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Inventory of the taxonomical composition of the plankton ciliates in the Curonian Lagoon (SE Baltic Sea)

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Abstract

Species composition of plankton ciliates was studied in the Curonian Lagoon in 2007–2008 and compared to long term investigations dating back to the 1980th. In total, 152 taxa were identified at the level of species or genera. More species (76 species/higher taxa) was found in the estuarine part of the Lagoon due to temporally unstable salinity and the presence of both freshwater and brackish/marine species. Some of the brackish/marine species: *Tintinnopsis baltica*, *Tintinnopsis kofoidi*, *Cothurnia maritima*, *Lohmaniella oviformis*, *Lohmaniella spiralis* and *Helicostomella subulatum* were recorded for the first time in the lagoon. The ciliate community at the freshwater sites was less diverse, containing 63 species/higher taxa in the central stagnant part of the Lagoon and 47 – in the Nemunas River avandelta. The comparison of present and past studies revealed that the use of a single live-counting method could lead to underestimation of small nanociliate species, whereas examination of Lugol fixed material provides relatively poor taxonomic information.

INTRODUCTION

Ciliates are an important component of the pelagic microbial food webs, both in the freshwater and marine systems. Numerous studies have reported ciliate feeding on bacteria, picoplankton and nanoplankton (Stoecker & Evans 1985, Porter et al. 1985, Bernard & Rassoulzadegan 1990, Šimek et al. 1998), making them a likely link in the transfer of energy from the microbial components to higher trophic levels (Azam et al. 1983, Sherr et al. 1986). Due to the high metabolic rates and short generation time, ciliates 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 et al. 1990).

Ciliate communities have cosmopolitan distribution and high sensitivity to pollutants; therefore ciliates are widely used for the biological evaluation of watercourses (Stossel 1979, Wiackowski 1981, Foissner 1988, Jiang & Shen 2005).

The numerous plankton ciliate studies in the Baltic Sea region cover a wide range of habitats from the open sea to the closed coastal areas (Smetacek 1981, Boikova 1984, Arndt 1991, Kivi & Setälä 1995, Uitto et al. 1997, Witek 1998, Setälä & Kivi 2003, Johansson et al. 2004, Samuelsson et al. 2006, Beusekom et al. 2007), however the knowledge of ciliate taxonomic composition in the transitory ecosystems with changing riverine discharges and salinity regimes are still scarce (Boikova 1989, Mironova et al. 2009, Telesh et al. 2009, Telesh et al. 2011).

Plankton ciliate studies in the Curonian Lagoon started from the description of 9 taxa by German scientist Schmidt-Ries (1940). The detailed freshwater ciliate taxonomical composition was provided by Mažeikaitė (1978 a, 2003). However, the brackish ciliate community has not been studied in

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the past. Also the previous studies included only fragmentary data on seasonality of the ciliate community (Mažeikaitė 1978b).

All previous studies in the Curonian Lagoon were based on live ciliate counts. In contrast to fixation methods, it provides the possibility of identifying the species by some taxonomically important characteristics, visible only in live cells: locomotion pattern, shape, color, contractile vacuole. It is well known that fixation and staining of ciliates can lead to reduction of cell numbers (Sime-Ngando et al. 1990, Leakey et al. 1994) and shrinkage, swelling up to the total cell destruction (Choi & Stoecker 1989, Dale & Burkill 1982, Stoecker et al. 1994). In live observations, rare, small and fast moving ciliate species can be overlooked or lumped with other species; moreover, longer transportation or storage time could lead to a loss of species due to changing temperature or water chemistry (Pfister et al. 1999). Considering all the reasons stated above, we decided to identify the ciliates by observing both the living and Lugol fixed material to provide comparable data for previous studies in the Curonian Lagoon and other parts of the Baltic Sea.

The main goal of this study is to revise the list of ciliate species in the Curonian Lagoon by combining the historical data and recent surveys, and to reveal the general biodiversity patterns (seasonal/spatial) of the ciliates under the changes in salinity and other environmental factors.

MATERIALS AND METHODS

Description of the study site

The Curonian Lagoon (SE Baltic Sea) is the largest transitional lagoon in Europe, influenced by the Nemunas River discharge and brackish water inflows from the Baltic Sea (Gasiūnaitė et al. 2008).

It is a shallow (the mean depth is 3.8 m) eutrophic water basin connected to the Baltic Sea by a narrow strait, which is Klaipėda harbor area (Fig. 1). The southern and central parts of the lagoon contain fresh water due to discharge from the Nemunas River, while the salinity in the northern part varies from 0 till 7 PSU. Seawater inflows with a residence time of 1-6 days are most common (Gasiūnaitė 2000); the seawater intrusions are usually restricted to the northern part of the lagoon, rarely propagating more than 40 km (Dailidienė & Davulienė 2008). In terms of hydraulic zonation, the northern part of the lagoon and Nemunas River avandelta are classified as

transitory, while the central part – as stagnant and intermediate (Ferrarin et al. 2008).

Water temperature shows a characteristic temperate seasonality ranging from 0.1 – 0.2°C in winter to 5 – 15°C in spring and reaching the highest values (up to 19.1 – 19.3°C) in July-August (Žaromskis 1996, Pustelnikovas 1998).

Sampling and sample treatment

To investigate seasonal changes of the plankton ciliate taxonomic composition, samples were collected weekly from March to November 2007 and twice a month from December 2007 to February 2008 at two sampling sites: Smiltynė and Nida, representing respectively the transitory northern estuarine and the stagnant central freshwater parts of the Curonian Lagoon (Fig. 1). In total, 76 samples from Nida and Smiltynė sites were taken. The water depth at both sites was 2.5 m.

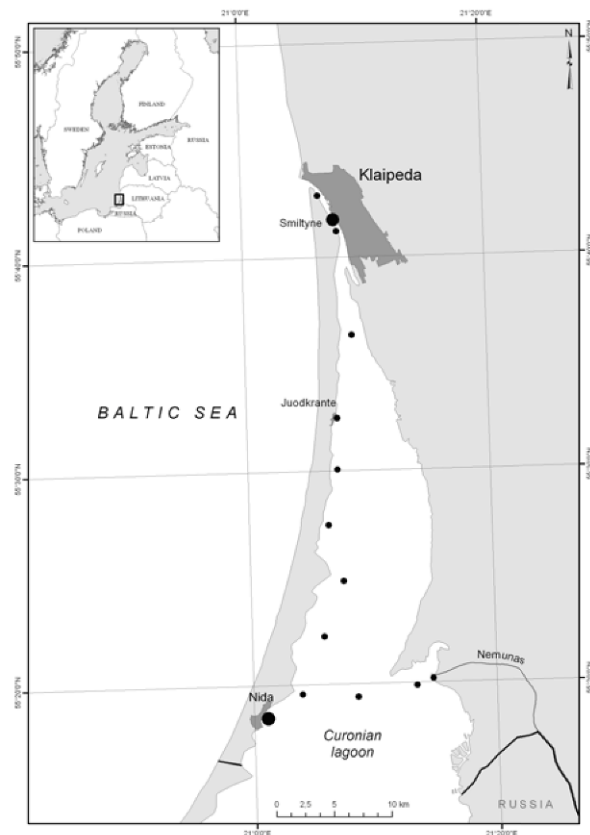


Fig. 1. Sampling sites. Large circles denote stations for seasonal sampling, small circles – spatial surveys on July 29-30, 2007; June 16-17, July 29-30 and October 7-8, 2008.

The spatial differences in taxonomic composition of ciliates were analyzed using the samples from 12 stations situated along the river-lagoon gradient during four cruises: July 29–30, 2007; June 16–17, July 29–30 and October 7–8, 2008. Two of those stations are located in Nemunas avandelta (Fig. 1). In total, 46 samples from cruises in 2007–2008 were taken for qualitative analysis of ciliates (i.e. live material examination).

Ciliate samples were taken with a 1 l sampling bottle from the near surface layer. 700–750 ml of a subsample was poured into a thermos bottle for live material examination of ciliates and transported to the laboratory within 6 h. The 250–300 ml subsample was preserved for quantitative analysis of ciliates. Acidified Lugol solution was added to each sample till 2% final concentration, samples were stored at 4°C in the dark. Secchi depth, temperature, salinity and chlorophyll *a* were measured on each sampling occasion. Temperature and salinity were measured with a portable temperature and conductivity meter (WTW MultiLine F/Set-3). Chlorophyll *a* concentration was determined fluorimetrically (FluorProbe II).

Qualitative analysis of ciliates

For live material examination, 50–500 ml of water from a thermos bottle was concentrated on a membrane filter (pore size 0.7 µm) till 10 ml volume above the filter surface by gravity filtration. The concentrated samples were analyzed in Bogorov's chamber in two or three portions under a stereomicroscope equipped with the bottom light source at 40× magnification. Individual ciliate cells were identified till the species or genus level with a microscope at 40–200× magnification.

Quantitative analysis of ciliates

We analyzed ciliate abundance and community composition in Lugol fixed samples by Utermöhl's (1958) method. Volumes of 10–50 ml were settled for at least 24 h in Utermöhl's chambers. Ciliates were counted and identified with an inverted microscope at 200–400× magnification. The entire content of each Utermöhl's chamber was surveyed and an additional subsample was counted if the total number was <150 organisms.

Ciliates were identified to the species or genera level consulting several works (Kahl 1930–1935, Small & Lynn 1985, Foissner & Berger 1996, Mažeikaitė 2003). Two species of the genera *Strobilidium* and *Cyclidium* and 2 unidentified species belonging to the order Prostomatida were pooled together.

Data analysis

The taxonomic list of ciliates provided in this work is based on compiled data of species presence/absence in live and fixed samples within each month for each freshwater and estuarine site separately.

The Shannon-Wiener diversity index (Krebs 1989) was calculated using species/taxa abundance in Lugol fixed samples. Nonparametric Spearman correlation coefficients between the environmental parameters and Shannon-Wiener diversity indices were calculated.

As a long term data set we used the latest species list published by Mažeikaitė (2003), which includes results from the earliest studies (Mažeikaitė 1978a, Antanyrienė et al. 1994). The taxonomic nomenclature was standardized following Corliss (1979). The sampling design is described in Table 1.

Table 1

Comparison of plankton ciliate surveys from the previous investigations and the present study: sampling methods, seasons and locations in the Curonian Lagoon.

Study year	Dates	Number of stations	Geographic range	Sampling method
1975 ^a	From end of May till 12–13 September (every 10 days)	5	Northern part of the lagoon	1 l bathometer, integrated sample
1991 ^b	June 7, July 14 and October 1	7	Northern (port area) and central parts including Nemunas River avandelta	1 l bathometer, integrated sample
2001 ^c	July	1	Nemunas River avandelta	1 l bathometer, integrated sample
2007–2008 ^d	July 29–30, 2007; June 16–17, July 29–30 and October 7–8, 2008	12	Northern and central parts including Nemunas River avandelta	1 l bathometer, from surface
	Weekly from March to November 2007; twice a month from December 2007 to February 2008	2	Northern and central parts	1 l bathometer, from surface

^aMažeikaitė (1978 a); ^bAntanyrienė et al (1994); ^cMažeikaitė (2003); ^dThis study

RESULTS

Taxonomic composition of ciliate community

During the study period, altogether 101 ciliate taxa were identified, assigned to 13 orders: Oligotrichida, Haptorida, Prostomatida, Peritrichida, Hymenostomatida, Heterotrichida, Pleurostomatida, Cyrtophorida, Scuticociliatida, Hypotrichida, Suctorida, Colpodida and Nassulida (Appendix 1).

Oligotrichida (including tintinnids and naked oligotrichs), prostomatids, haptorids and peritrichs dominated in the ciliate assemblage in terms of the species number. (Appendix 1). These groups were present in both Nida and Smiltynė sites throughout the sampling period, contributing in 85–90% to all recorded taxa. Tintinnids: *Tintinidium pusillum*, *Tintinnopsis tubulosa*, *Codonella cratera*, and naked oligotrichs: *Stobilidium* spp., *Lobmaniella spiralis*, *Strombidium viride* occurred in the samples most frequently. The most common representatives of haptorids were species of the genus *Askenasia*, *Mesodinium pulex*, *Monodinium* sp., peritrich *Vorticella microstoma* and prostomatids *Coleps birtus* and *Urotricha* sp. The brackish haptorid species *Myrionecta rubra* was frequently observed at the Smiltynė site (Appendix 1).

The species richness of other orders: Hymenostomatida, Heterotrichida, Pleurostomatida, Cyrtophorida, Scuticociliatida, Hypotrichida and Suctorida was much lower. All ciliate species, except for Scuticociliates (*Cyclidium* spp.), from those taxonomical groups were rare (Appendix 1).

In total, 9 brackish/marine ciliate species were found exclusively at the Smiltynė site: *Myrionecta rubra*, *Codonella relictia*, *Strombidium conicum*, *Strombidium styliferum*, *Tintinnopsis baltica*, *Tintinnopsis kofoidi*, *Cotburnia maritima*, *Frontonia marina* and *Helicostomella subulatum*. The other 3 brackish/marine species: *Lobmaniella spiralis*, *L. oviformis*, *Lobmaniella* sp. were common at both sites. The rare species, such as *Spirostomum teres*, *Prorodon ovum*, *Loxophyllum* sp., *Lembadion lucens* and *Phascolodon contractilis* were found only at the Nida site.

In total, 81 species were found at Nida (63 taxa) and Smiltynė (76 taxa) sites during the seasonal studies and 20 species were added to the list from the investigations at other lagoon stations (Appendix 1).

Different taxonomic composition of ciliates was observed in the Nemunas River avandelta. Altogether 47 species/higher taxa were found, 12 of them (*Paradileptus conicus*, *Hypotrichidium conicum*,

Holophrya atra, *H. hexatricha*, *Litonotus lamelata*, *Nassula* sp., *Cyclotrichium limneticum*, *Staurophrya elegans*, *Paruroleptus piscis*, *Frontonia leucas*, *Paramecium* sp., *Phascolodon vorticella*) typical of only this area. *Phascolodon contractilis* commonly found in the Nemunas avandelta was very rare in Nida and never observed at the Smiltynė site (Appendix 1).

Environmental factors and ciliate biodiversity

The Shannon–Wiener species diversity index (H') ranged from 0.96 to 2.65, and from 0.18 to 2.52 at Nida and Smiltynė sites, respectively (2 ± 0.41 and 1.9 ± 0.49 , no significant differences, paired t -test, $p > 0.05$). The highest H' values were recorded during spring, summer, the lowest during late autumn and winter (Fig. 2). Biodiversity was significantly related to temperature ($\rho = 0.43$, $p < 0.05$ Nida; $\rho = 0.55$, $p < 0.05$ Smiltynė) and chlorophyll a ($\rho = 0.67$, $p < 0.05$ Nida; $\rho = 0.68$, $p < 0.05$ Smiltynė).

The significant negative relation was found between Shannon–Wiener species diversity and salinity ($\rho = -0.45$, $p < 0.05$, Fig. 3). Salinity varied from 0 to 7 PSU; it was 0–2 PSU in 76% of cases (Fig. 3). H' reached the maximum value at 0–2 PSU and tended to decrease at >4 PSU. The minimum value of H' index was estimated for the ciliate assemblage at 7 PSU (Fig. 3). The same pattern was observed for the species number: the average number of taxa dropped from 18 at the salinity of <2 PSU to 11 at the salinity of >2 PSU ($\rho = -0.46$, $p < 0.05$).

Comparison of recent data with historical data

The complete list of plankton ciliate of the Curonian Lagoon comprises 152 species/higher taxa (Appendix 1). About one third of taxa (58) were the same in the present and past inventories, 52 were not identified in the present study (Fig. 4).

Representatives of 42 taxa were found for the first time during the present survey; 25 of them were identified to the level of genera and 17 – to the species level (*Enchebys pupa*, *Spirostomum minus*, *Helicostomella subulatum*, *Lobmaniella oviformis*, *L. spiralis*, *Tintinnopsis baltica*, *T. kofoidi*, *Cotburnia maritima*, *Paruroleptus piscis*, *Coleps birtus* subsp. *viridis*, *C. spetai*, *Holophrya atra*, *H. hexatricha*, *Prorodon discolor*, *Frontonia leucas*, *Marituja pelagica* and *Chilodonella cucullus*).

The present list of species comprises representatives of all orders, found in earlier studies, except for the species-poor order Odontostomatida

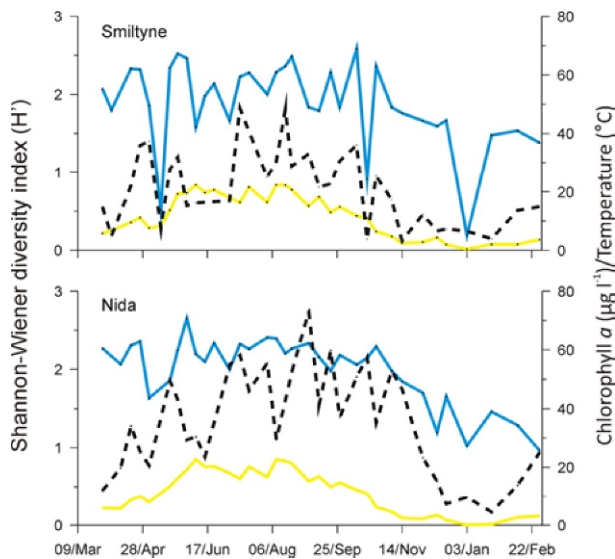


Fig. 2. Seasonal changes of ciliate species diversity (H' , Shannon-Wiener diversity index), chlorophyll a and temperature at Nida and Smiltyne sites.

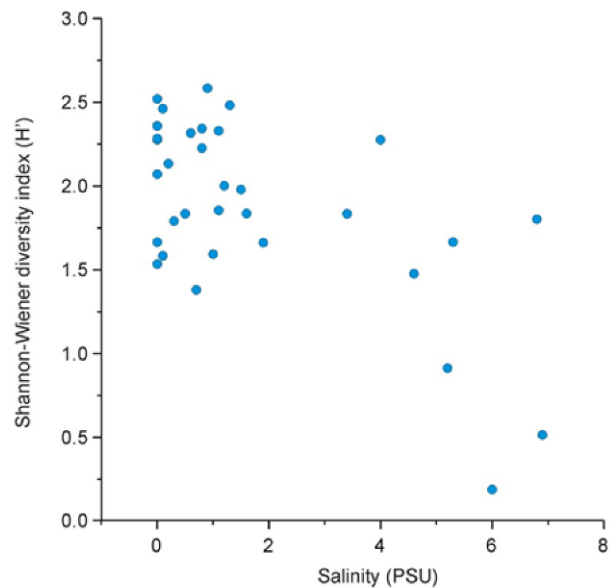


Fig. 3. Species diversity of ciliates (H' , Shannon-Wiener diversity index) versus salinity.

(1 species) (Fig. 4). The highest overlapping of both species lists was found for Peritrichida, Heterotrichida and Cyrtophorida (61, 63 and 67% of common taxa respectively). The hypotrichids and haptorids were better represented in Mazeikaite's (2003) list, whereas Oligotrichida has more representatives in this study than in the previous species list (Fig. 4).

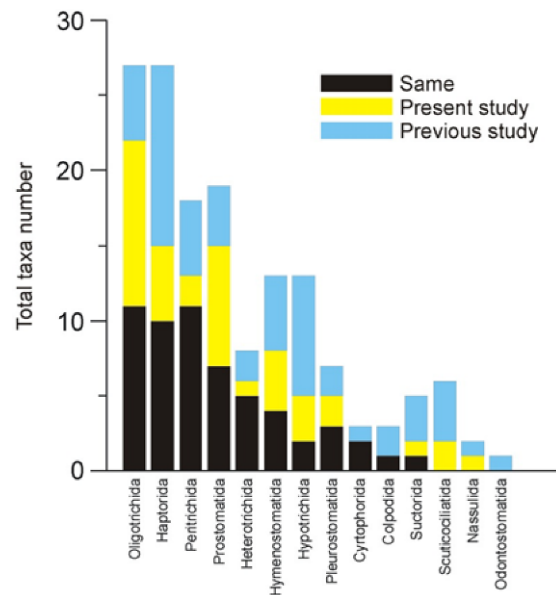


Fig. 4. The number of taxa found in the present and previous (Mažeikaitė 1978-2001) studies.

DISCUSSION

This study presents one of the most completed lists of ciliates in the coastal waters of the Baltic Sea, including the salinity gradient, seasonal surveys' and long term data records. To date, there are 152 species/higher taxa of ciliates identified in the Curonian Lagoon (Appendix 1). This number is comparable to 155 ciliate taxa identified in the Gulf of Finland, including the freshwater Neva Bay (Telesh et al. 2009). It is much higher than in the Archipelago and Bothnian Sea (41 taxa; Telesh et al. 2009), the Gulf of Riga (33 taxa; Boikova 1989) and the Gulf of Gdansk (40 taxa; Witek 1998).

The ciliates encountered in the Curonian Lagoon have been previously identified as common to freshwater lakes or to marine environments, including brackish estuaries. During the study period, the ciliate assemblage of the stagnant zone of the lagoon (site Nida) was less diverse (63 species), than the estuarine assemblage in the transitory zone of the northern part of the lagoon (76 species) due to brackish ciliate species (Appendix 1). Our findings of lower species number at the freshwater site, compared to the oligohaline site (Appendix 1), are consistent with Pfister et al. (2002), who observed a significantly higher number of taxa in brackish lakes due to mixture of common freshwater species and exclusively marine species. At the Smiltyne site, we found oligotrichs *Strombidium conicum* and

Strombidium styliferum, tintinnid species *Tintinnopsis baltica* and *Helicostomella subulatum*, as well as a unique photosynthetic haptorid – *Myrionecta rubra*, common for the brackish Baltic Sea (Smetacek 1981, Boikova 1984, Witek 1998, Johansson et al. 2004). We identified one brackish tintinnid species *Tintinnopsis kofoidii*, which was not found previously in the Baltic Sea and is not included in the newly updated ciliate list of the Baltic Sea, provided by Telesh et al. (2009).

Some brackish/marine species, particularly *Lobmaniella spiralis*, *Lobmaniella* sp. and *L. oviformis* were found both in the estuarine and freshwater part of the lagoon. The previous observations of these species were related to higher salinities. *Lobmanniella* sp. was found before in the Western Baltic Sea (Kieler Bight) and in the Southern Baltic Sea (Gdansk Basin and North-Rugian Bodden), *Lobmanniella oviformis* – in the Baltic Proper, the Western Baltic Sea (Kieler Bight) and the Eastern Baltic Sea (the Gulf of Finland, including the freshwater Neva Bay) (Telesh et al. 2009). *Lobmaniella spiralis* was identified in the Gulf of Riga, particularly at the mouth of the River Daugava (at the salinity range from 1.04 to 3.94 PSU) and the River Lielupe (the salinity range from 3.14 to 6.35 PSU). *Lobmaniella* sp. was found in the mouth of the River Gauja (the salinity range from 3.14 to 6.35 PSU) (Boikova 1989). Therefore our findings could extend the knowledge about the distribution range of these species.

The Nemunas River avandelta contained ciliate taxa, typical of large European rivers: Danube, Rhine and Loire (Lair et al. 1999, Scherwass & Arndt 2005, Kiss et al. 2009): *Paradileptus conicus*, *Hypotrichidium conicum*, *Holophryra atra*, *H. hexatricha*, *Litonotus lamelata*, *Nassula* sp., *Cyclotrichium limneticum*, *Staurophrya elegans*, *Paruroleptus piscis*, *Frontonia leucas*, *Paramecium* sp., *Phascolodon vorticella*, *Staurophrya elegans*, and *Nassula* sp. Recently, *Paradileptus conicus* was identified in the River Danube as a possible invasive species (Kiss et al. 2009). The lentic ciliate species *Phascolodon contractilis* was not listed in any of the mentioned rivers, possibly because it can be confused with *Phascolodon vorticella* (Mažeikaitė 2003). *Paruroleptus piscis*, *Frontonia leucas* and *Litonotus lamelata* were recorded in the Taro River (northern Italy) by Madoni & Zangrossi (2005).

The higher species number and biodiversity was observed during the vegetation season between April and October, and decreased substantially when the temperature and chlorophyll *a* dropped during the late autumn - early spring period (Fig. 2, Appendix

1). This seasonal biodiversity pattern is consistent with the other study, reporting a high relation between biodiversity indices and ciliate abundance (Xu & Cronberg 2010).

The large-scale, Baltic ciliate species richness maximum is estimated at the salinity of 5 to 8 PSU, which is a contrast to the species minimum range in classic Remanes's *Artenminimum* model (Telesh et al. 2011). According to Telesh et al. (2011), the possible evolutionary processes have resulted in the species adaptation in the horohaliticum (5 to 8 PSU) of the permanent salinity gradient in the Baltic Sea. In this study, we estimated the significant negative salinity effect on the species number and biodiversity. The biodiversity index, as well as the species number declined strongly above the salinity 4 PSU (Fig. 3). The low number of observations at the salinity 3-7 PSU in this study obscures the more detailed analysis of the pattern. Moreover, this study involves only one estuarine site with highly unstable salinity (the most common are 1-2 days brackish water inflows at a site) and the sampling design extended in time (not space).

The differences in the species lists from recent and past studies could be explained by two potential reasons: different sampling strategy and taxonomic resolution. The substantial mismatch of species from the order Hypotrichida could be explained by a different sampling strategy. Integral sampling, used in the earlier surveys by Mazeikaite (Table 1) enables to catch benthic ciliate species from hypotrichid genera *Euplotes*, *Aspidisca* and *Oxytricha*. These species tend to aggregate in the near-bottom layer (Telesh et al. 2009) and could be hardly found in the surface samples.

The Lugol fixation method gave a new insight into nanociliate taxonomic composition. Despite comparatively low taxonomic resolution of this method (only 65% of all species were found in Lugol samples), 8 new taxa/species were added to the species list due to Lugol fixed samples: *Lobmaniella spiralis*, *L. oviformis*, *L.* sp., *Strobilidium* spp. (2 species), *Cyclidium* spp. (2 species) and *Urotricha* sp. All the mentioned species (except for *L. spiralis*), having a small size (<20 µm), were missed in the live material examination in the present and past studies in the Curonian Lagoon (Appendix 1).

Underestimation of small ciliate species (<30 µm) in live counts was previously reported by Obolkina (2006). It is known that small oligotrichs, such as *Strobilidium* spp. (10 to 45 µm in length) are very sensitive to temperature changes induced by

microscope light and lose their motility once exposed for more than 10 min under the microscope light (Sime-Ngando et al. 1990). We used the underneath light source, which could have caused some temperature increase in the counting chamber. Another reason could be the mortality of species from the genus *Strobilidium* related to the examination in the Bogorov chamber (Boikova, pers. comm.), instead of using a plate with small wells (Dale & Burkill 1982). Therefore, the combination of live counts and fixed material is essential, since small nanociliates, including naked oligotrichs and scuticociliates, are the most productive and numerous in the pelagic ciliate community of the Baltic Sea (Mironova et al. 2009).

It could be concluded that a higher species number of plankton ciliates could be found in the estuarine part of the Curonian Lagoon due to mixture of freshwater and brackish/marine ciliate species. More detailed eco-taxonomic studies could help to clarify the remaining questions concerning the influence of salinity on the composition of ciliate communities in the future.

Seasonal studies of ciliate species composition revealed higher species diversity at both freshwater and brackish sites from April to October, and a drop of the species number during late autumn and winter related to a decrease in the temperature and chlorophyll *a* concentration.

Comparison of the present and past studies revealed that combination of live and Lugol fixed material counts improve the reliability of ciliate taxonomic studies. If the live counting method is applied alone, small nanociliate species could be underestimated, whereas the Lugol fixed material method without live material examination provides poor taxonomic information.

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