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Planktonic Ciliates of the Neva Estuary (Baltic Sea): Community Structure and Spatial Distribution

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Abstract. Ciliate communities in open waters of the meso-eutrophic Neva Estuary (the Baltic Sea) were studied in summer 2010. Abundance and biomass of ciliates were surprisingly low (0.03–1.9 ind ml^{-1} and 0.04–2.4 × 10^{-3} µg C ml^{-1}), especially in samples with high detritus content. During this study we detected four ciliate species which are new for the Baltic Sea. Mixotrophic ciliates dominated at the majority of stations (28–67% of overall ciliate numbers). Their contribution was significantly higher in the outfall area and northern part of the Neva Estuary (Resort District), where total density of ciliates was low. Medium-sized ciliates (30–60 µm) were the most diverse and abundant (average contribution 59% of total abundance). The two parts of the estuary, separated from each other by a storm-surge barrier, differed slightly in their community structure ($p < 0.05$) but did not significantly differ in ciliate numbers and biomass values.

Key words: Ciliates, mixotrophs, plankton, Neva Estuary, Baltic Sea.

INTRODUCTION

Ciliates are important consumers of phytoplankton and bacterial production (e.g. Gismervik *et al.* 1996, Šimek *et al.* 1998). They contribute significantly to the overall plankton diversity (Mironova *et al.* 2008; Telesh *et al.* 2011a, b) and the "microbial loop," which importance strongly increases in eutrophic conditions (Andersson *et al.* 2006). Therefore, the composition,

community structure and distribution of ciliates in the water basins which are stressed by pollution and eutrophication, such as the Neva Estuary of the Baltic Sea, are of special interest (Panov *et al.* 2002, Telesh *et al.* 2008). The Neva Estuary is one of the largest environmentally stressed Baltic estuarine ecosystems (Telesh *et al.* 2008).

The present-day ciliate communities of the entire Gulf of Finland and its eastern part – the Neva Estuary are still understudied, especially if compared to some other regions of the Baltic Sea (Mironova *et al.* 2009). In the Neva Estuary, earlier detailed investigation of ciliates was carried out more than 20 years ago, presumably in the freshwater Neva Bay (Khlebovich 1987). Since then, hydrological regime and environ-

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mental characteristics of the Neva Estuary have seriously changed, largely due to construction of the storm-surge barrier, which separated the freshwater upper inner estuary (the Neva Bay) from the brackish-water lower part – the eastern Gulf of Finland (Telesh *et al.* 2008). As a result, the ratio of primary production to the rate of decomposition of organic matter increased (Golubkov *et al.* 2003), and significant growth of phytoplankton biomass, changes in community structure, and cyanobacteria blooms were observed (Nikulina 2003). However, the alteration of hydrological conditions was not the only reason for those transformations, as nutrient regime of the estuary also changed a lot due to lower DOC inflow and higher input of biologically available nutrients in the inner part of the estuary (Golubkov *et al.* 2003).

Meanwhile, it remains unclear how ciliate communities can respond to such serious changes in the ecosystem. For example, it was shown that in the Kuryongpo Bay (the eastern coast of Korea) construction of the breakwater induced eutrophication of the inner area and caused significant differences in community structure and population dynamics of ciliates in the inner and outer parts (Kim *et al.* 2007).

Recently, the diversity and seasonal dynamics of ciliates in the near shore zone of the Neva Estuary were described (Mironova *et al.* 2012). According to those results, there were significant distinctions in structure of coastal ciliate communities between upper and lower parts of the estuary. However, seasonal changes in community structure were significantly larger than the differences between stations located in separated parts of the estuary; everywhere the main alterations occurred in April and October at water temperatures 5–12°C (Mironova *et al.* 2012).

In the present study, we investigate spatial variability of offshore ciliate communities in both parts of the inner Neva Estuary in summer period. Their taxonomic, size, and trophic structure, numbers, biomass and productivity were examined, which is necessary to evaluate the role of ciliates in pelagic ecosystems. We also set up and tested a hypothesis that the studied characteristics of ciliate communities significantly differed in parts of the Neva Estuary separated by storm-surge barrier.

MATERIALS AND METHODS

Study site and sampling procedure

The inner Neva Estuary is formed by the freshwater Neva Bay (upper inner estuary) and brackish-water eastern Gulf of Finland (lower inner estuary, salinity 1–5 psu) which are separated from each other by the storm-surge barrier (Fig. 1). Like many of the Baltic estuaries, the Neva Estuary is (i) shallow (mean depth of the Neva Bay is 4 m, in the eastern Gulf of Finland – 20 m), (ii) meso-eutrophic, (iii) characterized by intensive benthic-pelagic coupling (Telesh 2004). For the detailed information on hydrology, salinity regime, sediments and structure of pelagic communities of the study area see Telesh *et al.* (2008) and references therein.

Sampling was carried out during the research cruise in the inner Neva Estuary from 28 July till 2 August, 2010. Data about water temperature, salinity and water transparency (Secchi depth) are presented in Table 1. We collected samples at 17 offshore stations located in both parts of the inner estuary (Fig. 1). Water samples were taken with a Ruttner bathometer at 3–4 depths at each station from the upper mixed layer (0–5.3 m in the Neva Bay, 0–8 m in the lower inner estuary). Samples from each depth were poured in plastic bucket and gently mixed in order to obtain a single, integral sample from each station.

Sample processing

Immediately after sampling, we preserved 100 ml subsamples from each integral water sample with glutaraldehyde (final concentration 2%) and kept at 4ºC. These subsamples were filtered (pore size 2 μm); gentle vacuum was used (< 70 mm Hg) for minimizing cell damage during filtration (Caron 1983, Sherr and Sherr 1983). Then concentrated subsamples were stained with primulin and prepared for epifluorescence microscopy (Caron 1983). We used this method for enumeration of ciliates and their species identification,
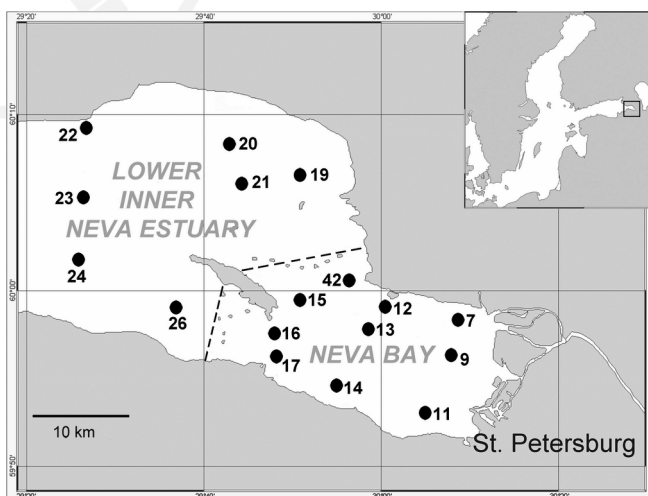
Fig. 1. Scheme of the inner Neva Estuary and location of sampling stations; modified from Telesh *et al.* (2008). Broken line indicates the storm-surge barrier.

Table 1. Characteristics of the sampling stations.

	Station	Depth (m)	Upper mixed layer depth (m)	Temperature (°C)	Salinity (psu)	Secchi depth (m)
Upper inner estuary	7	3.3	0–3.3	22.26	0.06	1.9
	9	3.4	0–3.4	21.6	0.06	1.8
	11	3.2	0–3.2	22.72	0.09	1.1
	12	2.7	0–2.7	23.77	0.06	1.4
	13	4.5	0–4.5	23.47	0.4	1.8
	14	4	0–4.0	22.78	0.1	1.1
	15	5.3	0–5.3	22.78	0.06	1.2
	16	5.2	0–5.2	24.79	0.11	1.6
	17	4.2	0–4.2	23.38	0.17	1
	42	5	0–5.0	22.54	0.16	1.6
Lower inner estuary	19	10.5	0–6.0	24.35	0.08	1.6
	20	12.3	0–8.0	24.67	0.74	2.1
	21	14	0–6.0	24.1	0.25	1.9
	22	19	0–8.0	25.18	0.44	1.7
	23	25	0–7.0	24.2	1.27	2.3
	24	21.6	0–6.0	22.98	0.93	1.6
	26	7.3	0–3.5	24.2	0.44	1.3

particularly for detecting small ciliates and mixotrophs. For more precise species identification we analyzed additional sub-samples, preserved with 2% acid Lugol's solution and formaldehyde (final concentration 2%). Species identification was carried out under a Leica DM 2500 microscope (× 600 to × 1350 magnification) and was supported by images taken with a Leica DFC 420 photo camera.

For species identification we used publications of Maeda and Carey (1985), Maeda (1986), Foissner *et al.* (1991, 1992), Foissner and Berger (1996), Agatha and Riedel-Lorjé (1997, 1998) and internet sources (Strüder-Kypke *et al.* 2003, Xu Kaiqin 2007). Information about feeding strategies and food preferences (Table 2) was taken from these sources and also from Fenchel (1968), Kivi and Setälä (1995), Stabell (1996), Montagnes (1996), Pitta and Giannakourou (2000), Kurilov (2004) and Myung *et al.* (2006). Additionally, mixotrophic ciliates were detected by epifluorescense microscopy.

Biovolume and wet weight of ciliates was calculated from measurements of their cell dimensions by comparing the organisms with geometrical figures. We did not apply any volume correction to our data, because information about volume changes of freshwater ciliates upon fixation with 2% glutaraldehyde is not available. Some studies considered the fixation effect of 2% glutaraldehyde on marine ciliates (Choi and Stoecker 1989) and the fixation effect of 1% glutaraldehyde (Ohman and Snyder 1991, Leakey *et al.* 1994). However, these results are inapplicable to our data, as effect of fixative greatly depends not only on its concentration, but also on salinity, ciliate species or strains, and prey size (e.g. Choi and Stoecker 1989, Menden-Deuer *et al.* 2001). For the calculation of carbon biomass, relationship proposed by Menden-Deuer and Lessard (2000) was used: pg C cell^{-1} = 0.216 × cell volume$^{0.939}$.

The potential maximum production of ciliates was estimated by multiplying the biomass (μg C l^{-1}) by the maximum growth rate (day^{-1}). The latter was calculated using the equation proposed by Muller and Geller (1993). In case of mixotrophic ciliates, we take into account that their maximum growth rates are 0.5 generations day^{-1} less than those for similar-sized heterotrophic ciliates (Perez *et al.* 1997). Ciliate clearance-rate calculations were based on the exponential biovolume-dependent equation (Kivi and Setälä 1995): y = 0.1493x$^{0.906}$, where x – ciliate spherical diameter (μm) and y is clearance rate (μl cell^{-1} h^{-1}).

Kivi and Setälä (1995) did not reveal temperature dependence of the clearance rates in the range of 10 to 18°C. As in our study temperature was higher (22–25°C), we applied temperature correction (Q$_{10}$ = 2.9) (Rychert 2011) to obtain maximum possible values of clearance rates.

Statistical analyses

For the statistical analysis of the data sets we used the program PRIMER 5 (PRIMER-E Ltd, Plymouth). Non-metric multi-dimensional scaling (MDS) using a Bray-Curtis dissimilarity matrix based on the relative abundance of ciliates according to taxonomic classes and size fractions was used to compare ciliate community structure in different samples. We applied square root transformation of data to weigh the contributions of common and rare species. The similarity/dissimilarity between groups of samples was tested using ANOSIM analysis (analysis of similarities). SIMPER (similarity percentage) procedure was used to examine the contribution of each species to the average dissimilarity between groups of samples. We used Spearman rank correlation because our data did not meet the assumptions of normality.

RESULTS

Species composition and community structure

We discovered four ciliate species which are new for the Baltic Sea: *Leegardiella sol*, *Strombidium wulffi*, *Strombidium emergens* and *Meseres cordiformis*. Ciliate communities were mainly composed of different oligotrichids, choreotrichs and haptorids (*Monodinium*, *Mesodinium*, *Lacrymaria*) (Table 2). Most common ciliates were *Rimostrombidium humile* and *Monodinium balbiani*; they were present in the majority of samples and reached 46% and 77% of total abundance, respectively. Among other common ciliates there were: *Lohmaniella elegans*, *Strombidium viride*, *Leegardiella sol, Strombidium conicoides*, *Myrionecta rubra*, *Lacrymaria* spp. and *Strobilidium* species. We found these ciliates in 30–50% of samples, and there they did not exceed 30% of total abundance.
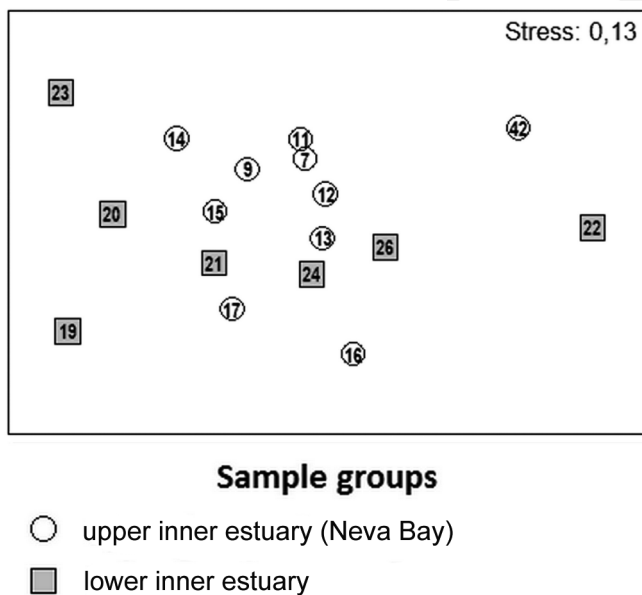
Ordination of all samples by similarity of their community structure and ANOSIM analyses revealed minor differences (Global $R = 0.163$, $p < 0.05$, $n_1 = 10$, $n_2 = 7$) between lower and upper parts of the inner Neva Estuary (Fig. 2). For example, we found *S. viride* almost in all samples from the upper part of the estuary, but never in the lower part. Additionally, average abundances of certain common ciliate species (*R. humile*, *L. elegans*, *L. sol*, *M. rubra*) in the Neva Bay were two to three times higher, than in the lower inner estuary.

According to results of SIMPER procedure, average similarity between samples from upper inner estuary ($n_1 = 10$) was nearly two times higher (20.59%), than between samples collected in the lower part of the estuary (11.24%, $n_2 = 7$). This fact demonstrated that ciliate community structure varied greatly in both parts of the estuary, but it was more uniform in the Neva Bay. Thus, samples from stations 16 and 42 located in the Neva Bay strongly differed from other samples of this group (Fig. 2). Such specificity of community structure was due to dominance of peritrichs *Epistylis* sp. at station 16 and the rare *Strombidium* species (e.g. *S. mirabile*, *S. wulffi*) at station 42.

Abundance and biomass

Total abundance and biomass of ciliates varied within the ranges 0.03–1.9 ind ml^{-1} and 0.04–2.4 × 10^{-3} µg C ml^{-1}, correspondingly (Fig. 3). We registered maximum values at station 13, located in the central area of the Neva Bay (1.9 ind ml^{-1}, 2.4 × 10^{-3} µg C ml^{-1}) and at station 24 in the southern part of the lower inner estuary (1.3 ind ml^{-1}, 1.2 × 10^{-3} µg C ml^{-1}). Ciliate numbers were surprisingly low (< 0.3 ind ml^{-1}) at half of the

Fig. 2. Two groups of samples, distinguished by ordination (MDS) on the basis of similarity of the ciliate community structure ($p < 0.05$). Upper and lower parts of the inner Neva Estuary (white and grey symbols) slightly differed by community structure (Global $R = 0.163$).
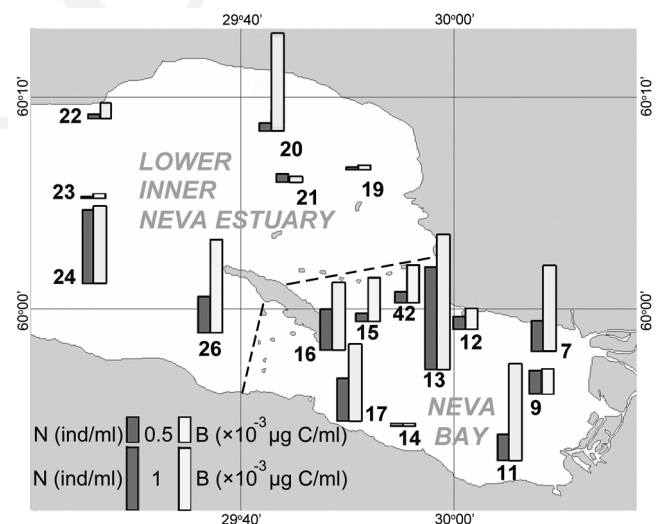
Fig. 3. Distribution patterns of ciliate abundance (ind ml^{-1}, dark bars) and biomass (× 10^{-3} µg C ml^{-1}, empty bars) in the Neva Estuary.

Table 2. Species list of ciliates, which were abundant in the open inner Neva Estuary. Feeding types are distinguished by mode of grazing (interceptors/filterers) and preferred prey size (pico-, nano-, micro-) based on literature data; "+ mix" – species capable of mixotrophic feeding (own observations, epifluorescense microscopy).

Taxa	Feeding type	Source
Lohmaniella elegans (Wulff, 1919) Kahl, 1932	pico/nano-filterer + mix	b
Rimostrombidium humile (Penard, 1922) Petz and Foissner, 1992	nano-filterer + mix	e
Strobilidium caudatum (Fromental, 1874) Foissner, 1987	pico/nano-filterer	e
Pelagostrobilidium spirale Petz *et al*. 1995	nano-filterer + mix	g, d
Strobilidium marinum (Fauré-Fremiet, 1910) Fauré-Fremiet, 1924	filterer (pico-, nano-?)	–
Leegardiella sol Lynn and Montagnes, 1988	nano-filterer + mix	k
Laboea strobila Lohmann, 1909	nano-filterer + mix	i
Strobilidium spp. (3 morphospecies)	pico-, nano-filterers + mix	a, b, g
Strombidium compressum (Leegaard, 1915) Kahl, 1932	pico/nano-filterer + mix	h
Strombidium crassulum (Leegaard, 1915) Kahl, 1932	+ mix	–
Strombidium epidemum Lynn, Montagnes and Small, 1988	pico-filterer + mix	k
Strombidium emergens (Leegaard, 1915) Kahl, 1932	pico-filterer + mix	k
Strombidium mirabile Penard, 1916	nano-filterer + mix	b
Strombidium sulcatum Claparède and Lachmann, 1858	pico/nano-filterer + mix	b
Strombidium viride (Stein, 1867) Krainer, 1995	pico/nano-filterer + mix	e
Strombidium conicoides (Leegaard, 1915) Kahl, 1932	pico/nano-filterer + mix	k
Strombidium vestitum (Leegaard, 1915) Kahl, 1932	pico/nano-filterer + mix	e, c
Strombidium wulffi (Wulff, 1919) Kahl, 1932	filterer (pico-, nano-?)	–
Strombidium spp. (2 morphospecies)	pico-, nano-filterers + mix	b
Paramecium bursaria (Ehrenberg, 1831) Focker, 1836	pico/nano-filterer	e
Epistylis sp.	pico-filterer	e
Vorticella sp.	filterer (pico-, nano-?)	–
Vorticella anabaena Still, 1940	pico-filterer	f
Codonella cratera Leidy, 1877	nano-filterer	e
Balanion comatum Wulff, 1922	nano-interceptor + mix	e
Lacrymaria sp.	nano/micro-interceptor	a
Lacrymaria olor O. F. Müller, 1776	nano/micro-interceptor	e
Monodinium balbiani Fabre-Domergue, 1888	nano/micro-interceptor	e
Mesodinium sp.	pico/nano-interceptor	e
Mesodinium pulex (Claparède and Lachmann, 1859) Stein, 1867	pico/nano-interceptor	e
Myrionecta rubra (Lohmann, 1908) Jankowski, 1976	pico/nano-interceptor + mix	l
Litonotus sp. (Vuxanovici, 1960)	nano/micro-interceptor	a

a – Fenchel (1968); b – Maeda (1985, 1986); c – Agatha and Riedel-Lorjé (1997); d – Kivi and Setälä (1995); e – Foissner and Berger (1996); f – Stabell (1996); g – Montagnes (1996); h – Pitta and Giannakourou (2000); i – Strüder-Kypke *et al.* (2003); k – Kurilov (2004); l – Myung *et al.* (2006).

studied stations and most of these stations (14, 15, 42, 19, 23) (Fig. 3) were characterized by high concentration of detritus in water column.

Ciliates were much more numerous in the southern part of the estuary (stations 11, 14, 17, 24, 26); average abundance 0.7 ind ml^{-1}, comparing to northern stations (stations 7, 12, 42, 19, 20, 21); average abundance 0.2 ind ml^{-1} (*t*-test; $t = 2.89$, $p < 0.05$) (Fig. 3). Average abundances and biomasses of ciliates in the Neva Bay (0.5 ind ml^{-1} and 1.04×10^{-3} µg C ml^{-1}) did not dif-

fer significantly (*t*-test; $t = 0.44$; $t = 0.88$, respectively; $n_1 = 10$, $n_2 = 7$, ns in both cases) from those in the lower inner estuary (0.4 ind ml⁻¹, 0.7×10^{-3} µg C ml⁻¹).

There were no significant correlations between abundance, biomass and environmental variables.

Size structure

Medium-sized ciliates (30–60 µm) dominated in the majority of samples (average contribution 59% of total abundance), especially in the upper inner estuary (Neva Bay) (Fig. 4). Small ciliates (20–30 µm) were the second abundant size class (average contribution 17%). Share of nanociliates (< 20 µm) was low at the majority of stations (average contribution 13%), but reached 47% and 61% of total abundance at stations with highest overall ciliate numbers (stations 13 and 24). Contribution of large (for plankton) ciliates (> 60 µm) was also negligible (11% on average), except for stations 16 and 20, where they constituted more than 60% of the overall ciliate numbers (Fig. 4).

Trophic structure

Mixotrophic ciliates (*M. rubra* included) were the most numerous trophic guild (average contribution 28%). They dominated in the majority of samples and formed up to 67% of overall ciliate numbers (Fig. 5). Contribution of mixotrophs was significantly (*t*-test; $t = 6.28$, $p < 0.001$) higher (47% of abundance) at sta-

tions located in the outfall area (7, 9, 11) and in the northern part of the Neva Estuary (stations 12, 42, 20, 21), than at other stations (7.7% of total numbers) (Fig. 5). Mixotrophic ciliates were represented mainly by various *Strombidium* species, choreotrichs and *Myrionecta rubra* (Table 2).

Nano-filterers were also common, but less abundant than mixotrophs (average contribution 16%). They reached 50 and 71% of total ciliate abundance at stations 13 and 24, correspondingly (Fig. 5).

Average contribution of pico-filterers and pico/nano-filteres was low (11 and 9%, correspondingly) (Fig. 5). However, our results on pico-, nano- and pico/nano-filterers may be underestimated because various oligotrichids, which formed the majority of "no data" group, potentially belong to one of these categories; however there is no precise information about their food spectrum so far.

We found predatory ciliates (e.g. genera *Monodinium*, *Lacrymaria*) almost at all stations (Fig. 5), but their contribution to total abundance was the lowest among all trophic groups in majority of samples (average share 13%), with the exception of stations 17 and 21 (77 and 30% of overall numbers, correspondingly). Abundance of predatory ciliates negatively correlated
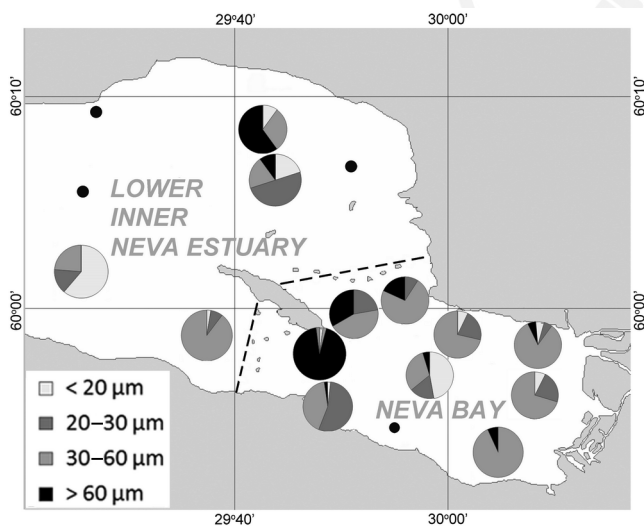
Fig. 4. Relative abundance (%) of different size groups of ciliates in the Neva Estuary. Data are not presented for four stations (indicated by points) with extremely low ciliate abundances (< 0.1 ind ml⁻¹).
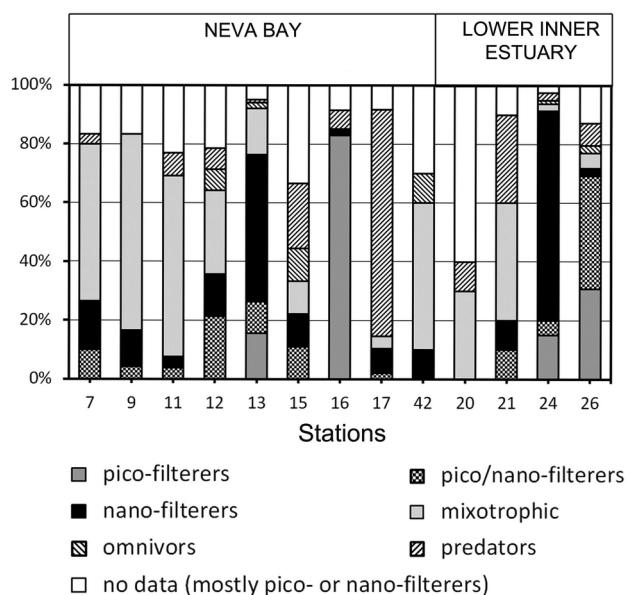
Fig. 5. Relative abundance (%) of different trophic guilds of ciliates in the Neva Estuary. Data are not presented for four stations with extremely low ciliate abundances (< 0.1 ind ml⁻¹).

with water transparency (Spearman's rho = –0.57, 9 d.f., $p < 0.05$) and number of mixotrophs (Spearman's rho = –0.60, 8 d.f., $p < 0.05$).

Functional characteristics of ciliate communities

Our calculations revealed that maximum growth rates of planktonic ciliates in the open part of the Neva Estuary constituted 1.08–2.35 day^{-1}. Maximum production of ciliate communities was less than 6.18 µg C l^{-1}day^{-1} (average 1.82 µg C l^{-1}day^{-1}). Clearance rates of ciliate communities in the lower and upper parts of the estuary constituted on average 0.03 and 0.04 l day^{-1} (correspondingly) and never exceeded 0.15 l day^{-1}.

DISCUSSION

The discovery of 4 ciliate species that are new records for the Baltic Sea during just one cruise in the Neva Estuary, in addition to 24 new records detected earlier in the near shore area of this water body (Mironova et al. 2012) suggest that ciliate diversity in the Baltic Sea is largely understudied. However, it is not surprising, when protistan biogeography is considered in general (Foissner et al. 2008), particularly concerning global diversity and distribution of aloricate Oligotrichea (Agatha 2011). The overall number of ciliate species presently known for the Baltic Sea is 814, which is relatively high (Telesh et al. 2011a, b; Mironova et al. 2012), if compared with other seas, e.g. 620 species in the Caspian Sea (Alekperov 2007), ca. 500 in the Black Sea (Kurilov 2007), and < 300 in the White Sea (Mazei and Burkovsky 2005).

Our results also showed that abundance and biomass of ciliates in the Neva Estuary were among the lowest values ever reported for the Baltic Sea (Smetacek 1981, Witek 1998, Garstecki et al. 2000, Setälä and Kivi 2003, Johansson et al. 2004). They were also significantly lower when compared with data from similar oligohaline and turbulent ecosystems of various other estuaries, such as the Elbe Estuary (Zimmermann 1997), Urdaibai Estuary (Iriarte et al. 2003), Nervión Estuary (Urrutxurtu et al. 2003), North Carolina's Neuse Estuary (Wetz and Paerl 2008) and Hudson Estuary (Lesen et al. 2010). As a rule, abundance of ciliates increased with trophic state of the environment (Beaver and Crisman 1982, Dolan and Pérez 2000). Low ciliate numbers which we registered in our study are typical for oligotrophic environments (e.g. Pitta and Giannakourou

2000) rather than for the meso-eutrophic Neva Estuary, especially in summer period. Ciliate numbers were negligible (at least in one third part of the samples) when detritus was evidently abundant, probably because of masking effect of detritus, which led to counting difficulties. On the other hand, ciliate numbers, which were reported in previous studies of the Neva Estuary (Khlebovich 1987, Mironova et al. 2012), were also lower, than in the other Baltic coastal ecosystems.

As such low ciliate abundances were revealed, we checked our methods by comparison with counts of several formaldehyde-preserved and 2% acid Lugol-fixed subsamples, collected from the same stations during present study. Formaldehyde-fixed subsamples were counted after filtration (for epifluorescence microscopy), while Lugol-fixed subsamples were processed using the Utermöhl method (to avoid cell losses during filtration). Use of these fixatives and different methods of sample concentration also gave low ciliate numbers, similarly to the present data. We conclude that it is unlikely that the detected low values of ciliate abundances were the consequences of any methodological problem. As the average loss of ciliates due to filtration is 15% (Sime-Ngando et al. 1990), the filtration process could not be the main reason for low ciliate abundances obtained in our study. It is not clear, however, what other factors (high pressure of meso-zooplankton predators, feeding conditions etc.) were the actual reasons for the low ciliate numbers in the Neva Estuary. Intensive hydrodynamics, for example, was reported as a limiting factor for development of phytoplankton in the open waters of the estuary (Panov et al. 2002), which could also be important for ciliates; however, further investigations are needed to clarify this issue.

Composition of ciliate communities in the Neva Estuary was also quite specific: e.g. *Rimostrombidium humile* which dominated in offshore and coastal zones of the Neva Estuary has never been found in other regions of the Baltic Sea, except for Tvärminne Storfjärden (Kivi 1986). Additionally, we found *Leegardiella sol* (a common ciliate in the open Neva Estuary) in the Baltic Sea for the first time during our investigations. However, numerous recent new records in the Neva Estuary clearly indicate insufficiency of knowledge about ciliate diversity in the Baltic Sea, rather than provide evidence of certain specificity of the Neva Estuary.

Ciliate communities in the open waters of the Neva Estuary were characterized by low abundances and

prevalence of medium-sized (30–60 µm) ciliates, mostly mixotrophs, in contrast to coastal area, where small heterotrophic pico- and nano-filterers dominated (Mironova *et al.* 2012).

The minor role of small ciliates in the open sea waters, comparing to coastal ecosystems, was already observed in other seas (e.g. Dolan and Marrase 1995), and most probably depended on concentration of picoplankton prey (Kissand and Zingel 2000, Bojanić *et al.* 2006) and size-specific grazing by mesozooplankton (Pérez *et al.* 1997, Zöllner *et al.* 2003). Other factors, such as turbulence, caused decrease in ciliate biovolume during storm period (Nielsen and Kiørboe 1994). As shown earlier (Telesh 2004), this factor is of special importance in the shallow wind-mixed Neva Estuary.

Our study indicated that mixotrophic ciliates were the most numerous trophic guild in open waters of the meso-eutrophic Neva Estuary, which confirms that mixotrophy in marine oligotrichs is not closely linked to the exploitation of oligotrophic environments, but probably serves a variety of purposes (Dolan and Pérez 2000). In majority of our samples from the open Neva Estuary contribution of mixotrophs (28–67% of total abundance) exceeded the average share of mixotrophs (about 30%), reported for various other marine and estuarine systems (Dolan and Pérez 2000, Pitta and Giannakourou 2000 and references therein). While contribution of mixotrophs is much lower in coastal zone, their absolute numbers are generally higher there (Mironova *et al.* 2012), if compared with the open Neva Estuary communities; in the latter case, mixotrophs dominated due to strong decrease in numbers of pico- and nano-filterers.

Significant prevalence of mixotrophs in the outfall area and northern part of the Neva Estuary indicated strong influence of the Neva River and Resort District zone (located northward of the sampling sites) on their distribution. These are the main sources of nutrients and organic matter in the estuary (Telesh *et al.* 2008), which are known as important factors controlling mixotrophs, along with light availability (Bouvier *et al.* 1998 and references therein). Moreover, nutrient conditions in these regions are very changeable, which probably also results in advantage of mixotrophic ciliates over strict heterotrophs (Bouvier *et al.* 1998).

However, our results on abundance of mixotrophic ciliates may be somewhat overestimated due to probing uncertainties because not only true mixotrophic organisms with kleptoplastids fluoresced, but also algivorous ciliates, which had recently ingested their algal prey (Sherr *et al.* 1986).

The present results along with our previous study in the Neva Estuary (Mironova *et al.* 2012) are likely the first reports about abundance of mixotrophic chloroplast-sequestering ciliates in the Baltic Sea. Mixotrophs dominated in the open Neva Estuary, but regretfully, comparison with other Baltic ecosystems is impossible, because other studies in this region provide quantitative data only about one mixotrophic species – *M. rubra,* known as indicator of eutrophication (Smetacek 1981, Olli *et al.* 1998, Witek 1998, Setälä and Kivi 2003, Johansson *et al.* 2004, Beusekom *et al.* 2007). Thereby, study of ecology of mixotrophic ciliates and their functional role in pelagic ecosystems can be considered a timely issue for future plankton research in the Baltic Sea.

Our new results confirmed the initial hypothesis only in part, showing minor differences in ciliate community structure between parts of the inner Neva Estuary, separated by storm-surge barrier. These results differ from, e.g. the consequences of breakwater construction in the Kuryongpo Bay at the eastern coast of Korea, which induced eutrophication in the inner waters and caused strong differences in ciliate communities between two parts of the bay (Kim *et al.* 2007). Our study indicated that significant spatial variability of ciliate community characteristics (abundance, biomass, size and trophic structure) was comparable in range to their temporal changes reported earlier (Mironova *et al.* 2012). In the freshwater Neva Bay, the structure of ciliate communities was more uniform than in the brackish waters of the lower estuary.

Interestingly enough, in both parts of the Neva Estuary, abundance and diversity of ciliates increased substantially from north to south. Similar tendency was reported in previous studies of the Neva Bay (Khlebovich 1987), but reasons for it were not obvious. In other respects, there are marked differences between our and previous data on ciliates in the Neva Bay (Khlebovich 1987). Namely, in the present days ciliate abundance is 4 to 10 times lower than it was in the earlier study; composition of dominants and ciliate distribution also differed (e.g. maximum abundance in 2010 was registered at the central station, in contrast to minimum values reported there previously). However, it is still unclear to what extent these differences are the results of high spatial and temporal variability of ciliate communities, or their response to recent environmental alterations of the Neva Bay ecosystem.

This study indicates that the meso-eutrophic Neva Estuary strongly differs from other regions of the Baltic Sea and various estuaries elsewhere by surprisingly low abundance and, consequently, production of ciliates. Estuarine ciliate communities are known as highly productive (Urrutxurtu *et al.* 2003), and physical and chemical conditions in the Neva Estuary are favourable for enhanced biological productivity (Telesh *et al.* 2008). However, the similarly low values of ciliate production were reported only for the open northern Baltic (Johansson *et al.* 2004). In addition, some part of this production is consumed by predatory ciliates, which input in total ciliate abundance in the Neva Estuary was relatively high, in comparison to various other environments (Muylaert and Vyverman 2006, Andrushchyshyn *et al.* 2006, Mieczan 2008, Quevedo *et al.* 2003, Kalinowska 2004).

Our results showed that non-predatory ciliates could filter less than 15% of estuarine open waters per day; so, the potential role of ciliates as consumers of pico- and nanoplankton in the open Neva Estuary is relatively low. For reference, in the upper layers of central Baltic Sea, planktonic ciliates are capable to clear about 50% of water volume per day, and sometimes their clearance rates reached 125% (Setälä and Kivi 2003). Thereby low potential production and consumption of planktonic ciliates obtained in our study is atypical for pelagic communities, where the functional role of ciliates is usually more significant (e.g. Lynn and Montagnes 1991).

These results put forward an urgent need for further research into the actual ciliate biodiversity and fine mechanisms behind the structure of ciliate communities and their functionality in the Neva Estuary ecosystem.

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