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ORIGINAL RESEARCH ARTICLE

Size-selective microzooplankton grazing on the phytoplankton in the Curonian Lagoon (SE Baltic Sea)

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Summary Dilution experiments were performed to estimate phytoplankton growth and microzooplankton grazing rates at two sites: freshwater (Nida) and brackish water (Smiltyne) in the Curonian Lagoon (SE Baltic Sea). Using the size-fractionation approach and dilution experiments, we found that the microzooplankton community was able to remove up to 78% of nanophytoplankton (2—20 μm) standing stock and 130% of the total daily primary production in the brackish waters of the lagoon, and up to 83% of standing stock and 76% of the primary production of picophytoplankton (0.2—2 μm) in the freshwater part. The observed differences were attributed to the changes in ciliate community size and trophic structure, with larger nano-filterers (30—60 μm) dominating the brackish water assemblages and pico-nano filterers (<20 μm and 20—30 μm) prevailing in the freshwater part of the lagoon.

1. Introduction

Microzooplankton (size category 20—200 μm) grazers, usually dominated by protists, are considered to be one of the most important phytoplankton mortality factors in aquatic systems. They can remove up to 60—75% (about 2/3) of daily primary production (PP), with the remaining 1/3 being chanelled directly through mesozooplankton or lost by viral lysis, settling and advection processes (Calbet, 2008; Landry and Calbet, 2004; Schmoker et al., 2013). Due to the high

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metabolic rate and short generation time, microzooplankton may play a pivotal role in determining the overall rates of grazing, nutrient regeneration and secondary production, especially during periods when they are most abundant (Weisse, 1990).

Ciliates tend to dominate microzooplankton communities in estuaries and reach very high abundances (up to 72 800 cells L^{-1}) (Gallegos, 1989; Quinlan et al., 2009). Even though their preferred prey falls within the 5–25 μm size-class, ciliates can feed even on the smallest phytoplankton, i.e. picofraction (<2 μm) (Hansen et al., 1994). Thus ciliates may be an important link in the transfer of carbon from picophytoplankton to higher trophic levels (Quinlan et al., 2009), especially during the summer when copepod production is at its highest (Dzierzbicka-Głowacka et al., 2015). In addition, a number of nano-sized (2–20 μm) ciliates are widening the effect of microzooplankton towards smaller prey size.

Size-selective grazing by ciliates has important implications for the food-web structure and nutrient cycling, especially in coastal regions, where efficient grazing on small-sized phytoplankton, called Fast-Growing-Low-Biomass, is observed (Sun et al., 2007). Moreover, different size groups of the phytoplankton community also have specific responses to grazing by ciliates.

Using the dilution technique (Landry and Hassett, 1982), the estimated grazing impact on phytoplankton is frequently masked by the abundant large phytoplankton fraction, not suitable for grazers, which is frequently dominant in coastal eutrophic waters (Gallegos et al., 1996). Therefore, the size-fractioning is suggested in coastal and estuarine areas, where the less abundant small phytoplankton fraction can have high turnover rates and contribute significantly to the secondary production of microzooplankton (Gallegos et al., 1996).

The information available on the trophic role of ciliates as grazers in the transitory ecosystems with changing riverine discharges and salinity regimes is limited. The dilution method for microzooplankton grazing estimation has been used only in a few Baltic Sea studies (Aberle et al., 2007; Lignell et al., 2003; Moigis and Gocke, 2003; Reckermann, 1996). Setälä and Kivi (2003) used field data combined with experimentally derived species-specific clearance rate information to assess ciliate community grazing in the open Baltic Sea. Reckermann (1996) estimated that microzooplankton carbon consumption rates exceeded mesozooplankton grazing in the Gotland Sea by 10 times, and in the estuarine Pomeranian Bay by 25–30 times.

The Curonian Lagoon is one of the most heavily eutrophicated coastal areas of the Baltic Sea (Gasiūnaitė et al., 2008). This transitory ecosystem is characterised by high primary production and the domination of toxic cyanobacteria during summer/autumn (Gasiūnaitė et al., 2005; Krevš et al., 2007; Sulčius et al., 2015). In the estuarine part the overall phytoplankton biomass markedly decreases with increasing salinity (Gasiūnaitė et al., 2008). An important feature of this system is the heterogeneity of the pelagic communities induced by the non-stable salinity gradient. The microzooplankton community in the lagoon is dominated by the ciliates, while heterotrophic dinoflagellates comprise only a minor fraction (<1%) of the total dinoflagellate abundance (Olenina I., personal communication). The detailed ciliate taxonomical composition of the Curonian Lagoon was described by Mažeikaitė (1978, 2003) and revised to include the brackish water ciliate assemblage by Grinienė et al. (2011). Recent observations show significant differences in the community structure of ciliated protozoan between the brackish water and freshwater parts of the lagoon (Grinienė, 2013). In this study it was demonstrated that very small nano-ciliates (<20 μm) compose more than 60% of total freshwater ciliate assemblage, while in the brackish water community the share of nano-ciliates is only 15% of the total abundance. The larger size fraction (20–60 μm) dominates the brackish water ciliate assemblage (Grinienė, 2013).

In this study we applied dilution experiments and phytoplankton size-fractionation to experimentally evaluate the differences in microzooplankton and phytoplankton community structures, grazing and growth rates between the freshwater and brackish water parts of the lagoon. The experiments were made with two communities representing the two extremes of the habitat: a high salinity sample from an area (Smiltyne) with extreme salinity variability, and a freshwater sample from an area (Nida) with constant low salinity regime. Our hypothesis is that the grazing efficiency varies according to the grazer community structure (size and grazing mode).

2. Material and methods

2.1. Study area

The Curonian Lagoon (SE Baltic Sea) is a shallow (mean depth 3.8 m) eutrophic water basin connected to the Baltic Sea by the narrow Klaipeda strait. The southern and central parts of the lagoon contain fresh water due to discharge from the Nemunas River. The salinity in the northern part varies from 0 to 7 due to seawater intrusions, which are usually restricted to the northern part of the lagoon, rarely propagating more than 40 km (Dailidienė and Davulienė, 2008). Seawater inflows with a residence time of 1–6 days are most common (Gasiūnaitė, 2000). In terms of hydraulic regime-based zonation, the northern part of the lagoon and Nemunas River avandelta are classified as transitory, while the central part is classified as stagnant and intermediate (Ferrarin et al., 2008).

According to the intensive weekly study in 2007–2008 the seasonal dynamics in the fresh water site (Nida) ciliates follows the model of temperate eutrophic lakes with four seasonal phases: winter, early spring, late spring and summer/autumn (Grinienė, 2013). Summarising, during the winter time ciliate growth is limited by low biomass of phytoplankton. In the early spring, when small sized phytoplankton prevails, ciliate assemblage is dominated by small naked oligotrichs and prostomatids. After the late spring diatom bloom, ciliate assemblage shifts to medium sized nano-filterers (tintinnids). The functional and taxonomic diversity of ciliates increases toward the summer, which points to the exploitation of a wide size range of food. Small sized naked oligotrichs (pico-nano fraction feeders) and peritrichs (mainly pico-fraction feeders) were most abundant in summer and autumn. Despite this ciliate community structure is homogenous during whole period (June–October) forming the same summer/autumn cluster (Grinienė, 2013).

The structural differences between the seasonal clusters were significant and shown by ANOSIM global *R* statistics

Table 1 Analysis of similarity (ANOSIM) of the four seasonal assemblages in Nida site (from Grinienė, 2013).

Assemblages	*R* statistics	Significance level, *p*
Winter, early spring	0.96	<0.01
Early spring, late spring	0.91	<0.01
Late spring, summer/autumn	0.74	<0.01
Summer/autumn, winter	0.88	<0.01

approaching 1 with the highest differences were observed between spring and winter assemblages, and also between the two spring assemblages (Table 1).

In the brackish water site (Smiltyne) significant negative salinity effect on the ciliate community is observed (Grinienė, 2013). Summarising, the total abundance of ciliates correlated negatively with salinity in this site ($r = -0.42$, $N = 34$, $p < 0.05$). Two ciliate assemblages, according to salinity intervals 0–2 PSU and ≥4 PSU, could be distinguished. The global *R* statistics from ANOSIM of these assemblages demonstrated that the overall differences between them were statistically significant (global $R = 0.939$, $p < 0.01$). Structurally, these assemblages were very different in species composition, size and feeding mode.

2.2. Dilution experiment setup and sample analysis

Water samples for the experiments were collected from two sites: freshwater (Nida) on 29 August and brackish water (Smiltyne) on 10 October 2009. Water was collected from a depth of 0.5 m into two 50 L carboys and transported to the laboratory.

The particle-free water (FW) was prepared by filtering lagoon water sequentially through a plankton mesh with a pore size of 20 μm, intermediate 2-μm and 0.7-μm GF/F filters and finally a 0.2-μm Millipore filter under slight air pressure. The length of the filtration process depends on the concentration of phytoplankton and suspended solids, and it took 20 and 5 h at the Nida and Smiltyne sites, respectively. The whole lagoon water (WW) was collected the next day in the Nida and Smiltyne experiments and was gently poured through a 150-μm mesh to remove mesozooplankton. Visual observation before experiments was conducted to assure that the 150-μm-sized mesh removed mesozooplankton and that the filtration through the mesh did not have a negative effect on the vitality of ciliates, especially aloricated forms. The WW was diluted by FW to four target dilutions in ratios of 1:0 (no dilution), 3:1, 1:1 and 1:3 (dilution factor or decimal fraction of WW: 1, 0.75, 0.5 and 0.25, respectively) in 3 L transparent plastic bottles. The incubation volume was 3 L and all treatments were carried out in triplicate. All bottles were incubated *in situ* at a depth of 0.5 m for 24 h. During the experiment on 10 October 2009, altogether 6 bottles out of 12 were lost during the night-time storm.

At the start and at the end of both experiments, 500 ml from each experimental bottle were sampled for nutrient (nitrate, nitrite, ammonium, phosphate and silicate) analysis, 25–30 ml for nano- and picofractions of chlorophyll *a* and 300 ml for microzooplankton counts.

The sample for nanophytoplankton (2–20 μm) chlorophyll *a* was filtered through a 20-μm mesh and concentrated onto a 2-μm Millipore polycarbonate filter. The remaining filtrate was concentrated on a 0.2-μm Millipore polycarbonate filter for picophytoplankton (0.2–2 μm) chlorophyll *a* measurement. All filters were kept frozen at −20°C and analysed within 2 months.

Total chlorophyll *a* concentration in the initial water samples was determined fluorimetrically (FluorProbe II). Pigments of nano- and picofractions were measured by high-performance liquid chromatography (HPLC) at the Baltic Sea Research Institute, Warnemünde, Germany. The samples were analysed according to Barlow et al. (1997). Pigments were detected by absorbance at 440 nm using a Biotek (545 V) diode array detector and identified by retention time and online visible spectra (350–750 nm) obtained from scans by the diode array detector. Chlorophylls were further detected by a Jasco (FP-1520) fluorescence detector (440 and 660 nm excitation and detection wavelengths, respectively). The chromatograms are processed using the Biotek Kroma 3000 software. Pigment concentrations were calculated by the peak area.

Nutrients were analysed at the Baltic Sea Research Institute (Warnemünde, Germany) according to standard methods (Grasshoff et al., 1983).

Ciliate counts were performed in Lugol fixed samples by Utermöhl's (1958) method. Volumes of 10–25 ml were settled for at least 24 h in Utermöhl's chambers. Ciliates were counted, measured and identified with an inverted microscope at 200× magnification. The entire content of each Utermöhl chamber was surveyed, and an additional subsample was counted if the total number was <150 organisms.

Rotifers and copepod nauplii were counted using a microscope at 40× magnification in the Bogorov chamber.

Ciliate-size groups (<20 μm, 20–30 μm, 30–60 μm and >60 μm) and trophic groups (pico-filterers, nano-filterers, pico/nano-filterers, omnivorous feeding on heterotrophic flagellates, algae or ciliates, and predators feeding on other ciliates) were distinguished according to Mironova et al. (2012). *Mesodinium rubrum* (*Myrionecta rubra*) was observed in the Smiltyne site experiment but not included in the total ciliate abundance counts, because it appears to function mostly as an autotroph.

2.3. Data analysis

Dilution experiment data analysis was performed according to Landry and Hassett (1982). The apparent growth rate of prey (*AGR*) was estimated using the function (1):

$$AGR\ (\mathrm{day}^{-1}) = \frac{\ln(Chla_t/Chla_0)}{t}, \quad (1)$$

where $Chla_t$ and $Chla_0$ are the final and initial concentration of chlorophyll *a* [μg L^{-1}] and *t* is the time of incubation [day]. *AGR* was estimated for both pico- and nanosize fractions.

The rates of growth and grazing mortality were calculated by the linear regression of *AGR* versus actual dilution factor. The absolute value of the slope of the regression is the grazing rate by microzooplankton *g* [day^{-1}] and ordinal intercept (y-intercept) of the regression is the growth rate of phytoplankton in the absence of grazing *k* [day^{-1}].

Significant negative slope (one-tailed t-test, $p < 0.05$) suggests a measurable grazer effect on phytoplankton growth. In cases of statistically non-significant regression, grazing rates were not determined and the phytoplankton growth rates were obtained from averaged *AGR* among all dilution treatments (Twiss and Smith, 2012).

The standing stock of phytoplankton biomass as chlorophyll *a* [μg L^{-1}] removed daily P_i [% day^{-1}] and phytoplankton potential production grazed daily P_p [% day^{-1}] were calculated using equations (2) and (3) presented in James and Hall (1998):

$$P_i = 1 - e^{-g}, \quad (2)$$

$$P_p = \frac{(e^k - e^{k-g})}{(e^k - 1)}, \quad (3)$$

where k is the growth rate of phytoplankton and g is the grazing rate of microzooplankton estimated from the linear regression.

To determine clearance rates (y) for total ciliate community and different trophic groups, a biovolume-dependent equation (4) established for the Baltic Sea (Setälä and Kivi, 2003) was applied:

$$y = 0.1493 \times x^{0.906}, \quad (4)$$

where x is the estimated spherical diameter (ESD) of the ciliate.

3. Results

3.1. Environmental parameters and nutrient concentrations

The different environmental parameters, nutrient concentration and microzooplankton abundances are given in Table 2. At the fresh water site (Nida) salinity was 0, whereas at the brackish water site (Smiltyne) it was 6.2. The difference in water temperature between sites was 7.6°C. Due to the high initial concentrations of both inorganic nitrogen (nitrate + nitrite + ammonium) and phosphorus (phosphate) as well as silicate no nutrient limitation happened during the incubations (Table 2). The lowest end values were above 1 μmol L^{-1} and 0.5 μmol L^{-1} for inorganic nitrogen and phosphorus, respectively (Table 2).

3.2. Phytoplankton community structure

We used high-performance liquid chromatography (HPLC) estimations of phytoplankton pigment signatures to determine the community structure of phytoplankton fractions. The total chorophyll *a* concentration was 6 times higher in freshwater (30.3 μg L^{-1}) than in brackish water (4.7 μg L^{-1}) (Table 2). The relative abundance of different phytoplankton size groups within the community, represented by the chlorophyll *a* concentrations, differed between freshwater and brackish water areas. In the freshwater site the share of >20 μm, nano- (2–20 μm) and picofraction (0.2–2 μm) was 47.7%, 46.4% and 5.9% of total chlorophyll *a* concentration, respectively. The nanofraction of chlorophyll *a* dominated the brackish water site with 59.8% of total chlorophyll *a*, while the share of >20 μm fraction was 38.2% and that of picofraction only 1.9% of the total.

Table 2 Environmental parameters and microzooplankton abundance at initial whole lagoon water (WW) at two research sites.

Parameters	Freshwater site	Brackish water site
Temperature [°C]	18.6	11
Salinity	0	6.2
Dissolved oxygen [mgO_2 L^{-1}]	16.6	10.1
Nitrate [μmol L^{-1}]	0.09	7.02
Nitrite [μmol L^{-1}]	0.03	0.31
Silicate [μmol L^{-1}]	1.95	11.81
Ammonium [μmol L^{-1}]	3.37	5.15
Phosphate [μmol L^{-1}]	1.88	0.98
Total chlorophyll *a* [μg L^{-1}]	30.3	4.7
Pico-fraction chlorophyll *a* [μg L^{-1}]	1.8	0.09
Nano-fraction chlorophyll *a* [μg L^{-1}]	14.1	2.8
Microzooplankton abundance[a]:		
Ciliates [ind. L^{-1}]	30 667	9800
Copepod nauplii [ind. L^{-1}]	115	24
Rotifers [ind. L^{-1}]	75	–

[a] Not including heterotrophic dinoflagellates and silicoflagellates.

The pigment composition gives an indication of the systematic composition of phytoplankton, but it cannot be considered quantitative. At both sites the picofraction of phytoplankton was represented only by chlorophyll *a*, whereas the nanofraction of phytoplankton contained additional pigments and varied between sites (Fig. 1). Lutein (green algae), alloxanthin (cryptophytes), β-carotene (for all phytoplankton taxonomic groups) and divinyl chlorophyll *a* (cyanobacteria) were found in the nanofraction at the freshwater (Nida) site, while at the brackish water (Smiltyne) site 19′-hexanoyloxyfucoxanthin (prymnesiophytes) and zeaxanthin (cyanobacteria) were recorded. In addition to chlorophyll *a*, fucoxanthin (diatoms) was detected in the nanofraction at both sites. Phytoplankton *AGR* calculations were performed using only chlorophyll *a* (as indicator of the whole phytoplankton community) data. Other pigments were detected only in undiluted water (dilution factor 1) or weakly diluted treatment (dilution factor 0.75), and they could not be used in *AGR* calculations. However, the pigment results indicate that the autotrophic communities remained stable during the experiments.

3.3. Microzooplankton community structure

At both experimental sites microzooplankton was dominated by ciliates (99% of total abundance), while the number of metazoans was very low, composing 1% of the total microzooplankton abundance at both experimental sites (Table 2). In the brackish water site nano-filterers feeding on nanosized phytoplankton were dominated by medium-sized (30–60 μm) tintinnid *Tintinnopsis* sp., large naked oligotrich *Strombidium*

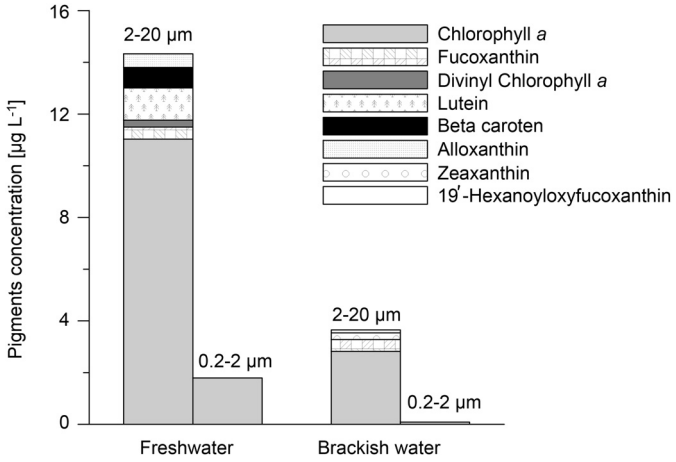

Figure 1 Pigments concentrations of pico- and nanophytoplankton at experimental sites.

conicum, *Strombilidium gyrans* and *Lohmaniella* sp. and large (>60 μm) ciliates (*Codonella relicta*, *Tintinnopsis kofoidi*): they shared 48% of the total ciliate abundance (Fig. 2). Small ciliates (<20 and 20—30 μm) were composed of *Mesodinium* cf. *acarus*, *Strobilidium* spp., *Urotricha* sp. and *Lohmaniella oviformis*.

In the freshwater site small-sized (<20 and 20—30 μm) pico/nano-filterers (*Strobilidium* spp., *Tintinnopsis* cf. *nana*, *Halteria* sp.) and pico-filterers (*Cyclidium* spp., *Vorticella* spp.) prevailed. Together these functional groups composed 77% of the total abundance (Fig. 2). Medium-sized ciliates (30—60 μm) were represented mainly by tintinnids *Tintinnidium pusillum*, *Tintinnopsis tubulosa* and *Codonella cratera*, and they composed 23% of the total ciliate abundance. Predators represented by *Didinium nasutum*, found only in the brackish water site, shared 4% of the total ciliate abundance. These could affect experiments by selectively preying on other ciliates, but at this low number the effect is considered to be minor.

3.4. Growth and grazing rates of phytoplankton

In the freshwater site the grazing rate (g = 1.8 day^{-1}) on the picofraction of the phytoplankton community exceeded the

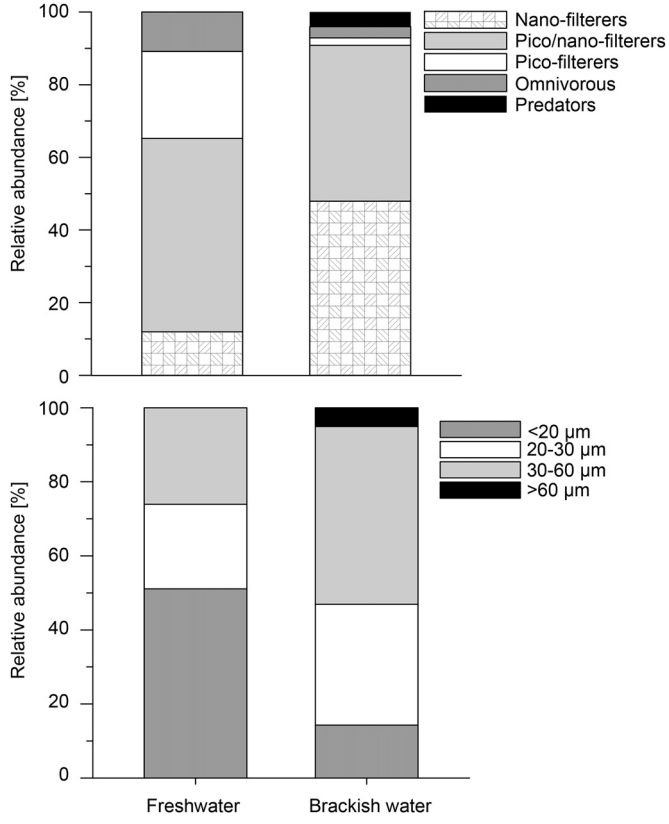

Figure 2 Relative abundance of ciliate functional and size classes at experimental sites.

Table 3 Growth rates of the phytoplankton pico- and nanofractions $k \pm$ SE [day^{-1}] and microzooplankton grazing rates $g \pm$ SE [day^{-1}] based on chlorophyll *a*. R^2, coefficient of determination; *N*, number of observations. The significance level of regression (i.e. slope, *g*, was significantly differed from zero, $p < 0.05$) is indicated by *p*-value; n.s., non significant.

Site	Fraction [μm]	*k*	*g*	R^2	*p*-value	*N*
Freshwater	0.2–2	1.33 ± 0.36	-1.83 ± 0.53	0.55	<0.01	12
	2–20	0.19 ± 0.19	-0.35 ± 0.29	0.15	n.s.	10
Brackish water	0.2–2	-1.09 ± 0.60	2.19 ± 0.90	0.59	n.s.	6
	2–20	0.92 ± 0.28	-1.52 ± 0.42	0.77	<0.05	6

prey growth rate ($k = 1.3$ day^{-1}) (Table 3), indicating high microzooplankton pressure on this size class. The microzooplankton grazing pressure on picoalgae expressed by the percentage of grazed biomass as standing stock (P_i) and percentage of grazed potential production (P_p) was 83% and 76%, respectively.

In the freshwater site the grazing rate of nanophytoplankton was not estimated, because no significant linear relationship was observed between the apparent growth rate (*AGR*) of this fraction and the dilution factor, i.e. the slope (microzooplankton grazing rate, *g*) did not differ significantly from zero (Fig. 3; Table 3). However, the growth rate of the nanofraction of phytoplankton can be calculated as the average of apparent growth rates among all dilution treatments (average ± SE) and replicates ($N = 10$), and it was near zero (-0.02 ± 0.08 day^{-1}).

The *AGR* of the picofraction increased linearly with the dilution factor at the brackish water site and regression analysis resulted in a positive slope, which did not differ statistically from zero (Fig. 3; Table 3); therefore the microzooplankton grazing rate (*g*) is not interpretable. However, the growth rate (0.28 ± 0.3 day^{-1}) was only less than ¼ of the growth rate calculated in the freshwater site, indicating significant differences in the activity of the picosize fraction.

The growth rate of nanoalgae at the brackish water site was 0.9 day^{-1} and largely exceeded the nanophytoplankton growth rate in the freshwater site. The grazing rate (1.5 day^{-1}) was higher than the growth of the prey community, however, while the actual values were lower (Table 3; Fig. 3). In the brackish water site microzooplankton grazed on 78% of the nanophytoplankton standing stock per day and 130% of potential daily production.

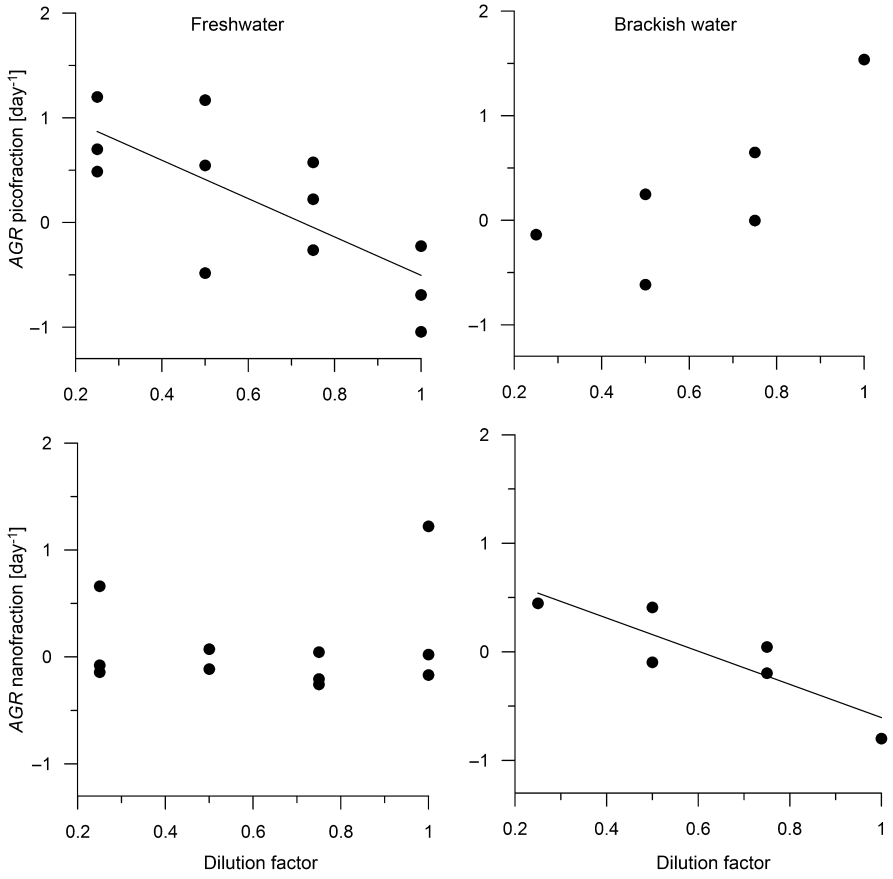

Figure 3 Relationship between dilution factor and apparent growth rate (*AGR*) of chlorophyll *a* of pico- and nanofractions at both sites. Only significant slopes are presented in the graph.

4. Discussion

Dilution experiments have been performed over the past three decades to examine the grazing impact of microzooplankton, ranging from the open sea to coastal zones and estuaries (data reviewed by Landry and Calbet, 2004 and Schmoker et al., 2013). This relatively simple and standard technique is useful for comparative microzooplankton grazing rate studies among the geographic regions, as well as revealing the role of microzooplankton in time series of ecological processes (Gallegos, 1989).

However, for the estimation of microzooplankton grazing on phytoplankton in the Baltic Sea the dilution technique has been applied to a lesser extent (Aberle et al., 2007; Lignell et al., 2003; Moigis and Gocke, 2003; Reckermann, 1996). Moigis and Gocke (2003) used the dilution method as an alternative to the ^{14}C and O_2 methods for primary production estimation, but they did not take into account the grazers' community. The grazing rate by microzooplankton varied from 0.21 to 0.41 day^{-1} in Kiel Fjord.

Reckermann (1996) found high microzooplankton grazing rates on ultraphytoplankton (<5 μm) both in the Gotland Sea and the Pomeranian Bay. In the Gotland Sea in 1994, the microzooplankton (<200 μm) grazing pressure on *Synechococcus* was higher than on eukaryotic pico- and nanophytoplankton. Generally, microzooplankton grazing on *Synechococcus* was over 100% of gross production grazed per day and pico- and nanoeukaryotic production was not completely grazed. In the Pomeranian Bay, microzooplankton grazing on ultraphytoplankton varied from considerably exceeding daily growth to rather low values (176–51%). In the study by Lignell et al. (2003) the microzooplankton grazing rate on the whole phytoplankton community varied between 0.05 and 0.30 day^{-1}. However, in both studies the total phytoplankton community rather than different size classes was measured, which may mask the effect of the size-selective microprotozoa grazing or even genus/species level as is evidenced by Aberle et al. (2007) in their mesoscosm study.

The significant estimates of ciliate grazing rates on phytoplankton pico- and nanofractions were obtained at freshwater (Nida) and brackish water (Smiltyne) sites, respectively. Grazing rates exceeded the growth rate of phytoplankton fractions ($g > k$), suggesting that phytoplankton production and biomass accumulation is controlled by microzooplankton, as it was frequently observed by other authors (Burkill et al., 1987; Landry et al., 1995; Lehrter et al., 1999; McManus and Ederington-Cantrell, 1992; Verity et al., 1993).

The grazing rate of the picofraction at the freshwater site is in the range reported in the other regions (Table 4). Ciliates consumed 76% of potential picophytoplankton production at this freshwater site. The dominance of small pico- and pico/nano-filterers in the freshwater site suggests that predation on the picophytoplankton fraction can be high, but it could be tested visually by observing autotrophic picofraction cells via epifluorescence microscopy or flow cytometry. The calculated clearance rate as the daily clearance percentages (% of the water volume cleared in 24 h) by pico/nano-filterers in this site was very similar (70%) to potential picophytoplankton production removed by ciliates calculated from the dilution experiment. This finding is in good agreement with the study by Rassoulzadegan et al. (1988) as they found that small ciliates (<30 μm) consist of 72% picoplankton and 28% nanoplankton.

In contrast, the dilution experiment provided no statistically significant estimates of grazing rate (g) for phytoplankton nanofraction at the freshwater site. The *AGR* of the nanofraction was very similar in all dilution treatments (Fig. 3), which indicates the absence of microzooplakton grazing. This is supported by the low number of nano-filterers in the initial water at the beginning of the experiment (Fig. 2). The low average value of *AGR* (-0.02 ± 0.08) indirectly points at a slowly growing nanophytoplankton community, which can be

Table 4 Published results of microzooplankton grazing in other regions. Growth rates of the phytoplankton pico- and nanofractions k [day^{-1}] and microzooplankton grazing rates g [day^{-1}], P_p, potential consumption of primary production [% day^{-1}]; N, number of dilution experiments.

Location	Salinity	Fraction [μm]	k	g	P_p	N	Reference
Curonian Lagoon	0	0.2–2	1.33	1.83	76	1	This study
	6.2	2–20	0.92	1.52	130	1	
Chesapeake Bay	20	0.2–2	2.10	1.92	97	1	Sun et al. (2007)
		2–20	0.61	0.41	73	1	
Delaware Inland Bay	15	0.2–2	2.05	0.7	58	1	
		2–20	0.81	0.77	97	1	
Delaware Bay	16	0.2–2	1.83	1.78	99	1	
		2–20	0.84	0.32	48	1	
Gulf of Alaska	–	<5	0.42	0.48 (0.02–1.07)	102 (±29)	39	Strom et al. (2007)
		5–20	0.34	0.39 (0.05–0.92)	102 (±32)		
Manukau estuary (New Zealand)	28–33	<5	0.2–1.8	0.3–1.3	30–230	12	Gallegos et al. (1996)
		5–22	0.2–1.8	0–0.8	0–98		
Upper St. Lawrence River (US)	–	0.2–2	0.2–1.8	0–1.1	–	12–38	Twiss and Smith (2012)
		2–20	0.1–1.3	0–1.2	–		

a result of viral lysis, the presence of toxic material or other unknown inhibitory metabolites that could be released during preparation of the filtered water (Stoecker et al., 2015).

The grazing rate of the nanofraction at the brackish water (Smiltyne) site exceeded grazing rates in other estuarine ecosystems by two- or threefold (Table 4). Ciliates consumed 130% of the nanophytoplankton production at the brackish water site. The calculated total ciliate community clearance rate as daily percentage was lower — 71%, but 41% was due to nano-filterers. This is not surprising as nanophytoplankton chlorophyll *a* concentration was 30 times (Table 2) higher than picophytoplankton chlorophyll *a*, and ciliate assemblage was dominated by medium-sized ciliates (Fig. 2) composed of naked oligotrichs *Strombilidium gyrans*, *Strombidium conicum* and tintinnid taxa *Tintinnopsis* sp.; all of them prefer to feed on small nano-sized algae (Appendix A, Table A1). Gallegos et al. (1996) used the dilution technique combined with size fractioning and found that the highest grazing rates of the phytoplankton fraction of 5—22 μm coincided with tintinnid abundance increase in ciliate assemblage. The tendency of higher consumption rates is usually reported in dilution experiments where nutrients are not added (Landry and Hassett, 1982). The adding of nutrients is recommended at the start of the experiment to keep the phytoplankton growth unlimited (Gallegos, 1989; Landry et al., 1995). In this study nutrients were not added, assuming high rates of N and P loading in the Curonian Lagoon during autumn, when experiments were conducted and to avoid increased mortality of delicate protists during experiments (Gifford, 1988; Landry and Hassett, 1982).

In the Smiltyne site, the *AGR* of the picofraction increased linearly with the dilution factor (theoretically impossible case); with the highest *AGR* values at nondiluted treatment (Fig. 3), similar results were reported previously (Gallegos, 1989; Lignell et al., 2003; Modigh and Franzè, 2009). Positive slopes are usually attributed to the complex cycling of nutrients between internal and external pools, mixotrophy or filtration contamination and trophic cascade effect (review by Calbet and Saiz, 2013). The last explanation could be the reason for the positive *AGR* of the picofraction trend along the dilution factor in our data, suggesting that nano-filterers, which dominated in the brackish water site (Fig. 2), intensively grazed not only on the autotrophic nanofraction of phytoplankton, but also on heterotrophic flagellates, which belong to the same size spectra (2—20 μm) and are one of the main pico-fraction feeders, thereby releasing the phytoplankton picofraction from predator control. Unfortunately, the number of heterotrophic nanoflagellates (HNF) was not estimated in this study, making the ciliate protozoa grazing estimates overestimations to some degree. HNF counts should also be included in the dilution experiments in the Baltic Sea. A similar food web effect was suggested to affect the dilution experiments in mesocosms (Lignell et al., 2003), but it was not found in the experiments conducted in the Baltic Sea by Reckermann (1996).

5. Conclusion

The dilution experiment approach revealed a significant ciliate grazing effect on the nano-fraction of phytoplankton in the brackish water, and on the pico-fraction in the freshwater community. This pattern is related to the differences in ciliate community size structure: larger nano-filterers dominate in the brackish water assemblages, whereas pico/nano-filterers prevail in freshwater. Thus it is important to monitor the species composition and/or size-class division of specific ciliate communities to estimate their size-selective grazing effect. This is also important for constructing more detailed carbon flow models in the Baltic Sea ecosystem.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.oceano.2016.05.002.

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