa, growth, aquatic food web, productivity, modeling, surface, near-bottom

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Introduction

In aquatic environments, energy from photosynthesis and allochthonous organic matter degradation flows through the classic food chain and the microbial food web (Azam et al. 1983; Sherr & Sherr 2002; Putland & Iverson 2007). Ciliates, which integrate energy flowing through bacteria, smaller protozoa, and the prevailing fraction of algae, occupy a special position within the microbial food web (Sherr & Sherr 2002; Calbet & Landry 2004; McManus & Santoferrara 2013). Ciliates are grazed upon by metazooplankton, which prefer them over large algae like diatoms and dinoflagellates (Stoecker & Capuzzo 1990; Sanders & Wickham 1993; Wiackowski et al. 1994a; Calbet & Saiz 2005; Calbet 2008; Stoecker 2013). Furthermore, ciliates are important, sometimes even crucial food for larval fish (Sanders & Wickham 1993; Montagnes et al. 2010; Stoecker 2013). Consequently, ciliates are incorporated into budgets (Lynn & Montagnes 1991; Leakey et al. 1992; Gaedke & Straile 1994; Hansen et al. 1996; Wallberg et al. 1999; Tanaka et al. 2004; Weitere et al. 2005; Stukel & Landry 2010; Montagnes 2013) and models describing ecosystem functioning (Sandberg 2007; Mooij et al. 2010; Davidson 2014; Kerimoglu et al. 2014; Mitra et al. 2014), including dynamic models based on differential equations like ERSEM (Baretta-Bekker et al. 1995), MP (Tett & Wilson 2000), PlankTOM5 (Buitenhuis et al. 2010), or CAEDYM (Mooij et al. 2010). To calculate the energy flow through ciliate communities, it is necessary to calculate ciliate production (Montagnes et al. 1988; Müller 1989; Leakey et al. 1992; Reiss & Schmid-Araya 2010). As was emphasized by Kiss et al. (2009), Buitenhuis et al. (2010), Reiss & Schmid-Araya (2010) and Kerimoglu et al. (2014), there are still insufficient numbers of datasets on ciliate production that can be used for parameterization and validation of biogeochemical, ecological, and fisheries models.

Generally, growth rates of ciliates depend on the cell size and temperature. Smaller ciliates grow faster than larger ones, whereas increases in temperature accelerate ciliate growth rates (Fenchel 1974; Finlay 1977; Verity 1986; Montagnes et al. 1988; Müller & Geller 1993; Hansen et al. 1997; Levinsen et al. 1999; Fenchel 2005; Rose & Caron 2007; Reiss & Schmid-Araya 2010; Mitra et al. 2014). The impact of temperature on the growth rates obviously holds true for physiologically suitable ranges for particular species (Montagnes & Lessard 1999; Weisse et al. 2001; Mitra et al. 2014); however, different species vary according to their preferred range of temperature and they replace each other within communities after changes in temperature (Montagnes et al. 2003; Aberle et al. 2007). These general regularities are described by allometric equations based on bulk data on ciliate growth rates in cultures (Finlay 1977; Montagnes et al. 1988; Müller & Geller 1993). All of them depict the "general ciliate growth" and are useful for estimating the growth rates of multi-species ciliate communities (Montagnes 1996). The formula by Müller & Geller (1993) is widely recognized (Macek et al. 1996; Montagnes 1996) as the best allometric equation for predicting the ciliate growth rates.

In field studies, ciliate growth rates have long been estimated by the size fractionation method, which entails observing ciliate growth after separating them from larger predators (e.g. Verity 1986; Taylor & Johannsson 1991; Nielsen & Kiørboe 1994; Seuthe et al. 2011). Studies performed with this method have suggested that ciliates grow much slower under natural conditions compared to the estimations made with the use of allometric equations (Leakey et al. 1994b; Macek et al. 1996; Wallberg et al. 1999; Carrias et al. 2001; Weitere et al. 2005). It was suspected (Verity 1986; Taylor & Johannsson 1991; Leakey et al. 1994b; Nielsen & Kiørboe 1994; Macek et al. 1996) that this difference resulted, at least partially, from the methodological constraints of the method, which did not relax grazing pressure efficiently. Further studies performed with the modified dilution method (Franzé & Modigh 2013; Rychert 2013) clearly demonstrated that the ciliate growth rates measured with the size fractionation method are significantly underestimated because of internal predation within microzooplankton communities. Consequently, the application of allometric equations for predicting the potential ciliate growth rates is much more reliable than previously thought.

The aim of this study was to estimate the productivity of pelagic ciliates in different temperate waters that could be used for constructing biogeochemical, ecological, and fisheries models. The calculations were based on bulk data on seasonal changes in water temperature, ciliate biomass, and ciliate community size distribution. The database comprised 11 complete year-long datasets for two sites located in coastal brackish waters and four lakes of varying trophic status. The surface and near-bottom waters of the lakes were studied separately. We computed the annual production to biomass (P/B) ratios after integrating the ciliate production estimated for particular sampling occasions. Such particular estimates were performed with the allometric equation by Müller & Geller (1993). To provide data for parametrization and validation of models, the data used in this study were gathered in supplementary material.

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Materials and methods

The annual production to biomass (P/B) ratios of pelagic ciliates were calculated for two sites in the coastal zone of the Baltic Sea (brackish waters, salinity at sampling sites ranged from 6.4 to 8.5 PSU), two mesotrophic lakes, one meso-eutrophic lake, and one eutrophic lake (Table 1). All sampling sites were located in the temperate zone in Poland. In the lakes, samples were collected from the surface and near-bottom waters at the deepest site of each lake. Samples were collected during all seasons of the year. The sampling periods and the number of samplings are presented in Table 1. Ciliate communities were examined in samples fixed with strong acid Lugol's solution (Edler 1979; stock solution: 5% iodine, 10% potassium iodide, 10% v/v glacial acetic acid; 1–2 ml of stock solution was added to 200 ml sample) with the standard Utermöhl method (Hasle 1978). Acid Lugol's solution was chosen as the fixative, because it does not cause loss of protozoan cells, which means it is the best fixative for quantitative studies of ciliates (Stoecker et al. 1989; Leakey et al. 1994a; Gifford & Caron 2000). The specimens were identified according to Marshall (1969), Foissner & Berger (1996), Patterson & Hedley (2003), and other guides. The functionally autotrophic ciliate *Mesodinium rubrum* was observed in the Baltic Sea coastal zone, but it was not included in this study, because it is functionally assigned to phytoplankton (Crawford 1989; Stoecker et al. 2009). Altogether 115 samples and almost five thousand ciliate cells were analyzed from all the sampling sites.

Ciliate volumes and biomass

All ciliate cells were measured, and their volumes (μm^3) were estimated by comparing their shapes to geometric figures. Cell volumes were necessary to calculate growth rates with the allometric equation. Additionally, ciliate biomass (carbon content) was estimated to characterize ciliate communities. The carbon content of naked (aloricate) ciliate cells (CC, pgC) was calculated from their volumes (V, μm^3) using the following equation by Menden-Deuer & Lessard (2000):

$$CC = 0.216 \times V^{0.939} \qquad (1)$$

The carbon content of tintinnid ciliates (CC, pgC) was calculated from the volume of lorica (V, μm^3) using the following equation by Verity & Langdon (1984):

$$CC = 0.053 \times V + 444.5 \qquad (2)$$

The biomass of individual ciliates was combined for a particular sample. Next, the mean annual ciliate biomass was calculated as the weighted mean taking into account periods between sampling occasions (see Fig. 1 for an example). Calculations were performed with the standard trapezoidal method.

Ciliate production

To calculate the ciliate community production, the potential growth rate (r, d^{-1}) was calculated for every cell in a given sample, according to the formula by Müller & Geller (1993):

$$\ln r = 1.52 \times \ln T - 0.27 \times \ln V - 1.44 \qquad (3)$$

where T (°C) is the ambient temperature and V (μm^3) is the cell volume. Calculations were more complicated for tintinnid ciliates. To take into account the energy

Table 1

Description of water bodies and sampling periods. Both surface and near-bottom zones were sampled in lakes on each occasion. Water bodies are arranged according to mean annual ciliate biomass.

Water body	Trophic status	Coordinates	Area (ha)	Maximum depth (m)	References – characteristics of water bodies	Sampling period (number of sampling occasions)
Lake Marszewo	mesotrophic	54°30.5′N 16°42.6′E	18	20	Jarosiewicz (2009)	Sept. 2007 – July 2008 (7)
Lake Mały Borek	mesotrophic	54°05.3′N 17°25.6′E	7.6	11	Witek & Jarosiewicz (2010)	Nov. 2010 – Dec. 2011 (12)
Lake Dobra	meso-eutrophic	54°23.4′N 17°20.3′E	28.5	12	Jarosiewicz & Hetmański (2009)	Sept. 2007 – July 2008 (7)
Baltic Sea in Ustka	coastal brackish waters	54°35.2′N 16°50.0′E	-	6–6.5	Rychert et al. (2013)	April 2006 – April 2008 (30)
Baltic Sea in Sopot	coastal brackish waters	54°26.9′N 18°34.7′E	-	6–6.5	Rychert et al. (2013)	April 2003 – March 2004 (12)
Lake Kociołek	eutrophic	53°45.6′N 19°31.8′E	0.4	6	Czychewicz & Rychert, unpublished	May 2009 – June 2010 (11)

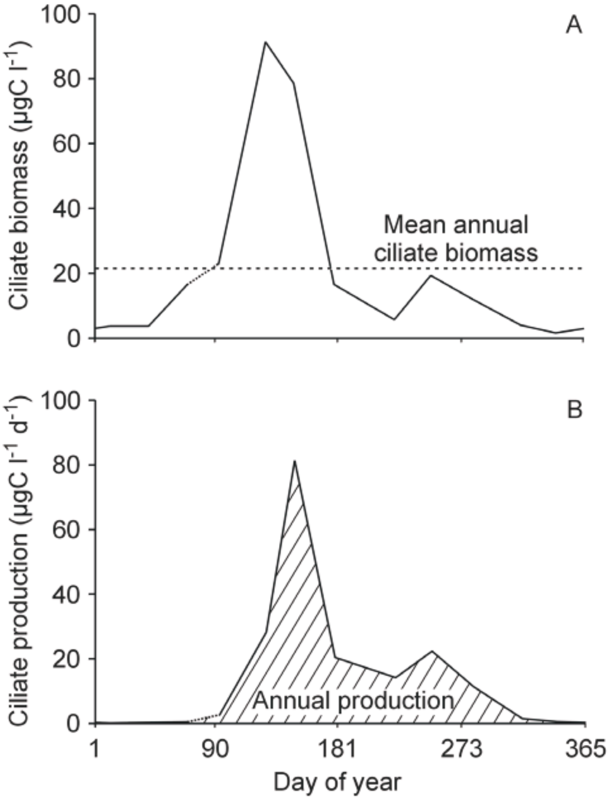

Figure 1

Data for the sampling site in the coastal waters in Sopot as an example of calculations performed for each water body. (A) Mean annual ciliate biomass – weighted mean calculated from seasonal changes in ciliate biomass. (B) Cumulative annual ciliate production – an integral calculated from seasonal changes in ciliate production rates.

allocated for the construction of lorica, the carbon content estimated by Verity & Langdon's (1984) equation was calculated back with Menden-Deuer & Lessard's (2000) equation to estimate the surrogate cell volume. This surrogate volume was used to estimate the potential growth rate with the formula by Müller & Geller (1993). The production of every ciliate cell was calculated as the product of its estimated growth rate and its biomass. Next, the production of organisms was combined for particular samples and expressed in µg C l^{-1} d^{-1}. Taking into account periods between sampling occasions, ciliate production was annual-integrated with the trapezoidal method (see Fig. 1 for an example).

During the warmer part of the year, anoxic conditions were encountered in the near-bottom zones of all the lakes studied. Each time, the anoxic conditions resulted in the development of communities of anaerobic ciliates. The fact that the growth efficiency of anaerobic protozoa is approximately 25% of the growth efficiency of aerobic protozoa is well documented (Fenchel & Finlay 1990; Fenchel & Finlay 1995). Thus, assuming the same grazing rates of both aerobic and anaerobic ciliates, the growth rates estimated for anaerobic ciliates were divided by four.

Production to biomass (P/B) ratio

The annual P/B ratios of pelagic ciliates were calculated as the quotient of cumulative annual production and the mean annual biomass. P/B ratios were calculated separately for the surface and near-bottom waters of the lakes and also for the surface waters at the two sites in the coastal zone of the Baltic Sea. Two of the eleven complete year-long datasets were obtained at the sampling site in Ustka for two consecutive years (Table 1). Consequently, the two P/B ratios calculated for this sampling site were averaged. In total, ten separate P/B ratios were estimated.

Results and discussion

Environmental conditions

Temperatures in surface water ranged from 0-3°C in winter to 20-24°C in summer, depending on the water body. Thus, temperature ranges were typical of the temperate zone (Müller et al. 1991; Carrick et al. 1992; Johansson et al. 2004; Pettigrosso & Popovich 2009; Kiss et al. 2009; Rychert 2009; Mironova et al. 2012; Rychert et al. 2012). Temperatures in near-bottom waters of the deeper lakes (depth 11-20 m, lakes: Marszewo, Mały Borek, Dobra, Table 1) ranged from 3-4°C in winter to 6-9°C in summer. In shallow Lake Kociołek (depth 6 m, Table 1), the temperature of the near-bottom water ranged from 4°C in winter to 15°C in summer. The surface waters were well oxygenated in the lakes and in the coastal zone of the Baltic Sea. Oxygen was depleted during the warmer part of the year in the near-bottom waters of all the lakes.

Ciliate communities

In all the lakes studied and in the brackish coastal waters in Sopot, seasonal changes in the ciliate abundance and biomass follow a bimodal pattern with a distinct spring peak and a less pronounced peak during fall. Such seasonal changes in the ciliate biomass are typical of temperate waters (e.g. Müller et al. 1991; Nielsen & Kiørboe 1994; Witek 1998;

Carrick 2005; Kiss et al. 2009). Seasonal changes were different in the coastal zone in Ustka with a summer peak only, which was explained by the irregular impact of fresh water from the mouth of the Słupia River located just 1.3 km from the sampling site (Rychert et al. 2013). Ciliate biomass peaks were less pronounced in the near-bottom waters than in the surface waters (data not shown).

Mean annual values of the ciliate biomass are presented in Fig. 2. The lowest mean annual biomass in surface waters was observed in the mesotrophic Lake Marszewo (3.24 μg C l^{-1}), while the highest – in the eutrophic Lake Kociołek (26.3 μg C l^{-1}). The ciliate biomass in the near-bottom waters was comparable to values in the surface waters (Fig. 2). The same pattern was exhibited by mean annual ciliate abundance, which ranged in the surface waters from 3.31 cells ml^{-1} in Lake Marszewo to 32.9 cells ml^{-1} in Lake Kociołek. Similar mean annual values of abundance were observed in the near-bottom waters: 3.65 cells ml^{-1} in Lake Marszewo and 29.3 cells ml^{-1} in Lake Kociołek. The mean annual values of abundance and biomass in the lakes are lower than values published by Beaver & Crisman (1982) for subtropical lakes of different trophic status, but are within the range of values reported for temperate lakes (Müller 1989; Taylor & Johannsson 1991; Kalinowska 2004; Lavrentyev et al. 2004; Chróst et al. 2009; Mieczan 2003; Xu & Cronberg 2010) when taking into account the biomass conversion factors used in particular studies. Ciliate abundances and biomasses at sampling sites in Sopot and in Ustka (Fig. 2) corresponded to those reported for temperate coastal waters (Smetacek 1981; Leakey et al. 1992; Garstecki et al. 2000; Urrutxurtu et al. 2003; Mironova et al. 2012).

The ciliate size observed in this study ranged from less than 10 μm to almost 200 μm. For the sake of size distribution analysis, the ciliates were divided into nanociliates (equivalent spherical diameter – ESD ≤ 20 μm) and microciliates (ESD > 20 μm) as was done in the study by Lynn et al. (1991). Nanociliates were more abundant than microciliates, but microciliates contributed the majority of the biomass in all the waters studied. Microciliates represented 68–95% of the ciliate biomass annually in the surface waters, while their contribution to the biomass was even higher in the lake near-bottom waters and ranged from 81 to 94%. No relationships between the trophic status and the size distribution of ciliate communities in either surface or near-bottom waters was detected and the detailed data are not presented. Generally, the size distributions resembled those reported in the literature for temperate (Johansson et al. 2004; Pettigrosso & Popovich 2009) and other waters (e.g. Lynn et al. 1991).

Ciliate communities occurring in the surface waters of lakes Marszewo, Mały Borek, and Dobra as well as in the coastal waters in Sopot and Ustka

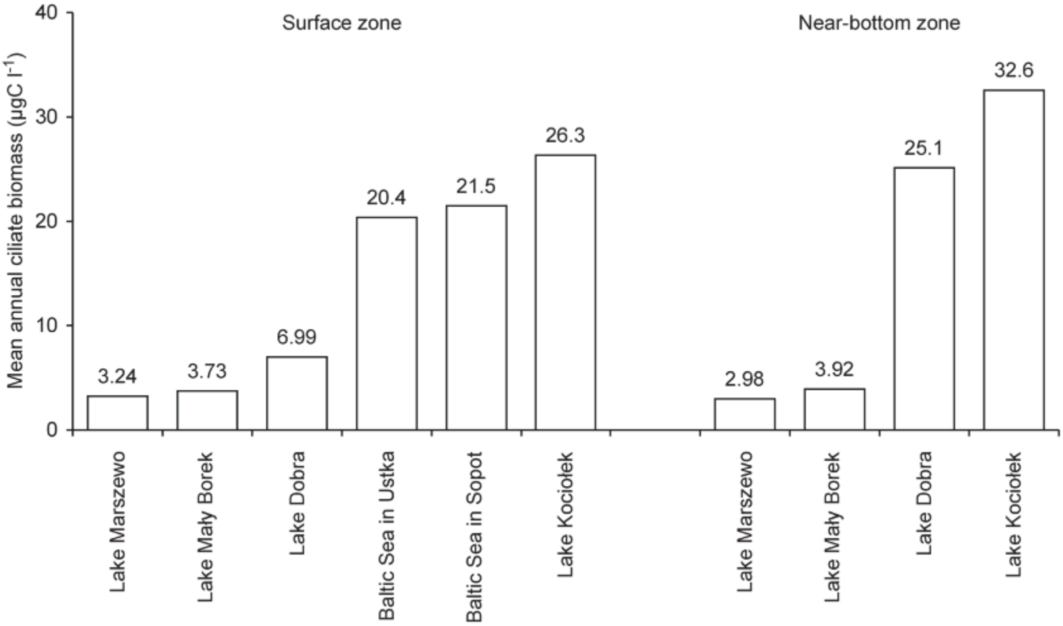

Figure 2

Values of mean annual ciliate biomass in surface and near-bottom waters of four lakes and at two stations located in the coastal zone of the southern Baltic Sea. All sampling sites were located in the temperate zone.

comprised oligotrichs and choreotrichs (43-85% of the mean annual ciliate biomass), prostomatids (2-31%), and haptorids (6-20%), whereas other orders were of lesser importance. The ciliate community in the surface water of Lake Kociołek was dominated by prostomatids, which contributed 72% of the mean annual ciliate biomass, whereas oligotrichs and choreotrichs accounted for 23%, and haptorids less than 1% of the mean annual ciliate biomass. Both types of ciliate communities, i.e. those dominated by oligo- and choreotrichs and those dominated by prostomatids, were previously reported as typical of temperate surface waters (Smetacek 1981; Müller 1989; Müller et al. 1991; Pfister et al. 2002b; Sonntag et al. 2006; Chróst et al. 2009; Lavrentyev et al. 2004; Xu & Cronberg 2010; Mironova et al. 2012). Ciliate communities in the near-bottom waters of the lakes were more diverse than those observed in the surface zone due to the occurrence of some benthic ciliates (observed also by e.g. Pfister et al. 2002b), and periodically also anaerobic ciliates. Oligotrichs and choreotrichs were less important than in the surface waters contributing 11-21% of the mean annual ciliate biomass. Other important ciliates belonged to the order Prostomatida (up to 43% of the biomass in Lake Marszewo), Peritrichida (up to 64% of the biomass in Lake Kociołek), and Scuticociliatida (up to 12% in Lake Marszewo). Haptorids contributed only up to 4% of the mean annual biomass in the near-bottom waters. As was mentioned above, the near-bottom waters of all the lakes studied were periodically anoxic. In the samples with oxygen-deficient water, we observed specialized microaerophilic and anaerobic ciliates belonging to different orders. The most frequently observed among them were ciliates from the genera *Prorodon* and *Metopus*. Generally, ciliate communities observed in the near-bottom waters resembled those described by Müller et al. (1991), Fenchel & Finlay (1995), Pfister et al. (2002a), Mieczan (2003), and Fenchel (2014).

To sum up, ciliate communities observed in the lakes and in the coastal zone of the Baltic Sea were typical of the temperate zone in terms of biomass, size distribution, and taxonomic composition.

Annual P/B ratio of pelagic ciliates

Annual production to biomass (P/B) ratios are presented in Fig. 3. The annual P/B ratios ranged from 240 to 447 yr^{-1} (308 ± 81 yr^{-1}, mean ± standard deviation) in the surface waters, which corresponded to a mean annual growth rate of 0.84 ± 0.22 d^{-1}, a mean annual doubling time of 19.7 h, and 1.22 doublings d^{-1}. There was no statistically significant

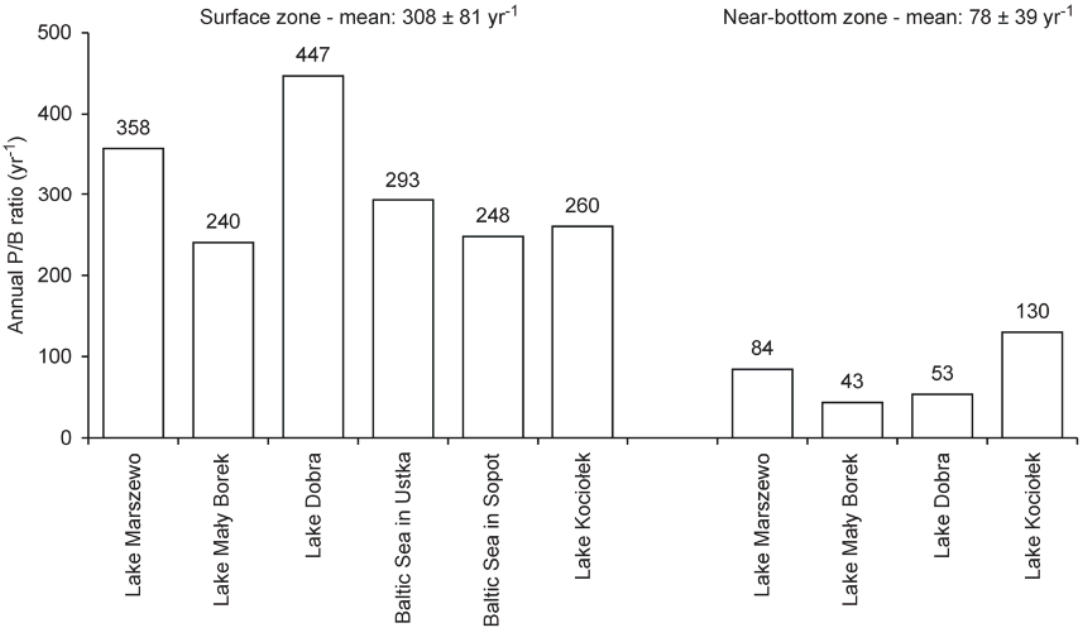

Figure 3

Annual production to biomass (P/B) ratios calculated for ciliate communities in surface and near-bottom waters of four lakes and at two stations located in the coastal zone of the southern Baltic Sea. Mean values and standard deviations are noted at the top of the graph.

relationships between ciliate biomass, used as a proxy for trophic status, and the annual P/B ratio of ciliates. Differences among growth estimates for particular water bodies were caused primarily by shifts between the period of maximum temperature (summer) and peaks of maximum ciliate abundance (spring and fall). Depending on the water body, spring peaks occurred between April and June, and fall peaks between September and November (see the supplementary material); thus, a spring peak that occurred early and a late fall peak resulted in the lower estimate of the mean P/B ratio. Small differences in the size distribution of ciliate communities observed in the studied waters were less important.

P/B ratios were also calculated for the near-bottom zone in the lakes, and they were lower in the near-bottom zone than in the surface zone (paired Student's t-test, $p = 0.02$), because of the lower water temperature (described above in the section on environmental conditions) and oxygen deficiency observed during the warmer part of the year in all the lakes studied. The mean annual P/B ratios of ciliates for the near-bottom waters were 78 ± 39 yr^{-1} (Fig. 3), which corresponded to a mean annual growth rate of 0.21 ± 0.11 d^{-1} or 0.31 doublings d^{-1}, and a doubling time of 78.1 h. The highest P/B ratio in the near-bottom zone was calculated in Lake Kociołek (130 yr^{-1}, Fig. 3), which was caused by the shallow depth of this lake (6 m, Table 1), which in turn resulted in the higher water temperature than in the near-bottom zones of the deeper lakes.

Annual P/B ratios calculated for surface waters are among the higher estimates reported in the literature for temperate waters (Table 2). On the other hand, all estimates for the temperate zone are lower than the annual P/B ratio calculated for tropical waters off Kingston, Jamaica (Table 2, Lynn et al. 1991), where water temperature (27-29°C) was stable and much higher than in temperate waters. The annual P/B ratios calculated for the near-bottom zones of the lakes were roughly four times lower and corresponded to the lowest estimates reported in the literature for temperate waters (Table 2). Some of the data gathered in Table 2 were calculated according to the allometric equation by Müller & Geller (1993), the same as in this study, but the equation by Montagnes et al. (1988) was applied for others. These data are presented together, because both of the allometric equations give comparable growth estimates for moderate temperatures (Rychert 2009; Rychert et al. 2012).

Table 2

Annual production to biomass (P/B) ratios for ciliates in different water bodies. When necessary, P/B ratios (yr^{-1}) were computed after the analysis of graphs published in the cited papers.

Water body	Layer	Annual P/B ratio (yr^{-1})	Reference
	Temperate zone		
Meso-eutrophic Lake Constance	Epipelagic water	77-84	Müller (1989)
Lakes of different trophic status	Periodically anoxic near-bottom waters	78	This study
Oligo-mesotrophic Lake Michigan	Surface water	117	Carrick et al. (1992)
Gulf of Gdańsk	Epipelagic water	120-193	Witek (1998)
Gulf of Maine	Entire water column	135	Montagnes et al. (1988)
Gulf of Gdańsk	Oxygenated near-bottom water	139-153	Witek (1998)
Open northern Baltic Sea proper	Epipelagic water	141	Johansson et al. (2004)
Southampton water	Surface water	164-183	Leakey et al. (1992)
River Rhine	Main flow	238*	Weitere et al. (2005)
Bahía Blanca Estuary	Surface water	250	Pettigrosso & Popovich (2009)
Słupia River	Main flow	252	Rychert (2009)
Southern Kattegat	Entire water column	~256	Nielsen & Kiørboe (1994)
Lakes of different trophic status and coastal waters	Surface water	308	This study
Lake Gardno	Surface water	325-332	Rychert et al. (2012)
Oligo-mesotrophic Lake Ontario	Epilimnetic water	~475	Taylor & Johannsson (1991)
	Tropical zone		
Waters off Kingston, Jamaica	Entire water column	621**	Lynn et al. (1991)

* P/B ratio without assumption made by authors (Weitere et al. 2005) that ciliate growth was limited in summer, fall, and winter.

** Aloricate ciliates only.

Possible constraints

The estimated annual P/B ratios can be used when constructing biogeochemical, ecological, and fisheries models, as was mentioned in the Introduction. The complete data were provided in the supplementary material. However, there are four constraints that could cause the overestimation of the actual ciliate production in different water bodies. The first constraint is that the approximation of ciliate growth rates with allometric equations assumes the lack of food limitation (Müller 1989; Leakey et al. 1992; Leakey et al. 1994b). It is generally acknowledged that ciliates in hypertrophic and eutrophic lakes, estuaries, and in most coastal marine waters are not food-limited (Smetacek 1981; Beaver & Crisman 1982; Nielsen & Kiørboe 1994; Jürgens et al. 1999; Weisse et al. 2001; Weisse et al. 2002; Urrutxurtu et al. 2003; Pettigrosso & Popovich 2009). However, in oligotrophic and mesotrophic lakes and also in offshore marine waters, food resources for ciliates are permanently or temporarily limited (Beaver & Crisman 1982; Müller et al. 1991; Taylor & Johannsson 1991; Macek et al. 1996; Weisse et al. 2001; Weisse et al. 2002; Johansson et al. 2004). The effect of food limitation on ciliate growth in such waters occurs mainly in summer, when water temperatures exceed 18–20°C (Müller et al. 1991; Gaedke & Straile 1994; Weisse & Müller 1998; Wiackowski et al. 2001; Pfister et al. 2002a; Weisse et al. 2002; Johansson et al. 2004). Additionally, the ciliate growth could be limited by food in winter (Tirok & Gaedke 2007). To check the importance of summer and winter production in our estimates, we computed percentages of annual production for each season of the year (Fig. 4). In surface waters, summer production accounted for about one third of the cumulative annual production (33%, Fig. 4), while in winter – only 5%. Almost two third (62%) of the annual production occurred in spring and fall (Fig. 4). This is not surprising because ciliate biomass peaks during spring and fall (described above, see also Fig. 1). Assuming that ciliates were limited by food in winter and summer, and realized only half of the production theoretically possible, the cumulative annual production would be 81% of the potential production estimated by the allometric equation. In the near-bottom zone, the distribution of ciliate production between seasons was different (Fig. 4). The fraction of ciliate production, realized during a particular season, gradually increases from winter (15%) to fall (33%). In summer, the ciliate production was inhibited by anoxic conditions and the highest portion of the annual ciliate production in the near-bottom waters was realized during fall. Assuming that the actual ciliate production during summer and winter was half of the potential, the cumulative annual production would be lower at 79% of that estimated by the allometric equation.

The second constraint is the permanent reconstruction of ciliate communities as a result of changing environmental conditions, e.g. temperature, pH, etc.

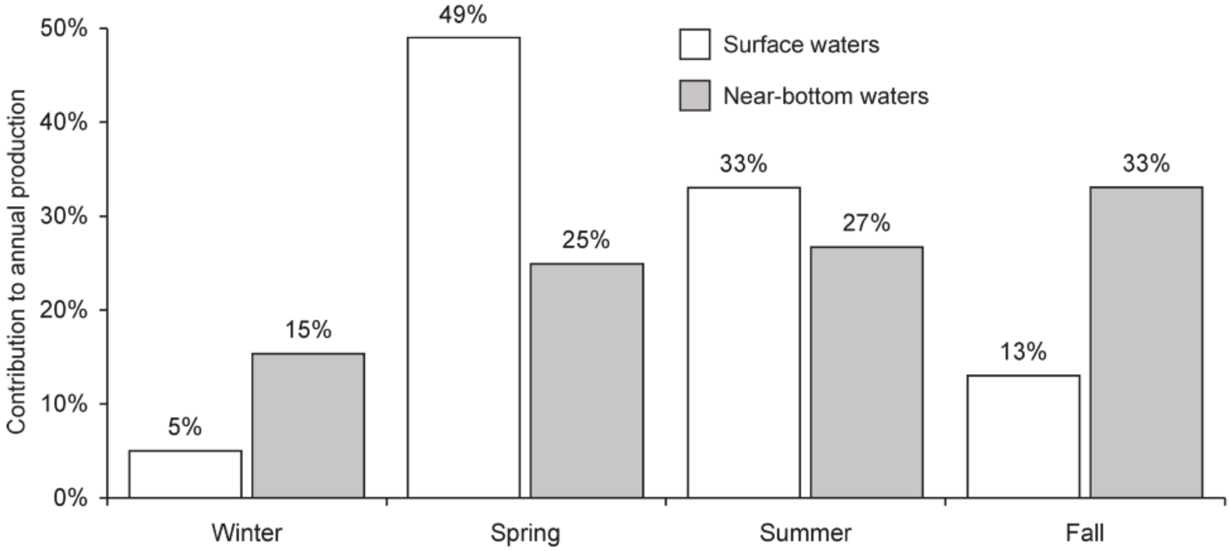

Figure 4

Distribution of annual ciliate production among the seasons of the year. Calculations were performed separately for surface and near-bottom waters of four lakes and at two stations located in the coastal zone of the southern Baltic Sea. All sampling sites were located in the temperate zone.

(Weisse & Stadler 2006; Montagnes et al. 2008) or the changing composition of food resources (Wiackowski et al. 2001; Franzé & Lavrentyev 2014). At any point in time, only a fraction of the ciliate community grows at the maximum growth rates, whereas the remaining ciliates grow slower because of sub-optimal conditions. A review of the literature indicates that the fraction of ciliates growing at the maximum rates can vary greatly from a minority (37% on average, Franzé & Lavrentyev 2014) to the majority of the ciliate community (e.g. Nielsen & Kiørboe 1994). Due to the methodological problems, estimation of the actively growing fraction of ciliates is very difficult and most probably underestimated. Conservative assumptions that one third of ciliates grows at the maximum rates, one third grows at half of the maximum rates, and one third does not grow at all indicates that the annual P/B ratio of ciliates reaches only 50% of the potential value estimated in this study. This is the lowest limit of the P/B ratio expected under environmental conditions. It would be useful to develop methods to assess the nutritional status of individual ciliate cells, which would enable the estimation of the fraction of the ciliate community that grows at the maximum rates, which in turn could be approximated by allometric equations. This would allow estimation of the importance of both the first constraint – possible food limitation, and the second one – the permanent reconstruction of ciliate communities.

The third constraint is that ciliate growth rates estimated by allometric equations depend on cell volumes, which can either shrink or swell after fixation with acid Lugol's solution. Changes in cell volume vary with concentrations of fixative agents, the species in question, and even the nutritional state of the cells (Choi & Stoecker 1989; Ohman & Snyder 1991; Montagnes et al. 1994; Wiackowski et al. 1994b). The correction was not applied in this study, because it would have introduced an additional error (Levinsen et al. 1999; Menden-Deuer & Lessard 2000; Sonntag et al. 2006). However, to check the importance of such corrections for growth rates estimated by allometric equations, P/B ratios were calculated simultaneously with cell volumes that were typically corrected. A review of the literature indicated that Lugol's solution typically causes shrinkage of ciliate cells from the initial live volumes to 64-74% (Choi & Stoecker 1989; Müller & Geller 1993), 63-89% (Wiackowski et al. 1994b) or 55-80% (Jerome et al. 1993). Consequently, similarly to Müller & Weisse (1994), Macek et al. (1996), and Carrias et al. (2001), we decided to assume that fixation with Lugol's solution caused cell shrinkage to about 70% of the initial volume, which meant that all cell volumes were multiplied by 1.4 prior to parallel calculations of annual P/B ratios. This additional analysis provided an annual ciliate P/B ratio for surface waters that was 282 yr^{-1}, which was lower by 9% than the P/B ratio calculated without corrections for shrinkage. The corrected annual P/B ratio for near-bottom waters was 71 yr^{-1}; therefore, it was also lower by 9% than the P/B ratio calculated without correction for shrinkage. In conclusion, the problem of volume correction was of minor importance.

The fourth constraint concerns the possible mixotrophy of some ciliates, because growth rates of mixotrophic ciliates correspond to about 70% of that of the heterotrophic ciliates of the same size (Pérez et al. 1997). This is true for typical temperatures observed in the temperate zone, because mixotrophs can grow faster than heterotrophs at temperatures around 0°C (Franzé & Lavrentyev 2014). Mixotrophy was observed in many oligotrichs and naked choreotrichs (Stoecker et al. 1989; Pérez et al. 1997; Stoecker et al. 2009), but separation of mixotrophs from heterotrophs was generally impossible in this study after fixation with Lugol's solution. In the temperate zone, both in freshwater and marine environments, mixotrophic ciliates contribute a variable fraction to the ciliate biomass, which can be as much as half of the ciliate biomass during some periods in spring or summer (Macek et al. 1996; Stoecker et al. 1994; Kalinowska 2004; Chróst et al. 2009; Stoecker et al. 2009). However, their annual contribution is not higher than 30% (Stoecker et al. 2009; Mironova et al. 2012). Thus, assuming that 30% of ciliates were mixotrophic and their growth rates were lower than that of the heterotrophic ciliates (70%), the actual annual P/B ratios would be 91% of the P/B values calculated with the assumption that all the ciliates were heterotrophic. This is a rough estimation, because it assumes there is a stable fraction of mixotrophs among the ciliates in all seasons; however, it indicates that this constraint is not substantial. Mixotrophic ciliates are insignificant in near-bottom waters due to the lack of irradiance.

Application in models

Among the constraints discussed above, the first two, i.e. (i) possible food limitation and (ii) permanent reconstruction of the ciliate community, are interrelated and of major importance. When they are taken into account, the annual ciliate P/B ratio in models describing the surface waters should range from 154 to 308 yr^{-1} (50-100% of estimated P/B ratio). Lower P/B ratios are expected in less nutrient-enriched waters (oligotrophic and mesotrophic lakes, offshore marine waters) and higher ones in hypertrophic and eutrophic lakes, estuaries, and in coastal marine waters. The proposed range of the P/B ratio corresponds well with that presented in the study by Weitere et al. (2005), in which the assumption of food limitation led to an estimated ciliate P/B ratio in the River Rhine of 140 yr^{-1}, whereas calculations with the allometric equation by Müller & Geller (1993) indicated a value of 238 yr^{-1} (Table 2). P/B ratios in the near-bottom waters should range from 39 to 78 yr^{-1}. The ciliate P/B ratio in the near-bottom zones of oligotrophic lakes, which are permanently oxygenated, would be higher than that estimated in this study for near-bottom waters and would resemble P/B ratios estimated for surface waters.

The last two constraints, i.e. a possible underestimation of cell volume after fixation and lower growth rates of mixotrophic ciliates, are of lesser importance. Mixotrophy is insignificant and food limitation is less likely in the near-bottom waters.

The mean annual P/B ratios calculated in this study can be applied to ecosystem modeling in typical temperate waters, that is, those with typical ciliate community size distributions and typical temperature changes. In lakes with a strong dominance of nanociliates (e.g. oligo-mesotrophic Lake Jasne, Czychewicz & Rychert 2011) or lakes included in open cooling systems of heat and power stations in which water is artificially heated (e.g. Ejsmont-Karabin & Hutorowicz 2011), the annual P/B rates calculated in this study would underestimate the actual ciliate productivity. The P/B estimates are also not applicable to acidified (e.g. Packroff 2000) or sulfurous lakes (e.g. Gasol et al. 1991) in which ciliate communities are atypical and subjected to unfavorable environmental conditions.

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Supplementary Material

Mesotrophic Lake Marszewo

Date	Surface water				Near-bottom water (20 m)			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1} d^{-1}]	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1}d^{-1}]
17-Sep-2007	14.3	4.00	6.42	6.07	7.5	0.60	0.14	0.09
23-Oct-2007	9.7	1.14	1.04	0.61	7.4	2.20	2.02	0.79
20-Nov-2007	5.2	1.71	2.35	0.45	5.3	5.07	5.30	1.21
13-Feb-2008	2.9	3.20	1.15	0.14	3.0	3.12	1.34	0.17
13-May-2008	16.6	5.44	6.65	8.18	5.2	3.12	2.04	0.50
17-Jun-2008	17.5	2.80	4.63	5.93	5.5	12.00	12.55	2.96
23-Jul-2008	19.6	3.80	0.95	2.23	5.5	1.20	0.55	▨ 0.04

▨ anaerobic ciliates – low production

Mesotrophic Lake Mały Borek

Date	Surface water				Near-bottom water (11 m)			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1} d^{-1}]	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1}d^{-1}]
7-Nov-2010	7.3	5.12	8.17	2.73	7.2	3.84	3.52	1.30
21-Feb-2011	0.3	0.83	0.59	0.00	4.4	0.66	0.32	0.07
4-Apr-2011	6.4	12.00	15.93	4.24	4.6	11.60	3.49	1.02
18-Apr-2011	9.9	9.12	17.26	9.35	4.9	0.21	0.42	0.08
9-May-2011	15.4	2.48	4.27	4.80	5.3	0.19	0.55	0.11
6-Jun-2011	22.2	3.44	2.21	5.51	5.8	0.13	0.57	0.11
5-Jul-2011	20.7	1.20	0.93	1.93	6.2	3.60	6.65	▨ 0.37
8-Aug-2011	21.8	0.72	0.56	1.21	6.5	2.32	27.59	▨ 1.18
5-Sep-2011	18.8	2.00	1.34	2.58	6.8	0.70	0.68	0.26
10-Oct-2011	14.2	2.70	1.67	2.13	7.2	1.90	1.60	0.70
7-Nov-2011	8.7	1.28	1.07	0.58	7.8	1.70	1.22	0.57
3-Dec-2011	4.6	0.60	0.83	0.15	4.7	0.30	0.46	0.08

▨ anaerobic ciliates – low production

Meso-eutrophic Lake Dobra

Date	Surface water				Near-bottom water (12 m)			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1} d^{-1}]	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1}d^{-1}]
17-Sep-2007	14.5	57.49	20.30	27.99	10.1	40.00	30.25	18.35
23-Oct-2007	9.3	7.49	4.78	3.10	9.4	12.60	17.45	8.07
20-Nov-2007	4.2	3.20	6.36	0.85	4.2	2.40	66.98	0.80
13-Feb-2008	2.2	4.08	2.33	0.16	2.9	-	-	-
13-May-2008	16.8	7.60	4.54	7.69	7.7	5.00	3.29	1.48
17-Jun-2008	17.4	37.00	13.46	24.28	8.1	4.20	2.23	▨ 0.31
23-Jul-2008	19.5	8.63	3.32	7.66	8.5	0.08	0.27	▨ 0.03

▨ anaerobic ciliates – low production

Baltic Sea in Ustka

Date	Surface water			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1} d^{-1}]
9-Apr-2006	5.0	60.35	199.48	29.77
7-May-2006	8.0	16.30	49.41	14.89
11-Jun-2006	17.0	43.60	36.24	50.69
19-Jun-2006	14.0	13.36	15.44	14.22
5-Jul-2006	10.8	6.01	6.78	4.42
2-Aug-2006	20.6	6.30	23.76	29.86
18-Aug-2006	19.0	12.05	33.26	39.26
3-Oct-2006	16.9	1.40	2.07	2.61
24-Oct-2006	5.0	18.01	6.40	1.88
24-Nov-2006	6.6	10.05	8.87	2.98
26-Dec-2006	3.0	2.88	4.47	0.42
25-Jan-2007	2.0	0.48	0.24	0.02
26-Feb-2007	4.0	4.00	5.33	0.78
13-Mar-2007	6.9	23.69	35.03	9.76
29-Mar-2007	8.7	15.91	19.45	9.62
14-Apr-2007	10.0	16.73	15.76	10.28
30-Apr-2007	9.4	15.11	30.63	12.06
20-May-2007	15.0	6.24	17.28	15.36
1-Jun-2007	15.9	30.81	57.06	62.93
8-Jun-2007	13.6	10.13	16.19	13.53
14-Jul-2007	20.6	46.45	71.87	113.98
28-Jul-2007	20.5	8.23	3.69	8.53
12-Aug-2007	19.0	5.60	2.83	5.70
28-Aug-2007	17.7	56.67	12.77	28.44
30-Sep-2007	15.0	5.40	5.31	6.63
13-Oct-2007	11.8	9.11	5.85	5.91
14-Dec-2007	5.4	3.51	12.05	2.29
18-Jan-2008	3.2	2.60	2.59	0.31
3-Mar-2008	5.2	2.57	1.00	0.31
14-Apr-2008	6.4	4.12	2.15	0.86

Baltic Sea in Sopot

Date	Surface water			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[µgC l^{-1}]	[µgC l^{-1} d^{-1}]
3-Apr-2003	3.9	9.00	22.79	2.42
8-May-2003	8.2	24.60	91.35	28.21
29-May-2003	13.7	149.60	78.53	81.38
26-Jun-2003	14.5	28.20	16.58	20.43
12-Aug-2003	21.3	14.30	5.69	14.17
8-Sep-2003	17.3	9.48	19.31	22.38
9-Oct-2003	13.2	13.44	12.06	11.35
14-Nov-2003	7.3	3.08	4.03	1.48
10-Dec-2003	5.5	3.66	1.63	0.55
12-Jan-2004	1.6	2.55	3.73	0.13
10-Feb-2004	2.6	2.94	3.74	0.29
11-Mar-2004	1.4	11.00	16.61	0.46

Eutrophic Lake Kociołek

Date	Surface water				Near-bottom water (6 m)			
	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production	Temp.	Ciliate abundance	Ciliate biomass	Ciliate production
	[°C]	[cells ml^{-1}]	[μgC l^{-1}]	[μgC l^{-1} d^{-1}]	[°C]	[cells ml^{-1}]	[μgC l^{-1}]	[μgC l^{-1}d^{-1}]
16-May-2009	17.0	24.80	4.45	11.07	9.0	5.80	2.70	1.96
30-May-2009	18.0	139.20	99.88	184.64	13.0	103.20	59.67	69.20
14-Jun-2009	18.0	5.87	0.96	2.72	11.0	9.31	7.16	5.44
27-Jun-2009	22.0	11.00	2.25	7.52	13.0	3.60	4.85	0.90
12-Aug-2009	24.0	8.20	1.58	6.52	14.0	30.60	22.60	6.61
13-Sep-2009	17.0	38.40	8.30	18.94	12.0	4.40	6.61	4.15
11-Nov-2009	10.0	55.20	82.49	26.87	9.0	72.00	105.22	28.31
23-Feb-2010	3.0	24.00	10.15	1.40	4.0	5.40	3.21	0.60
18-Mar-2010	5.0	11.20	3.64	1.20	8.0	16.20	20.46	1.96
30-Apr-2010	19.0	31.80	4.26	13.65	15.0	26.00	3.98	8.40
4-Jun-2010	18.0	0.53	0.07	0.20	12.0	0.90	0.39	0.38

anaerobic ciliates – low production