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OF LATVIA**

FACULTY OF BIOLOGY

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**THE INFLUENCE OF ENVIRONMENTAL FACTORS  
AND PHYTOPLANKTON FUNCTIONAL GROUPS  
ON THE SEASONAL DYNAMICS OF PRIMARY  
PRODUCTION IN THE GULF OF RIGA**

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Form of the thesis: a collection of scientific publications in biology, hydrobiology

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The thesis is available at the Library of the University of Latvia, Raiņa blvd. 19, Riga.

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## SUMMARY

Primary production in an aquatic ecosystem depends on the process of photosynthesis carried out by autotrophic organisms such as phytoplankton, phytobenthos and macroalgae. This doctoral thesis focuses on a scarcely studied issue in the Gulf of Riga – the seasonal dynamics of primary production and its relationship to abiotic and biotic environmental factors. The obtained knowledge provides an opportunity for a more in-depth comprehension of the effects of eutrophication processes both locally and regionally. It also adds to the body of knowledge about the amount of energy available in the pelagic trophic food web and its impacting environmental factors, allowing for a more accurate assessment of the pelagic habitat and ecosystem. The main results indicate that the annual primary productivity in the Gulf of Riga reached 353–376 g C m<sup>2</sup>. During the productive period (from April to October), the source of substances available to phytoplankton changes seasonally, thus primary production follows seasonal cycle. The highest net (NPP) and total (GPP) primary production were observed in the spring, while autumn was the least productive period during the growth season. Based on the isotopic signals, the spring measurements differed from the rest of the period implying greater influence from terrestrial and anthropogenic sources. Diatoms, dinoflagellates and the ciliate *Mesodinium rubrum* showed the strongest positive relation to isotopic changes in the Gulf of Riga, indicating their role in the creation of new production, supplementing the system with new energy. It should be emphasized that the mixotrophic ciliate *M. rubrum* dominated in all seasons, showing a significant correlation with increased productivity. On the other hand, diatoms were identified as the main producer of new production in spring, and diazotrophic cyanobacteria *Aphanizomenon flosaquae* – in summer, during the nutrient regenerating system. The main results of the doctoral thesis are published in four publications.

**Key words:** Primary production, *Mesodinium rubrum*, Baltic Sea, Gulf of Riga

## ANOTĀCIJA

Pirmprodukcija ūdens ekosistēmās ir atkarīga no fotosintēzes procesa, ko veic autotrofie organismi, piemēram, fitoplanktons, fitobentoss un makroaļģes. Šajā promocijas darbā apskatīts Rīgas līcī maz izpētīts aspekts – fitoplanktona pirmprodukcijas sezonālā dinamika un tās saistība ar abiotiskajiem un biotiskajiem vides faktoriem. Šādu zināšanu iegūšana sniedz iespēju padziļināti izvērtēt eitrofikācijas procesu ietekmi gan reģionāli, gan vispārīgi, kā arī papildina zināšanu kopumu par pelaģiskajā trofiskajā barības tīklā pieejamo enerģijas apjomu un to ietekmējošajiem vides faktoriem. Galvenie rezultāti norāda, ka ikgadējā pirmproduktivitāte Rīgas līcī sasniedza 353–376 g C m<sup>2</sup>. Produktīvā perioda laikā (no aprīļa līdz oktobrim) fitoplanktona pieejamo barības vielu avots sezonāli mainās, līdz ar to pirmprodukcijai novērojama izteikta sezonālitate. Augstākā tīrā (NPP) un kopējā (GPP) pirmprodukcija ir novērojama pavasarī, savukārt rudens periodā vērojama viszemākā fitoplanktona produktivitāte. Pamatojoties uz izotopu signāliem, pavasara mērījumi atšķirās no pārējā perioda, pieļaujot lielāku ietekmi no sauszemes un antropogēnajiem avotiem. Kramaļģēm, dinoflagelātiem un ciliātam *Mesodinium rubrum* vērojama visciešākā pozitīvā saistība ar izotopiskajām izmaiņām Rīgas līcī, norādot uz minēto taksonu lomu jaunās produkcijas veidošanā, papildinot sistēmu ar jaunu enerģiju. Jāuzsver, ka miksotrofais ciliāts *M. rubrum* dominēja visos gadalaikos, uzrādot būtisku korelāciju ar paaugstinātu produktivitāti. Savukārt kramaļģes tika identificētas kā galvenais jaunās produkcijas veidotājs pavasarī, bet diazotrofās cianobaktērijas *Aphanizomenon flosaquae* – vasarā, barības vielas reģenerējošā sistēmā. Promocijas darba galvenie rezultāti ir publicēti četrās publikācijās.

**Atslēgas vārdi:** Pirmprodukcija, *Mesodinium rubrum*, Balijas jūra, Rīgas līcis

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# LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following published research papers, which are referred to in the text by Roman numerals:

- I Labucis A., Purina I., Labuce A., Barda I., Strake S.  
**Spring seasonal pattern of primary production in the Gulf of Riga (Baltic Sea) after a mild winter.**  
*Environmental and Experimental Biology* (2017) 15:247–255 ..... 38
- II Purina I., Labucis A., Barda I., Jurgensone I., Aigars J.  
**Primary productivity in the Gulf of Riga (Baltic Sea) in relation to phytoplankton species and nutrient variability.**  
*Oceanologia* (2018) 60, 544–552 ..... 47
- III Tunēns J., Aigars J., Poikāne R., Jurgensone I., Labucis A.,  
Labuce A., Liepiņa-Leimane I., Buša L., Vīksna A.  
**Stable carbon and nitrogen isotope composition in suspended particulate matter reflects seasonal dynamics of phytoplankton assemblages in the Gulf of Riga, Baltic Sea.**  
*Estuaries and Coasts* (2022) 45, 2112–2123 <sup>1</sup> ..... 57
- IV Labucis A., Labuce A., Jurgensone I., Barda I., Andersone I.,  
Ikaunieca A. **Seasonal variation in size structure and production of autotrophic plankton community in eutrophied, low-light environment: A focus on *Mesodinium rubrum*.**  
*Oceanologia* (2023) 65, 398–409 ..... 70

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<sup>1</sup> A part of Publication III is included in this doctoral thesis – included are the results that describe the source (marine or terrestrial) of the phytoplankton assimilated biomass based on signals of stable carbon and nitrogen isotopes.

# 1 INTRODUCTION

Primary production in an aquatic ecosystem depends on the photosynthetic process carried out by autotrophic organisms, e.g. phytoplankton, phytobenthos, and macroalgae. Up to date, four approaches have been used widely to quantify the photosynthetic process over time: (a) changes in oxygen, (b) changes in carbon dioxide, (c) the formation of organic matter, and (d) the time-dependent changes in consumption of light (change in chlorophyll fluorescence) (Falkowski et al., 2003). Here, in this doctoral thesis, oxygen is used as a proxy for the synthesis of organic material by autotrophic phytoplankton (microalgae).

Phytoplankton are the main contributor to primary production in the pelagic habitat (Ask et al., 2016; Henriksen, 2009). It is comprised of a taxonomically diverse group of mainly single-celled and photosynthetic organisms (numerous mixotrophic and heterotrophic phytoplankton species also exist). Phytoplankton communities, thus predominant characteristics of phytoplankton, successively change throughout the productive season. The spring bloom period can be named as one example representing the contrasts between different phytoplankton communities and the impacts of environmental conditions. In the northern temperate and boreal seas, the spring bloom sustained by the nutrient winter pool lasts approximately one month but contributes up to 40–60% of the annual carbon fixation (Heiskanen, 1998). The rest of the productive season is lower in production efficiency.

Noteworthy that the majority of temperate and boreal spring primary production is new production (Falkowski et al., 2003). New production, in brief, is all primary production associated with newly available nitrogen (e.g.,  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{N}_2$ ). In a confined system such as the mixed productive layer of pelagic habitat, new production is a part of primary production formed from external nitrogen input (definition from Dugdale & Goering, 1967; Williams et al., 1989). Hence, new production is supported by physical and chemical processes, *inter alia*, water mixing, upwelling, riverine and atmospheric inputs. On the contrary, “regenerated production” is organic matter fueled by nutrients sustained within the confined system, e.g., ammonium (Berg et al., 2003).

The Baltic Sea is a continental inland sea with limited water exchange and a large-scale gradient from temperate marine to subarctic limnic ecosystems. Pelagic primary production in the Baltic Sea is variable (Table 1) between sub-basins and their trophic condition (mesotrophic to eutrophic) along the mentioned gradient due to differences in environmental and ecological factors. In the Baltic Proper (central Baltic) average gross primary production is estimated to be approximately  $172 \text{ g C m}^{-2} \text{ y}^{-1}$  (Samuelsson et al., 2006). The coastal areas and plumes, usually, are higher in primary production than open Baltic Sea waters. Although, in

very turbid waters such as Klaipeda Strait (Secchi depth < 1 m) it can be lower due to limited light availability in surface waters.

The Gulf of Riga is a semi-isolated, shallow Baltic Sea sub-basin with high riverine input resulting in increased eutrophication compared to the Baltic Proper (Kotta et al., 2008). Also, the reported primary production values attribute it to eutrophic conditions (as defined in Wasmund et al., 2001). Surveys of primary production in the Gulf of Riga have been sporadic in recent decades (Andrushaitis et al. 1992, Wassmann, Tamminen 1999; Olesen et al. 1999; Wasmund et al. 2001), mainly covering the period from 1989 to 1997. Overall, the estimated annual gross primary production in the Gulf of Riga is one of the highest for the Baltic Sea (Table 1). Its reported values vary between 250 and 350 g C m<sup>-2</sup> y<sup>-1</sup> which lies within the general range defined for coastal transitional water systems by Nixon (1982), i.e., 200–400 g C m<sup>-2</sup> y<sup>-1</sup>.

The knowledge base for the Gulf of Riga primary production dynamics and its driving factors show that plankton community production and respiration are predominantly limited by nutrient supply during spring and summer (Olesen et al., 1999), whereas the combination of low light levels and deep vertical mixing lead to light limitation during the autumn season. However, stabilizing (solar heating) and destabilizing (wind) forces strongly affect the Gulf of Riga pelagic habitat throughout the entire productive season due to its shallowness, and day-to-day primary production can vary by up to two times (Wassman, Tamminen, 1999). Variability is enhanced by local mixing events, such as upwelling, that usually result in a decreased primary production in the short term but an overall increase in the long term (Lehmann, Myrberg, 2008). Previous studies (Lundsgaard et al., 1999; Olesen et al., 1999; Olli, Heiskanen, 1999) point to high pelagic recycling efficiency in the Gulf of Riga and marks that new production is mostly limited to the southern part of the gulf and regenerated production predominate the system.

Still, the majority of previous studies were focused on abiotic factors affecting primary production and information about the linkages between ecological aspects and primary production is scarce for the Gulf of Riga and the Baltic Sea in general. It is known that pelagic primary production may respond to taxonomical composition, community structure, and physiological and ecological characteristics of phytoplankton (e.g., Chavez et al., 1990; Falkowski et al., 2003; Spilling et al., 2019; Barmejo et al., 2020).

**Table 1.** Primary production values reported for different Baltic Sea and North Atlantic areas.

Area	Gross primary production, g C m <sup>-2</sup> year <sup>-1</sup>	Layer sampled	Period	Season	Reference
<b>Baltic Sea</b>					
<b>Gulf of Riga</b>	353–376	0–10 m	2011–2012	Apr–Oct	This study ( <b>Paper II</b> )
<b>coastal Gulf of Riga</b>	350	1, 5, 10 m	1993–1995	May–Sep	Olesen et. al., 1999
<b>Gulf of Riga</b>	290	NA	NA	Jan–Dec	Kotta et al., 2008
<b>Gulf of Finland</b>	74–111	NA	NA	Jan–Dec	Pitkänen, 2008
<b>Bothnian Bay</b>	18	0, 1, 2, 4, 8, 15, 20 m	2000	Jan–Dec	Samuelsson et al., 2006
<b>Bothnian Sea</b>	47	0, 1, 2, 4, 8, 15, 20 m	2000	Jan–Dec	Samuelsson et al., 2006
<b>Baltic Proper</b>	172	0, 1, 2, 4, 8, 15, 20 m	2000	Jan–Dec	Samuelsson et al., 2006
<b>Gulf of Gdańsk</b>	225	NA	1993–1997	Jan–Dec	Witek et al., 1999
<b>Kattegat</b>	135–165	2, 7, 15 and 22 m	1981–2000	Jan–Dec	Rydber et. al., 2006
<b>Belt Sea</b>	185–220	2, 7, 15 and 22 m	1981–2000	Jan–Dec	Rydber et. al., 2006
<b>Other regions</b>					
<b>North Sea, German Wadden Sea</b>	124–176	3 m	1995–1996	Jan–Dec	Tillmann et. al., 2000
<b>Chesapeake Bay, USA</b>	300–500	from 0.5–1m	1995–2004	Jan–Dec	Harding et. al., 2020
<b>Atlantic Ocean</b>	83.9	0–200	NA	Jan–Dec	Eppley, Peterson, 1979

The phytoplankton taxonomical composition and community structure in the Gulf of Riga follow the classical succession of boreal phytoplankton development (Yurkovskis et al. 1999; Jurgensone et al. 2011; Olli et al. 2011). Typically, diatoms *Pauliella taeniata* and *Thalassiosira baltica* dominate the vernal period from April to May. Diatoms *Chaetoceros* spp. becomes prevalent towards the end of the spring bloom accompanied by dinoflagellates *Peridiniella catenata*, and mixotrophic ciliate *Mesodinium rubrum*. Summer blooms of cyanobacteria can be observed in July and August when *Aphanizomenon flosaquae* dominates. Chlorophytes and cryptophytes are often accompanying cyanobacterial blooms.

The second bloom of diatoms completes the phytoplankton succession in September–October after the disruption of the thermocline due to convective mixing of the water column (Jurgensone et al., 2011; Yurkovskis et al., 1999). In autumn and winter, wind-induced mixing of the water column brings up nutrients from the bottom to the surface, supporting the growth of phytoplankton in the following spring (Rydberg et al., 1990) that is hindered during winter time due to the significant shortening of daylight and windy conditions or ice sheet resulting in overall low light intensity (Vihma, Haapala, 2009).

Lastly, the role and impact of environmental factors vary regionally and, to an even higher extent, locally in the Baltic Sea (Snoeijs-Leijonmalm, Andrén, 2017). Therefore, the estimates of planktonic primary production and the identification of its main contributors are crucial to the comprehension of regional carbon flow dynamics and ecosystem functioning, both being relevant to ecosystem-based management. Moreover, the recently proposed mixotrophic-centric paradigm for marine ecology highlights the need for detailed ecological characterization of functional groups, especially mixotrophic organisms (Mitra et al., 2014, 2016), to provide sufficient data for their meaningful inclusion within regional food web models.

**The main objective** of the doctoral thesis was to examine the seasonal variation of planktonic primary production in the Gulf of Riga identifying the major impacting environmental factors and the most contributing phytoplankton groups.

**The main tasks** of the doctoral thesis were

- to identify the environmental factors impacting primary production and their effects;
- to estimate the amount of “new” and “regenerated” production;
- to determine the source of substances assimilated in primary production (land or marine environment);
- to pinpoint the important phytoplankton functional groups in the formation of primary production.

### **Scientific novelty and practical importance of the research**

This doctoral thesis focuses on a scarcely studied issue in the Gulf of Riga – the seasonal dynamics of primary production and its relationship to abiotic and biotic environmental factors. The obtained knowledge provides an opportunity for a more in-depth comprehension of the effects of eutrophication processes both locally and regionally. It also adds to the body of knowledge about the amount of energy available in the pelagic trophic food web and its impacting environmental factors, allowing for a more accurate assessment of the pelagic habitat and ecosystem. Moreover, this study is the first to estimate the new and regenerated primary production and identify the origin of substances assimilated in the biomass based on isotope signals. Hence, the results provide an analysis of the circulation of nutrients and

their involvement in biological processes, which is essential information for a more complete understanding of biogeochemical cycling and food web processes – information that can be practically applied in environmental management, focusing on ecosystem-based approaches.

**The results were presented at three national and three international conferences:**

1. 73<sup>rd</sup> scientific conference of the University of Latvia. February 2–6, 2015. Section “Research and protection of Latvian water environment”, Department of Hydrobiology, Faculty of Biology. Oral presentation: **Development of limit values of phytoplankton ecological quality element indicator classes for the coast of Latvia.** *Atis Labucis, Ieva Barda, Iveta Jurgensone*
2. 74<sup>th</sup> scientific conference of the University of Latvia. February 1, 2016. Section “Research and protection of the Latvian water environment”. Oral presentation: **Seasonal dynamics of phytoplankton primary production in the Gulf of Riga in 2015.** *Atis Labucis, Iveta Jurgensone, Anda Ikauniece*
3. ICES Annual Science Conference. September 19–23, 2016, in Riga. Oral presentation: **Phytoplankton community and controlling factors of primary production in the Gulf of Riga (Baltic Sea).** *Atis Labucis, Iveta Jurgensone, Anda Ikauniece*
4. ICES/PICES Early Career Scientist Conference in Busan, Republic of Korea. May 30–June 2, 2017. Oral report: **Phytoplankton community and controlling factors of primary production in the Gulf of Riga (Baltic Sea).** *Atis Labucis, Iveta Jurgensone, Ieva Barda, Anda Ikauniece*
5. 3<sup>rd</sup> scientific conference of the National Research Program EVIDEnT. November 24, 2017, in Riga. Oral presentation: **Dynamics of primary production in the Gulf of Riga.** *Atis Labucis, Iveta Jurgensone, Ieva Barda, Lelde Ozoliņa.*
6. European Space Agency’s 2019 Living Planet Symposium. May 13–17, 2019, in Milan, Italy. Poster Presentation: **Sentinel-3 Ocean and Land Colour Instrument data comparison with in situ chlorophyll a.** *Atis Labucis*

**The doctoral thesis includes results obtained in the following projects:**

- European Union LIFE+ Nature & Biodiversity programme project “Innovative approaches for marine biodiversity monitoring and assessment of conservation status of nature values in the Baltic Sea (MARMONI)”
- project “Development of a mechanistic model of the Gulf of Riga ecosystem in support of efficient national policy to ensure the protection of the Baltic Sea and to promote the sustainable use of its ecosystem (LIMOD)” funded by the European Regional Development Fund
- National Research Programme “The value and dynamic of Latvia’s ecosystems under changing climate (EVIDEnT)” funded by the Latvian state research programme for 2014–2017

## 2 MATERIALS AND METHODS

### 2.1 Study area

The Gulf of Riga is situated in the north-eastern part of the Baltic Sea and covers an area of 16 330 km<sup>2</sup> with an average depth of 26 m and a maximum depth of approximately 60 m. Its drainage basin (135 700 km<sup>2</sup>) covers more than eight times the surface area of the Gulf itself (Kotta et al. 2008), and 86% of the freshwater inflow comes from the southern part of the Gulf (Kļaviņš et al. 2002) resulting in a north-westward salinity gradient, from 0.5–2.0 practical salinity units (henceforth: PSU) in surface layers of the southern regions to 7.0 PSU at the Irbe Strait. The mean salinity in most parts of the Gulf of Riga is between 5.0 and 6.0 PSU. Surface water temperature varies seasonally from 0 to 20 °C. Temporal and spatial patterns of ice coverage and duration vary annually, and the average ice season lasts approximately 80 days (Kotta et al. 2008). In the coldest season (November to April) water layers of the Gulf are mixed, such that the temperature, salinity and nutrient concentrations throughout the water column are similar (Stigebrandt 1996). From April to mid-October seasonal stratification restricts vertical water exchange and promotes oxygen depletion and nutrient accumulation in the bottom layer until the entire water column is mixed again in autumn (Yurkovskis 2004).

### 2.2 Sample collection and analysis

Samples were collected at national monitoring stations in the open and coastal Gulf of Riga (Figure 1) between 2011 and 2017 (**Table 2**). At each sampling occasion, physical, chemical and biological parameters were surveyed and collected following the recommendations of the HELCOM COMBINE Manual (HELCOM, 2017), except for the study described in **Publication I**, where water samples were collected by Ferry box installed on passenger ferry *Tallink MS Romantika* (pumping water from 5 m depth).

Nutrient concentrations were determined according to Grasshoff et al. (1983). All laboratory analyses were performed in an accredited laboratory of the Latvian Institute of Aquatic Ecology (ISO/IEC 17205). Chlorophyll a and phytoplankton taxonomical composition and biomass were analyzed according to the standard method of the Manual for Marine Monitoring in the HELCOM COMBINE Program (HELCOM, 2017). Stable isotope analysis was conducted in the Laboratory of Analytical Chemistry at the Faculty of Chemistry, University of Latvia.

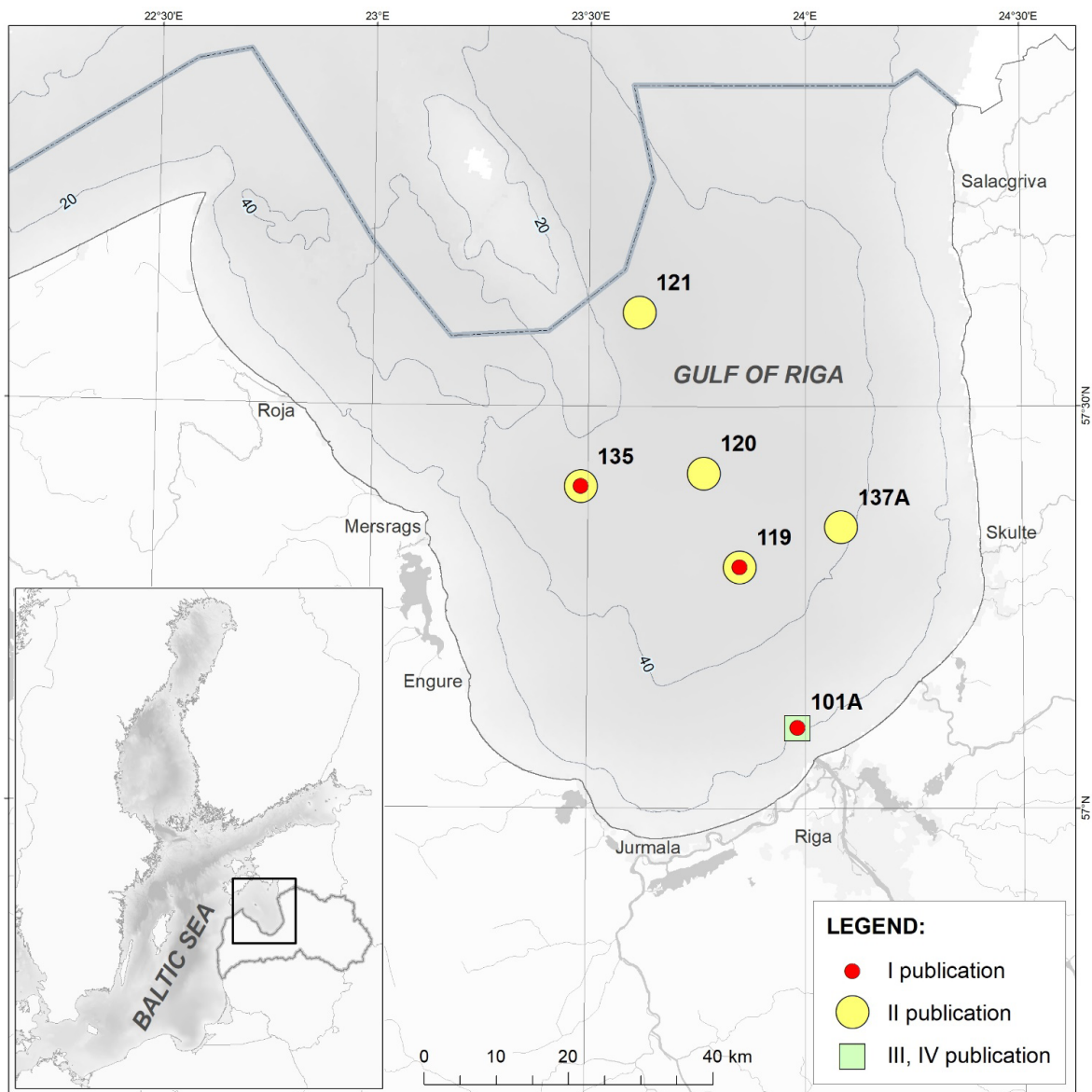
The light and dark bottle oxygen technique (see Olesen et al., 1999 for details) was used to estimate the primary production of the study area. Initial and post-incubation oxygen concentrations were determined by titration with sodium thiosulfate according to ISO 5813:1983. Photosynthetically active radiation (PAR) was measured on board using the LI-1400 Data Logger and the LI-190 Quantum Sensor during experimental incubation.

New production was calculated using the nutrient depletion approach (see Wasmund et al. 2005 for details) for the year 2012. April was considered the starting month of the spring bloom. Deposition of nitrogen from air was considered in the calculations; the air deposition over the whole surface area of the Gulf of Riga was 9973 t of nitrogen in 2010 (HELCOM, 2013). Averaging the deposited amount over the surface area of the Gulf of Riga, the rate at which nitrogen deposits from air was assumed to be  $0.12 \text{ mmol m}^{-2} \text{ day}^{-1}$ .  $\text{PO}_4^{3-}$  excess production as described by Rahm et al. (2000) was assumed irrelevant for the calculations.

Size-fractionation (**Publication IV**) was conducted immediately after sampling prior to the analysis of the samples. The phytoplankton samples were divided by reverse fractionation: passed through a sieve with a mesh size of  $56 \mu\text{m}$  (henceforth:  $< 56$ -fractionated). The  $56 \mu\text{m}$  sieve was chosen for fractionation based on the observed distribution of *M. rubrum* size classes in the long-term data collected at Station 101A (see database <https://latmare.lhei.lv/>).

## 2.3 Statistical methods

Multivariate techniques (i.e., principal component analysis, and partial least squares regression) were applied to analyse relationships between environmental factors and primary production variables. Before the analysis, phytoplankton biomass was normalized and all variables were centred and scaled to unit variance. The analysis was performed using StatSoft© STATISTICA 7 (**Publication I**) and R software v.3.6.1 (R Core Team 2019) (**Publications III and IV**). A comparison of NPP between the unfractionated community and  $<56$ -fractionated community was conducted by the Wilcoxon signed-rank test.



**Figure 1.** Study sites in the Gulf of Riga.

**Table 2.** Station coordinates, depths and studied periods.

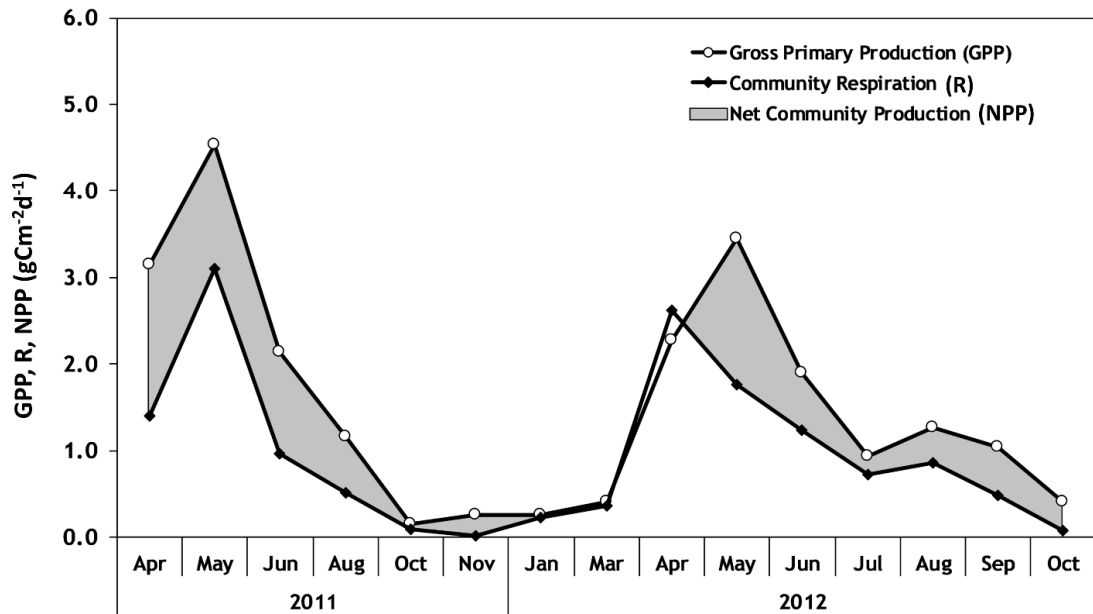
Station	N latitude	E longitude	Depth, m	Data period
101A	57.1000	23.9833	22	Mar – May 2014 (I), Mar – Nov 2017 (III, IV)
119	57.3000	23.8500	44	Mar – May 2014 (I), Apr 2011 – Oct 2012 (II)
120	57.4167	23.7667	45	Apr 2011 – Oct 2012 (II)
121	57.6167	23.6167	56	Apr 2011 – Oct 2012 (II)
137A	57.3500	24.0833	42	Apr 2011 – Oct 2012 (II)
135	57.4000	23.4833	44	Mar – May 2014 (I), Apr 2011 – Oct 2012 (II)

## 3 RESULTS AND DISCUSSION

### 3.1 Gross primary production

The estimated annual GPP of the Gulf of Riga was relatively high (353–376 g C m<sup>-2</sup> y<sup>-1</sup>; **Publication II**) compared to other Baltic Sea regions (**Table 1**). For the period 1993–1995, the annual GPP was estimated to range between 250–255 g C m<sup>-2</sup> y<sup>-1</sup> (Andrushaitis et al., 1992; Wasmund et al., 2001). However, this estimate was based on measurements that did not include the most productive period of phytoplankton succession, i.e., from the end of March until the end of April. Olesen et al. (1999), on the other hand, approximated that annual primary production in the Gulf of Riga can exceed 350 g C m<sup>-2</sup>, impeding to the values obtained in this study (**Publication II**). Noteworthy, that despite the maintained eutrophication and high P and N inputs (HELCOM, 2018), primary production has not changed notably since the early 90'ties (Andrushaitis et al., 1992; Wasmund et al., 2001) in the Gulf of Riga.

Overall, GPP in the Gulf of Riga mirrored phytoplankton biomass with the highest values (from 4.53 to 3.45 g C m<sup>-2</sup> d<sup>-1</sup>) in the spring period and the lowest values during the autumn-winter time (**Publication II**), which is consistent with the boreal seasonal succession. A rapid increase in GPP during early spring (typically March – early April; **Figure 2**) is directly linked to increasing solar irradiance and high nutrient concentrations after the winter convection period, also spring flooding and heavy precipitation events sustain nutrient enrichment supporting high GPP (**Publication I, II**). Moreover, the dynamics of stable carbon and nitrogen isotopes in suspended particulate matter (SPM) (**Publication III**) did not eliminate sources of terrestrial or anthropogenic origin during winter and early spring (i.e., March). All through the remaining productive period (April–October), however, the main drivers of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variability were established to be the succession of phytoplankton species, marking limited impact from terrestrial or anthropogenic sources on primary production. Diatoms, dinoflagellates and mixotrophic ciliate *Mesodinium rubrum* showed the strongest positive relation to isotopic changes in the Gulf of Riga, whereas the diazotrophic cyanobacteria had an evident but statistically insignificant negative effect on  $\delta^{15}\text{N}$  values.



**Figure 2.** Average daily production rates (GPP, NPP and R) in the central part of the Gulf of Riga (depth > 30 m) during 2011 and 2012. Figure from **Publication II**.

The annual GPP in coastal waters was 8.5–14.1% lower (i.e., 323 g C m<sup>-2</sup>; **Publication IV**) than estimated for the euphotic layer of the central part of the Gulf of Riga (**Publication II**). This spatial difference most likely is a result of lowered water transparency due to coastal water turbidity and a direct impact of opaque freshwater discharge at the coastal study site. Indeed, Secchi depth varied between 2.3 and 5.1 m in the central part of the Gulf of Riga (**Publication II**), whereas it did not exceed 2.5 m in studied southern coastal area (station 101A; **Publication IV**). Based on National monitoring data (see database <https://latmare.lhei.lv/>) the euphotic layer depth differed notably between coastal and open areas; during period 2011–2014, on average it was 5.9 m and 9.7 m for coastal (101A) and open waters (119), respectively. In contrast to this observation, a 20% higher primary production was observed in the Daugava plume compared to the open Gulf of Riga during 1994–1997 (Wasmund et al., 2001; Olesen et al., 1999). However, differences in the approaches of sampling and calculations between this study (**Publication II, IV**) and the previous studies (Wasmund et al., 2001; Olesen et al., 1999) have to be noted. The most important difference is that the plankton community of the upper mixed layer was sampled and used for the oxygen metabolism incubations by Olesen et al. (1999) and Wasmund et al. (2001), hence the layer depth compared between open and coastal waters differed (ranging from 15 – 25 m for station 119 and 10 m for the coastal station 101A). In this doctoral thesis, plankton community from euphotic layer (assumed to be 10 m) was sampled and used in the dark-and-light-bottle incubations (**Publication II, IV**). The difference in the sampling approach, when including also non-euphotic layer where naturally is lower autotrophic organism biomass (Andersson, Rudehäll, 1993; Lugioyo et al., 2007), potentially has resulted

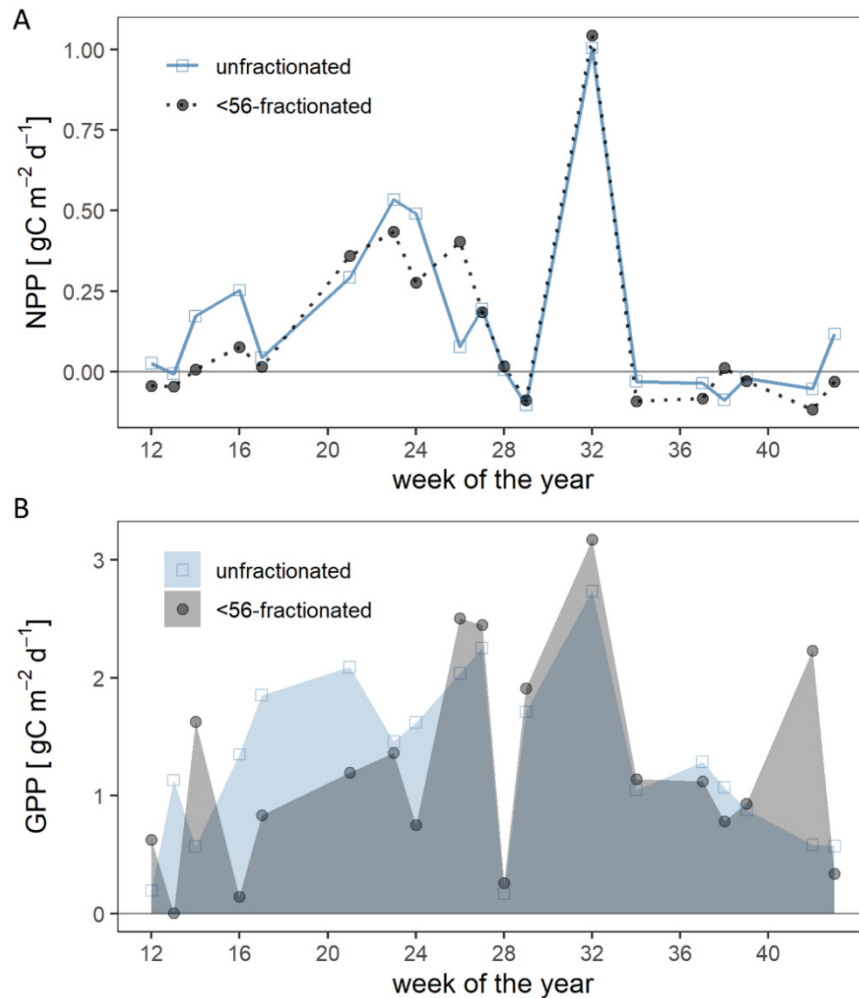
in more diluted integrated samples. Subsequently, incubations by Olesen et al. (1999) and Wasmund et al. (2001) resulted in lower gross primary production values in open water stations compared to coastal waters where only euphotic layer was sampled, thus limiting direct comparison of the studies considering open waters and spatial differences; the results from the coastal areas, though, are directly comparable.

### 3.2 Planktonic community metabolism

Plankton community respiration varied between 0.01 and 3.12 g C m<sup>-2</sup> d<sup>-1</sup> (on average 1.01 g C m<sup>-2</sup> d<sup>-1</sup>) and accounted for a vast portion (40.4% to 68.5%; **Figure 2**) of GPP. Size-fractionation exposed unfractionated communities as inefficient net producers due to their high respiration rates (**Figure 3**). Hence, unsurprisingly, the amount of both NPP and GPP of the unfractionated community was not significantly different from the amount produced by the <56-fractionated community (for NPP  $V_{\text{Wilcoxon}} = 205.00$ ,  $p = 0.488$ ,  $n = 19$ ; for GPP  $V_{\text{Wilcoxon}} = 194.00$ ,  $p = 0.708$ ,  $n = 19$ ). Moreover, the results of this doctoral thesis demonstrate strong covariation between NPP and <56-fractionated community taxa, particularly the small-sized (16–33 μm) *M. rubrum* biomass (**Figure 4**). This implies that the majority of NPP stems from the lower end of the size spectrum.

Notably, the respiration of the unfractionated community was high because it also included heterotrophic protozoans (e.g., tintinnids) and metazoans (e.g., rotifers and Copepoda nauplii). In general, respiration of microzooplankton is estimated to reach 35–43% on average of daily primary production (Calbet, Landry, 2004), whereas Witek et al. (1997) calculated zooplankton respiration to contribute 1% of the total respiration during winter and 20% (on average) in the remaining period. Anyhow, due to the size overlap, it was impossible to filter out zooplankton before incubation without removing diatoms, cyanobacteria filaments, and dinoflagellates. Hence, the results of respiration (and GPP) should be interpreted with caution if compared to estimates obtained by a different method (i.e., other than the light-dark bottle oxygen technique).

The productive layer of the central Gulf of Riga was net heterotrophic (NPP < respiration) during winter and early spring (**Figure 2**), whereas coastal plankton communities were more dynamic and shifted from net autotrophic to net heterotrophic throughout the year (**Figure 3A**). An increase in planktonic community respiration towards river mouths has been also observed in, e.g., the Gulf of Gdańsk (Witek et al., 1997). In transitional water systems, a high rate of allochthonous matter boosts bacterial activity increasing the planktonic community respiration (Alongi, 1998; Smith, 1993; Hopkinson, 1985), and, consequently, causes the shifts in community metabolism (varying between net autotrophic and net heterotrophic).

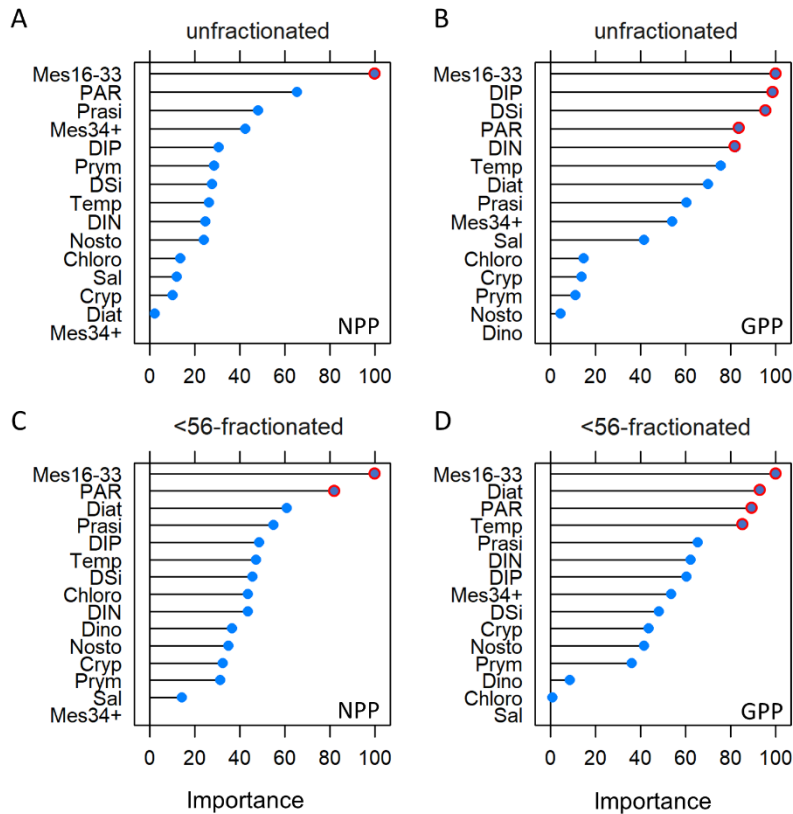


**Figure 3.** Average daily A) net primary production (NPP) and B) gross primary production (GPP) of unfractionated and <56-fractionated autotrophic communities from the top 10 m layer in the coastal Gulf of Riga (Station 101A). Figure from **Publication IV**.

The observed dynamics of pelagic habitat metabolism are in contrast to the study by Olesen et al. (1999) which identified mid-to-late spring (April–May) as the only period when the productive layer of the Gulf of Riga was net autotrophic. Albeit, the study by Olesen et al. (1999) denoted respiration measurements as hampered for the summer season; the community respiration was overestimated in their study resulting in an underestimation of summer NPP, hence net autotrophic periods throughout the rest of the productive season might have been left unidentified. Also, studied layers differ between this study and the study by Olesen et al. (1999). Here, planktonic communities from 0–10 m are described whereas in Olesen et al. (1999) sampling was conducted from the upper mixed layer (varying from 10 to 25 m depth). The differences between sampled communities could be the reason for the discrepancies.

All things considered, the euphotic layer of the Gulf of Riga, most likely, is net autotrophic during spring and early summer and in many instances during the rest of the year

depending on environmental factors and autotrophic biomass. Multivariate analysis showed that the NPP was influenced by fewer environmental factors than the GPP (Figure 4) in the Gulf of Riga. The partial least squares regression analysis identified small-sized *M. rubrum* and PAR as the most important influencing parameters for NPP in both unfractionated and <56-fractionated communities (Figure 4A, C), whereas for GPP, besides small-sized *M. rubrum* and PAR, also nutrients, temperature, and carbon mass of diatoms were identified as significant factors (Figure 4B, D).



**Figure 4.** Variable Importance for the projection (VIPs) for explanatory variables of partial least squares regression (PLSR) model. A) VIPs of NPP of unfractionated community, B) VIPs of GPP of unfractionated community, C) VIPs of NPP of <56-fractionated community, and D) VIPs of GPP of <56-fractionated community. The most influential variables (VIPs > 80%) are marked with a red circle. Figure from **Publication IV**.

### 3.3 New and regenerated primary production

Primary production in the euphotic layer can be viewed as dependent on two different sources of nitrogen supply. One is the dissolved nitrogen compounds derived from the metabolism of heterotrophic organisms, e.g., ammonia, urea and to a lesser extent amino acids. The primary production resulting from this recycled nitrogen is called “regenerated” production. In a theoretical balanced steady-state system, the recycling of

nutrients can continue indefinitely. However, in real ecosystems, there are losses such as the flux of sinking material, migration and extraction of organisms (fishing) and most importantly, denitrification. The losses have to be replaced by external inputs of nutrients to avoid a decline in the productivity of the system. Nutrient replenishing is a natural process occurring during convective mixing – a period when nutrients accumulated in deeper layers are brought upwards to the euphotic layer. Although this is the dominant process of nitrate renewal, other processes of external nitrogen inputs exist, e.g.,  $N_2$  fixation by diazotrophs and inputs of terrestrial and anthropogenic origin. The primary production based on external nitrogen input in the form of  $N_2$  or oxidized nitrogen forms is called “new” production (Eppley, Peterson, 1979).

The nutrient concentration decrease pattern suggested that the new production period in the Gulf of Riga lasts until June and shifts to the predomination of regenerated production afterwards. That coincides with the previous studies (Lundsgaard et al., 1999; Olesen et al., 1999; Olli, Heiskanen, 1999) which revealed high pelagic recycling efficiency in the Gulf of Riga during the productive period, especially summer. The average new production, calculated from the nutrient consumption, was equivalent to 51.8% of NPP during the studied period. Still, it varied together with phytoplankton succession. New production exceeded 75.0% of NPP during diatom bloom, whereas during the dinoflagellate dominance period, it was approximately 50% (**Publication II**). If new production is calculated from the consumption of  $SiO_4$  (following Wasmund et al., 2013), it gives an average estimate of  $1.95 \text{ g C m}^{-2} \text{ d}^{-1}$  for the diatom bloom period (April–May). This implies the significant role of diatoms in primary production during the spring bloom period. Also, the results of **Publication III** indirectly support the importance of diatoms during the spring bloom, but no direct linkage between diatoms and primary production rates was identified in other studies of this doctoral thesis (**Publication I, II, IV**).

In the summer nutrient-regenerating system, diazotrophic cyanobacteria are well known for bloom formation in the Baltic Sea (Kahru et al., 1994; Eigemann et al., 2019; Liepina-Leimane et al., 2022) despite the low concentrations of inorganic nutrients. Ploug et al. (2010) showed that cyanobacteria *A. flosaquae* was highly productive in the Baltic Sea with high rates of C and N assimilation and the capacity to release a large fraction (35.5%) of newly assimilated N. This suggests that the population of *A. flosaquae* sustains the observed high biomass values in the Gulf of Riga (**Publication II**) by rapid recirculation of phosphorus upon the death of phytoplankton cells and the assimilation of nitrogen via  $N_2$  fixation. This is supported also by the isotopic signals (**Publication III**) that displayed a negative effect (although, insignificant) of diazotrophic cyanobacteria on  $\delta^{15}N$  values during the summer period. Dugdale and Goering (1967) noted that nutrient recycling is almost non-measurable due to its continuous and fast nature that prevents the detection of changes in concentrations of nutrients and makes causal linkages ambiguous.

### 3.4 Importance of phytoplankton community on primary production

SPM is a dynamic pool of both living and non-living particles that can have a role in the functioning of food webs, nutrient and contaminant cycling, and system productivity, especially in coastal and estuarine environments (Cresson et al. 2012; Golubkov et al. 2017; Jędruch et al. 2017; Xu et al. 2019). The amount and composition of SPM in such environments are affected by various external sources like riverine inflows, coastal erosion, and atmospheric deposition, as well as internal processes like primary production and mineralization of organic matter.

In the Baltic Sea surface layer, SPM isotopic content is generally controlled by the presence or absence of phytoplankton that incorporates dissolved nutrients into SPM (Winogradow et al. 2019; **Publication III**). As dissolved nutrient concentrations in the water column decrease, autotrophic organisms, i.e., phytoplankton, exhibit less discrimination to absorbing isotopically enriched and energetically more consuming dissolved carbon compounds, e.g., bicarbonate or atmospheric CO<sub>2</sub> (Golubkov et al. 2017). Detailed examination of phytoplankton species along with primary production rates revealed key species governing the nutrient fluxes and the productivity of the Gulf of Riga. The mixotrophic ciliate *M. rubrum* prevails in all seasons and significantly correlates with elevated GPP, while diazotrophic cyanobacteria *A. flosaquae* contributes to new production in the summer nutrient-regenerating system (**Publication II, III**).

As mentioned previously, the dynamics of stable carbon and nitrogen isotopes in SPM (**Publication III**) strongly indicate that the cause of the variability in carbon and nitrogen isotope fractionation is biological processes. The co-variation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with seasonally changing abiotic factors, e.g., temperature and nutrient concentrations, establishes the seasonal nature of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (as observed in Savoye et al., 2003), reflecting the seasonal succession of phytoplankton species (**Publication III**). It defines carbon and nitrogen isotopic ratios of SPM during the spring bloom and substantially affects isotope ratios until mid-autumn. Diatoms, dinoflagellates, and *M. rubrum* show the strongest positive relation to isotopic changes in the Gulf of Riga, meaning that their growth enriches <sup>13</sup>C and <sup>15</sup>N implying new production (hence, coinciding with the results of **Publication II**). Whereas the lower values of  $\delta^{15}\text{N}$  during summer can be explained by isotopically depleted atmospheric nitrogen ( $\delta^{15}\text{N} = 0\text{‰}$ ) fixation by diazotrophic cyanobacteria. However, the negative correlation between cyanobacterial biomass and  $\delta^{15}\text{N}$  values is not evident in summer (**Publication III**), plausibly, because the cyanobacteria were never the main and only dominating taxa during the non-vernal phytoplankton community.

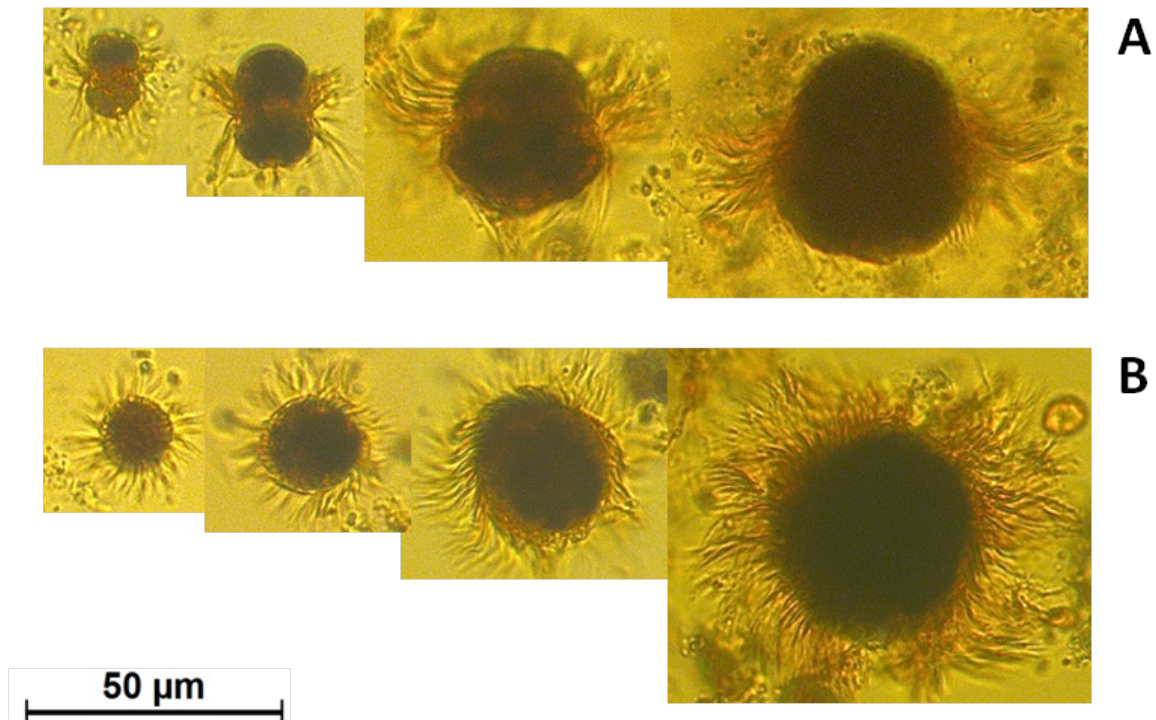
By the end of the spring bloom, in April,  $\delta^{15}\text{N}$  values barely exceeded the range of values that are typical for deep ocean inorganic nitrogen (Jędruch et al., 2017; Pantoja et al., 2002) or Baltic Proper surface SPM  $\delta^{15}\text{N}$  values (Winogradow et al., 2019) suggesting a limited impact of the riverine inflows, which delivers inorganic nitrogen of terrestrial and anthropogenic

origin. Presumably, in the absence of a significant external nitrogen pool by the end of spring bloom, the  $\delta^{15}\text{N}$  value in SPM would be determined by the recycling of an already assimilated nitrogen pool. Contrary to this assumption, the highest  $\delta^{15}\text{N}$  value was observed in May when no further phytoplankton carbon biomass increase could be detected. Most likely, the shift in phytoplankton species composition, e.g., successive diatom replacement by actively migrating ciliate *M. rubrum* in May, was an important factor. It is highly possible that the vertical migration ability of this ciliate causes  $\delta^{15}\text{N}$  value increase from utilizing the bottom layer nitrogen pool (Lips, Lips, 2017). Additionally, it is possible that unfiltered water, as sampled in the study, contains other taxa, e.g., small-sized zooplankton that could increase the overall  $\delta^{15}\text{N}$  values of bulk SPM as shown by Rolff (2000).

*Mesodinium rubrum* is a mixotrophic Litostomatea ciliate that possesses plastids preying upon cryptophyte algae (Johnson et al., 2016; Fenchel, Juel Hansen, 2006). It is highly productive in turbid waters and at low light irradiance (Crawford, 1989; Herfort et al., 2012; Johnson, Stoecker, 2005; Moeller et al., 2011). *Mesodinium rubrum* is often abundant in estuarine-coastal waters (Cloern et al., 1994; Leles et al., 2017; Sanders, 1995), including the brackish Baltic Sea (Lips, Lips, 2017; Rychert, 2004). The next section delves into *M. rubrum*'s behaviours and strategies that allow it to dominate in the Gulf of Riga and beyond.

### **3.5 Mixotrophic ciliate *Mesodinium rubrum* – an efficient key player**

*Mesodinium rubrum* has been identified as an important contributor to the primary production of the Gulf of Riga in **Publications I, II, and III** of this doctoral thesis. Hence, its role was investigated more closely in **Publication IV** by assessing the production of size-fractionated autotrophic communities, including small-sized (length of 16–33  $\mu\text{m}$ ) and large-sized *M. rubrum* (length  $\geq 34 \mu\text{m}$ ). *Mesodinium rubrum* displays a wide size distribution (from 15 to 70  $\mu\text{m}$ ; **Figure 5**); therefore, size distinction is introduced in numerous studies on *M. rubrum* (e.g., Johansson, 2004; Johnson et al., 2016; Montagnes et al., 2008), revealing different ecological responses to environmental changes between size classes.



**Figure 5.** A) Lateral and B) superior view of *Mesodinium rubrum* cell demonstrating its wide size distribution.

*Mesodinium rubrum* showed significant covariation to primary production rates in both open and coastal areas (**Publication I, II, IV; Figure 4**) despite the different underwater light conditions, expressing its flexible nature. Additionally, in several other Baltic sub-basins, *M. rubrum* has been stated as a significant contributor to primary production (Höglander et al., 2004; Johansson, 2004; Lips, Lips, 2017; Nielsen, Kiørboe, 1994), implying its essential role as one of the main producers in the Baltic Sea and beyond as it has been noted as highly productive organisms in various turbid waters and, especially, at low light irradiance (Crawford, 1989; Herfort et al., 2012; Johnson, Stoecker, 2005; Moeller et al., 2011). Hence, *M. rubrum* is often abundant in estuarine-coastal waters (Cloern et al., 1994; Leles et al., 2017; Sanders, 1995) and under certain conditions, it forms blooms (red tides) (Taylor et al., 1971), yet *M. rubrum*-induced red tides are not reported from the temperate Baltic Sea region – the research area of the present study.

Notably, *M. rubrum* has been recently recognised as a species complex consisting of at least two described and accepted species – *M. rubrum* and *Mesodinium major* (cf. Garcia-Cuetos et al., 2012; Johnson et al., 2016). Both have similar morphology and they possess plastids of the same origin (red plastid cryptophytes), but they show differences in cell length and ecology. However, as molecular methods were not utilised in this doctoral thesis all *M. rubrum*-like ciliates are referred to as *M. rubrum*. However, studies on the genetic diversity of *M. rubrum* should follow to continue expanding the understanding of the processes, including primary production in the Gulf of Riga and the Baltic Sea in general.

*Mesodinium rubrum* is known to have migration behaviour based on the response of phototaxis (Crawford, Lindholm, 1997) and has wide temperature, salinity and light tolerances (Lindholm, Mörk, 1990; Olli et al., 1996). It can migrate vertically over tens of meters per day (Hajdu et al., 2007), exploiting the nutrient-rich lower layers. *Mesodinium rubrum* benefits from this behaviour under stratified conditions in the shallow Baltic Sea (Lips, Lips, 2017). Due to the focus on the surface layer in the present study, subsurface accumulations of motile phytoplankton are most likely missed, potentially resulting in an underestimate of the overall abundance and biomass of *M. rubrum*. However, the efficient production of *M. rubrum* is attributed to mixotrophy and photosynthetic machinery of cryptophyte-originated chloroplasts that are well adapted to dim light (Daneri et al., 1992; Herfort et al., 2012) rather than the ability of vertical migration. In general, mixotrophy is an advantage under nutrient-limited conditions (Mitra et al., 2014), and it is an important feeding strategy during the decline of spring blooms and during summer or other periods when the system shifts from net autotrophy to net heterotrophy (Haraguchi et al., 2018). However, the ecological flexibility of *M. rubrum* and its implications for its phototrophic production remain poorly understood and require further in-depth research.

The photosynthetic activity of *M. rubrum* increases with the availability of cryptophytes, although with a 7-day lag (Gustafson et al., 2000). Cryptophytes are common in the Gulf of Riga during summer (**Publication IV**). Hence, the acquisition of chloroplasts does not limit the growth of *M. rubrum*, allowing it to reach the highest efficiency in photosynthetic activity without notable limitations. Although no direct linkage between cryptophytes and primary production rates was detected in this study, cryptophytes are known to prevail in biomass and contribute significantly to production in the polar regions, especially in the upper mixed layer under stratified conditions (e.g., Moline et al., 2004; Mendes et al., 2018). Polar regions are not the main distribution area of *M. rubrum*, hence under low predation pressure cryptophytes thrive there (Anschütz et al., 2022).

Cryptophytes affect various aspects of the performance of *M. rubrum*. In addition to the aforementioned physiological components, the availability of *Teleaulax* cryptophytes results in a reduction of the average size and volume of *M. rubrum* cells, as the high prevalence of cryptophytes promotes cell division (Gustafson et al., 2000). This is a potential explanation for a shift to small-sized *M. rubrum* during summer in the Gulf of Riga as cryptophytes were the most abundant in the period between weeks 26 and 32. A shift from larger to smaller *M. rubrum* during summer has also been observed in other Baltic Sea regions. This is, however, explained by increased grazing pressure (Johansson, 2004; Rychert, 2004; Witek, 1998), higher temperature (Garcia-Cuetos et al., 2012; Haraguchi et al., 2018), and low DIN values (Haraguchi et al., 2018) during the summer.

### 3.6 Primary production under changing environmental factors

Primary production is a fundamental process in any ecosystem as it provides energy for the entire food web. Therefore, it is critically important to understand and quantify the regulatory effects that environmental factors have on rates of important biogeochemical processes such as primary production. Realistic biogeochemical rates are fundamental for properly calibrating coupled hydrodynamic-biogeochemical models (Kuliński et al., 2022) which are the foundation of successful ecosystem-based management implementation.

New production, in theory, is directly dependent on mixing and vertical advective processes. Gross primary production, on the other hand, in a long term depends on new production to replace the lost material due to sinking, extraction or other processes. Thus, climate change can have an impact on the primary productivity of shelf seas by affecting meteorological and thermohaline situations in the systems. In line with future global projections, climate change scenarios for the Gulf of Riga region foresee a continuation of already occurring air temperature and precipitation increases. A consequent drop in frost and ice days will follow (BACC II, 2015). Overall, the winters will become milder and sea-ice cover will decrease, but the summers will become more pronounced. The changes, most likely, will promote stratification and inorganic nutrient (especially nitrogen) limitation in the surface layer, as well as potentially decrease the light availability even further (Skudra, Lips, 2017; Sommer et al., 2012; Winder, Schindler, 2004). From an ecological perspective, organisms with coping mechanisms to nutrient deficiency in the euphotic layer (e.g., motile *M. rubrum*, diazotrophic cyanobacteria) will outperform others under such conditions (Griffiths et al., 2016; Spilling, Markager, 2008; Wasmund, Uhlig, 2003). Moreover, future climate conditions appear non-detrimental to cryptophytes (Gaillard et al., 2020), thus predicting the continuous availability of cryptophyte-originated chloroplasts ensuring autotrophy of mixotrophic ciliate *M. rubrum*. Several studies have revealed that *M. rubrum* is able to remain photosynthetic and survive for months at low irradiance (Johnson, Stoecker, 2005) considering its efficient inorganic nutrient uptake rates (Stoecker et al., 1991; Tong et al., 2015; Wilkerson, Grunseich, 1990). Therefore, an increase in the prevalence of *M. rubrum*, along with a consequent rise in its contribution to primary production, can be expected in the region, especially during the summer period. However, shifts in communities at the first trophic level, plausibly, will affect the following food chains – a topic that requires further studies.

Projected climate change (BACC II, 2015) is likely to exacerbate eutrophication effects in the Baltic Sea due to (i) increased runoff that will consequently increase external nutrient loads and (ii) water temperature rise that will reduce oxygen flux from the atmosphere and intensify internal nutrient cycling (e.g., Meier et al., 2011, 2012a; Neumann et al., 2012; Omstedt et al., 2012; Meier et al., 2018). In the Baltic Proper, phytoplankton growth and consequent primary production will likely increase as a result of the projected

intensification of nutrient cycling in the euphotic zone. Also, bacterial activity will rise (e.g., Wulff et al., 2001) and enhance nutrient flow from the sediments (Meier et al., 2012b) amplifying the effects. On the other hand, in the northern Baltic Sea, primary production may be reduced due to increased land runoff of allochthonous organic matter that will favor heterotrophic bacteria (Andersson et al., 2015). The potential changes in the primary production of the Gulf of Riga have not yet been the focus of the modelling studies, hence it requires more attention in future research disentangling the effects from eutrophication and brownification (decrease of water transparency due to increased optically-active allochthonous material, e.g., dissolved humic compounds). However, a recent study revealed that brownification affects phytoplankton community composition shifting from larger to smaller organisms but it did not affect primary productivity in eutrophic coastal waters (Spilling et al., 2022).

Another environmental aspect that unavoidably is changing in global oceans, including the Baltic Sea, is ocean acidification (decrease in pH) due to increased CO<sub>2</sub> concentration in the atmosphere. Ocean acidification is known to have both direct and indirect impacts on pelagic species, communities and entire ecosystems. Noteworthy that the responses of Baltic phytoplankton to acidification are highly variable and species-specific (Havenhand et al., 2019). Cyanobacterial species display positive, negative or no response (western Baltic, Bergen et al., 2016; Gulf of Finland, Hornick et al., 2017), *M. rubrum* also seem tolerant (Nielsen et al., 2010), whereas diatoms increased under acidification treatment in experimental studies (Kremp et al., 2012). Additionally, mesocosm studies revealed that ocean acidification can influence phytoplankton community structure, but overall phytoplankton productivity tends to increase under acidification treatment, although with seasonal variation in the response (Skagerrak, Eberlein et al., 2017).

In conclusion, primary production fuels the pelagic food web, and, consequently, climate-driven shifts in primary production will have major impacts on carbon cycling and the structure and functioning of the entire marine biome. Understanding the underlying processes and drivers will help to improve the conceptual understanding of the complex marine system and its functioning. The results of this doctoral thesis identified phytoplankton functional groups (i.e., small-sized *M. rubrum* and diatoms) that play a key role in primary productivity in the Gulf of Riga. Hence studies focusing on their responses to climate change and their specific interactions with other trophic guilds (e.g., competition or predator-prey relationships) are required to understand the Gulf of Riga food web dynamics and ecosystem as a whole.

## CONCLUSIONS

- The annual primary production in the Gulf of Riga was 353–376 g C m<sup>-2</sup> in our study. It showed no evident increase in productivity compared to the period 1993–1995.
- The highest net (NPP) and total (GPP) primary production can be observed in the spring, while autumn was the least productive during the growth season. New production prevailed during spring, accounting for 51.80% of spring NPP.
- During the productive period in the Gulf of Riga, the source of substances available to phytoplankton changes seasonally. Based on the isotopic signals, the spring measurements differed from the rest of the period, implying greater influence from terrestrial and anthropogenic sources.
- Diatoms, dinoflagellates, and *Mesodinium rubrum* show the strongest positive relation to isotopic ratio changes in the Gulf of Riga, implying their contribution to new production.
- Mixotrophic ciliate *Mesodinium rubrum* prevailed in all seasons and significantly correlated with elevated productivity. Whereas diatoms were identified as the main contributors to new production in spring and diazotrophic cyanobacteria *Aphanizomenon flosaquae* – in a nutrient-regenerating system of the summer.
- *Mesodinium rubrum* is identified as a significant contributor to primary production in the Gulf of Riga. A close covariation between small-sized (16–33 µm) *M. rubrum* and NPP was detected in the coastal waters of the Gulf of Riga.
- The primary production dynamics of both the unfractionated and <56-fractionated plankton community in the Gulf of Riga is directly influenced by the biomass of *Mesodinium rubrum* small-sized (16–33 µm) cells and the amount of available light, while the primary productivity of the <56-fractionated plankton is also affected by temperature and diatom biomass.

## THESIS FOR DEFENCE

- The amount of primary production has decreased in the Gulf of Riga compared to the period 1993–1995 due to the changed management of the Gulf of Riga (due to decreased P and N loads).
- Phytoplankton taxonomical composition, size structure and biomass affect primary production dynamics.
- The new production in the Gulf of Riga is formed by diatoms in spring and diazotrophic cyanobacteria – in summer.
- The ciliate *Mesodinium rubrum* is an important contributor to net primary production of the Gulf of Riga.

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## **PUBLICATIONS**



I

## Spring seasonal pattern of primary production in the Gulf of Riga (Baltic Sea) after a mild winter

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### Abstract

**ABSTRACT:** Spring phytoplankton production was estimated by a light and dark bottle oxygen technique in the southern part of the Gulf of Riga during spring 2014. The main study objective was to determine factors that define the spring primary productivity by analyzing the pattern of primary production and changes in succession of phytoplankton species along with environmental factors. The productivity was measured after an untypically mild winter without ice-cover on the Gulf of Riga and spring flooding, which resulted in large amounts of terrestrial nutrients retained in the catchment area. At that time precipitation played a major role in river-originated nutrient inflow in the study area. A vague diatom bloom was stretched throughout April, followed by increase in biomass of dinoflagellate *Peridiniella catenata* and autotrophic ciliate *Mesodinium rubrum*. The total amount of spring primary production (March–May) reached 116.3 to 127.2 g C m<sup>-2</sup> in the southern region of the Gulf of Riga. Our results suggest that diatoms contributed most to the “new” production and net primary production, whereas dinoflagellate *P. catenata* and autotrophic ciliate *M. rubrum* vernal production was mostly based on regenerated nutrients, although showing also the highest respiration rates.

**Key words:** eutrophication, Gulf of Riga, nutrients, phytoplankton, productivity, vernal succession.

**Abbreviations:** Chl, chlorophyll *a*; DIN, sum of nitrite, nitrate and ammonium; DIP, dissolved inorganic phosphate; DSi, dissolved silicate; GPP, gross primary production; *k*, attenuation coefficient; NPP, net primary production; PCA, principal component analysis; PSU, practical salinity units; R, planktonic community respiration; TN, total nitrogen; TP, total phosphorus.

### Introduction

Baltic Sea is a shallow intra-continental brackish sea, affected by large nutrient inputs from densely populated drainage areas. The eutrophication status of nine of its seventeen sub-regions has been assessed as bad (Andersen et al. 2011), based on several environmental parameters, including winter concentrations of phosphorus and nitrogen, phytoplankton biomass and concentration of chlorophyll *a*. Although the amount of primary production is a good proxy of eutrophication, it is not included as indicator in eutrophication assessments due to lack of data in the Baltic Sea region (Wasmund et al. 2001). Even in the Baltic Sea Environmental Proceedings of Eutrophication (HELCOM 2009; HELCOM 2014), almost no long-term annual means of phytoplankton biomass and in situ primary production were reported.

In The Gulf of Riga is one of the Baltic Sea sub-regions marked with bad eutrophication status (Andersen et al. 2011; HELCOM 2014) and it has one of the longest phytoplankton time-series in the world, covering almost four decades (Jurgensone et al. 2011). Still, only two studies have been carried out to estimate primary production of the area (Olesen et al. 1999; Wassmann, Tamminen 1999; Wasmund et al. 2001) and both of them were carried out

almost 20 years ago, during a period when one of the lowest concentration of dissolved inorganic nitrogen was observed in the region (Jurgensone et al. 2011). Simulated annual primary productivity showed a slight decrease in 1990-ies as well, reaching up to 206 g C m<sup>-2</sup> (Müller-Karulis, Aigars 2011), consistent with the results of previously mentioned studies that estimated annual primary production in the range of 255 (Wasmund et al. 2001) to 350 g C m<sup>-2</sup> (Olesen et al. 1999). However, the concentration of nitrogen started to increase from early 2000-ies, and the concentration of phosphorus increased steadily at least until 2007 (Müller-Karulis, Aigars 2011), therefore we expect an increase in primary production as well.

The phytoplankton dynamics in the Gulf of Riga follows the classical scenario of boreal phytoplankton development (Yurkovskis et al. 1999; Jurgensone et al. 2011; Olli et al. 2011). Expressed spring blooms can be observed after ice melting, in April–May, which are usually dominated by diatoms *Achnanthes taeniata* and *Thalassiosira baltica*. Towards the end of the spring bloom, the diatom *Chaetoceros* spp. and dinoflagellate *Peridiniella catenata* become the dominating ones. The development of thermal stratification favours motile dinoflagellates, but promotes sinking of large-sized, non-motile diatoms. As a result, the majority of spring bloom production leaves the euphotic

zone and settles in sediments. In the second half of summer, a bloom of cyanobacteria (mainly *Aphanizomenon flos-aquae*) accompanied by chlorophytes and cryptophytes can occur, but succession of phytoplankton is terminated by the second bloom of diatoms in September-October, after the disruption of the thermocline (Yurkovskis et al. 1999; Jurgensone et al. 2011 and the references therein). As the bulk of annual primary production is produced during spring bloom and summer/autumn production contributes in a lesser way (e.g. Hällfors et al. 1981), the chosen study period was the spring season, covering the period from early March to the end of May. The main study objective was to determine factors that define the spring primary productivity by analyzing the pattern of primary production and changes in succession of phytoplankton species along with environmental factors in the southern part of the Gulf of Riga.

## Materials and methods

### Study area

The Gulf of Riga is situated at the north-eastern part of the Baltic Sea and covers an area of 16 330 km<sup>2</sup> with average depth of 26 m and maximal depth of approximately 60 meters. Its drainage basin (135 700 km<sup>2</sup>) covers more than eight times the surface area of the Gulf itself (Kotta et al. 2008), and 86% of the freshwater inflow comes from the southern part of the Gulf (Kļaviņš et al. 2002) resulting in a north-westward salinity gradient, from 0.5 – 2.0 practical salinity units (PSU) in surface layers of the southern regions to 7.0 PSU at the Irbe Strait. The mean salinity in the most parts of the Gulf of Riga is between 5.0 and 6.0 PSU. Surface water temperature varies seasonally from 0 to 20 °C.

Temporal and spatial pattern of ice coverage and duration varies annually and the average ice-season lasts approximately 80 days (Kotta et al. 2008 and references therein). In the coldest season (November to April) water layers of the Gulf are mixed, such that the temperature, salinity and nutrient concentrations throughout the water column are similar (Stigebrandt 1996). From April to mid-October seasonal stratification restricts vertical water exchange and promotes oxygen depletion and nutrient accumulation in the bottom layer until the entire water column is mixed again in autumn (Yurkovskis 2004).

### Sampling

Sampling was performed weekly in three stations (Fig. 1) during spring (March-May) 2014. Sampling was conducted using a Ferry Box Sampling Module (Ferry Box) installed aboard the Tallink passenger ferry “MS Romantika”, travelling between Riga and Stockholm. “MS Romantika” is 192.9 m long with draught of 6.5 m. The Ferry Box was used for the measurements and collection of water from 5 meter depth at the end of the ferry. Long term (1993-2012) mean depth of the vernal upper mixed layer of the

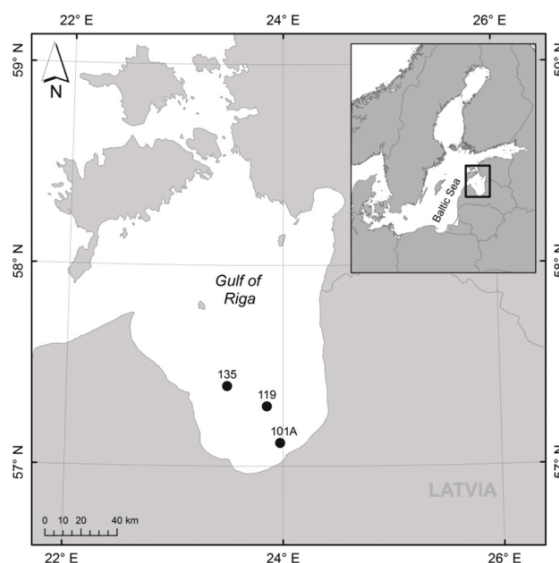


Fig. 1. The location of three sampling stations (black dots) in the Gulf of Riga.

Gulf of Riga is approximately 8 m deep with no evident trend over last twenty years (Skudra, Lips 2016), thus the assumption was made that samples collected from 5 m depth in general represents environmental conditions and planktonic community prevailing in upper mixed layer of studied locations.

Water samples for evaluating chemical parameters (nutrients), phytoplankton biomass and phytoplankton species composition, concentration of chlorophyll *a* (Chl) and community production/respiration were pumped via system of the Ferry Box as the ferry travelled. In a couple of hours samples were transported to laboratory for further execution. The data of water temperature and salinity were obtained from the system of the Ferry Box (PT-100 sensor). Meteorological data (maximal wind speed and dominating wind direction, precipitations and incident radiation) were obtained from the Latvian Environment, Geology and Meteorology Centre database for station located on building of University of Latvia in Riga (<https://www.meteo.lv>).

### Analysis of field samples

The samples were treated for analysis of chemical parameters, phytoplankton biomass and concentration of Chl according to HELCOM COMBINE Manual (HELCOM 2015). Concentration of ammonium (NH<sub>4</sub>) and phosphate (DIP) were measured by the indophenol blue and molybdenum blue methods (Grasshoff et al. 1983). The sum of nitrate and nitrite (NO<sub>2+3</sub>) was determined by nitrite reaction with an azo dye after reduction of nitrate to nitrite in a copper coated cadmium column. The nitrite was determined by reaction with an azo dye and nitrate

was determined as difference between nitrite and sum of nitrate and nitrite. In further text, DIN refers to for the sum of nitrite, nitrate and ammonium. Concentration of dissolved silicate (DSi) was determined colorimetrically according to the procedure described by Grasshoff et al. (1983). Concentrations of total nitrogen (TN) and total phosphorus (TP) were analyzed as nitrate and phosphate after wet digestion with persulfate.

To Chl was collected on glass fiber filters (Whatmann GF/F), extracted in 96% ethanol for 24 h and further analyzed by a spectrophotometer (Cary 100 Conc UV-Visible Spectrophotometer). Phytoplankton samples (300 mL) were fixed with acid Lugol's solution. Subsamples of 10 and 25 mL of fixed samples were settled in a sedimentation chamber for 12 h and counted according to Uthermöl technique with an inverted microscope Leica DMI3000 at 200× and 400× magnification. The number of counted cells in all subsamples exceeded 500 (Uthermöl 1958; HELCOM 2015; Olenina et al. 2006). The cell volume for phytoplankton wet weight biomass determination was determined using geometrical formulae for various suitable geometrical shapes (Edler 1979).

#### Estimation of primary production

Light and dark bottle oxygen technique (Olesen et al. 1999) was used to evaluate net primary production (NPP) and planktonic community respiration (R). Oxygen concentrations were determined by the Winkler titration method. Water was filled in 15 glass bottles with volume of 100 mL. Winkler reagents (1 mL manganese chloride and 1 mL alkaline iodide) were added to three bottles immediately for estimation of initial oxygen concentration, and then they were stored in darkness at room temperature, while the others were divided in four groups with different transparency level and placed in an incubator for further analysis.

Specific light transmittance to each group was provided using GAM optical filters: no filter for 100% transparency, 1514 GAM optical filter for 66% transparency, 1516 GAM optical filter for 23% transparency and aluminum folium for 0% transparency. All the vials were fixed on a rotating device and were submerged in water to ensure the least

possible changes in ambient temperature during incubation that lasted for 24 h. After the incubation, 1 mL sulphuric acid was added to the samples. Sodium thiosulphate was added gradually during the titration in order to determine oxygen concentration.

Oxygen consumption in the dark bottles (0% transparency) was used as a proxy of R, but the other three groups were used to evaluate NPP rates. R and NPP were transformed to carbon units. Daily produced carbon was calculated for each transparency group that was equalized to specific depths according to the attenuation coefficient (k). The attenuation coefficient was calculated based on the long-term average of Secchi depth in May for each sampling station. Then, a trapezoidal integration method was used to estimate daily NPP ( $\text{g C m}^{-2} \text{d}^{-1}$ ) rates of the upper 10 m layer. Gross primary production (GPP) was calculated by summing NPP and R (expressed as positive values).

#### Statistical analysis

A Principal Component Analysis (PCA) based on correlation coefficients was applied to the primary production variables and the environmental parameters to identify the main sources of data variability and detect which factors responded similarly both spatially and temporally. Five of the environmental parameters (DIP, DIN,  $\text{NH}_4$ ,  $\text{NO}_{2+3}$ , DSi) were used as supplementary parameters in order to avoid creation of a non-essential third component, as these five variables did not show strong relation to any primary production variables, though showed strong inter-correlations. The analysis was performed using StatSoft® STATISTICA 7. Data were Box-Cox (Wessa 2015) transformed prior to analysis to ensure similar (normal) distribution for all variables.

## Results

#### Meteorological conditions

The spring weather conditions were untypical for northern Europe in the year 2014. The winter was mild with little or no snow coverage on land (Table 1), and the Gulf of Riga was completely ice-free for the whole winter season. Accordingly, spring flooding did not occur. Wind conditions were moderate during spring 2014. The maximal wind speed was  $5 \text{ m s}^{-1}$  on average, and it exceeded  $8 \text{ m s}^{-1}$  only on several occasions (Fig. 2).

Due to absence of spring flooding, precipitation played the critical role in increasing river runoff and bringing fresh nutrient inputs to the Gulf of Riga. While riverine waters mainly affected the southern coastal regions of the Gulf, increased concentrations of nutrients were detected after rainfall in the farther station as well (e.g. station 135; see the next chapter). Regular precipitation occurred in the middle of March and beginning of May, but the heaviest rainfall occurred during the second week of April (Fig. 2).

**Table 1.** Meteorological observations during winter 2013/2014. Data were obtained from the Latvian Environment, Geology and Meteorology Centre database for station located on building of University of Latvia in Riga (<https://www.meteo.lv>)

Month	Average air temperature (°C)	Days below 0 °C (number)	Total precipitation (mm)
Dec 2013	2.7	5	57.4
Jan 2014	-5.5	20	33.7
Feb 2014	1.1	5	24.9

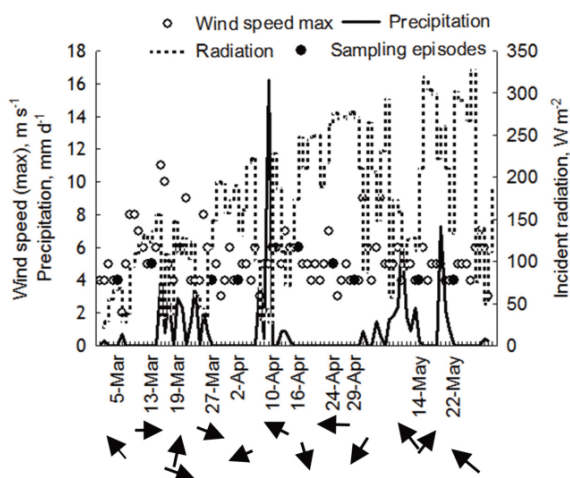


Fig. 2. Spring meteorological conditions (max. wind speed, dominating wind direction at sampling days, precipitations and incident irradiance) during spring 2014. Black arrows indicate the dominating wind direction during sampling day. Data obtained from the Latvian Environment, Geology and Meteorology Centre database for station located on the building of the University of Latvia in Riga.

#### Nutrients and hydrology

Initial spring concentrations of DIN, DIP and DSi were relatively low: 9.5, 0.6 and 17.8  $\mu\text{mol L}^{-1}$ , respectively (Fig. 3). Despite the slow development of phytoplankton during March (Fig. 4), concentrations of nutrients were almost constant. Windy weather and convective mixing ensured instability of water column in this period, constantly supplying fresh nutrients to the upper water layer. Evidence of consumption of nutrients started in the beginning of April. The concentration of DIN began to decrease slightly in the farther stations (135 and 119), whereas the continuous supply of fresh DIN input from the River Daugava was observed in station 101A. The concentration of DIN doubled in this station after heavy rainfall on April 10, reaching 17.9  $\mu\text{mol L}^{-1}$ . Subsequently it started to decrease in all stations, meeting the minimal value just slightly above 0.5  $\mu\text{mol L}^{-1}$  on May 15 (Fig. 3). However, a rainstorm on May 20 caused enrichment of DIN in two stations (101A and 119) closest to three large river plumes inflowing in the southern part of the Gulf.

Consumption of DIP started on April 2, but in contrast to DIN it did not vary with river runoff. Concentration of DIP reached a minimum that neared the analytical detection limit (0.1  $\mu\text{mol L}^{-1}$ ) on April 24 (Fig. 3) in farther stations 135 and 119. At the same time a rapid increase in values of Chl were detected (Fig. 4). Mean concentration of DSi was 17.9  $\mu\text{mol L}^{-1}$  when the diatom bloom started, and it never become depleted in any of sampled sites. In coastal station 101A, the River Daugava ensured fresh supply of DSi with runoff, which was especially evident after rainfalls (Fig. 3). Concentrations of TN and TP did

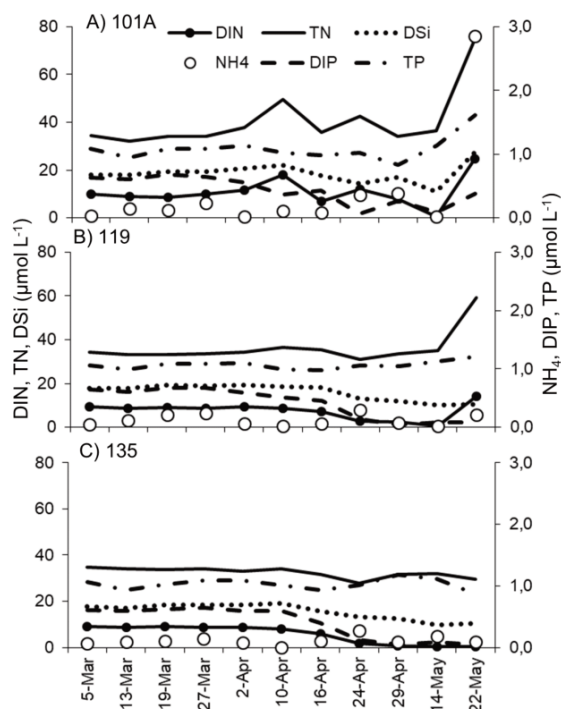


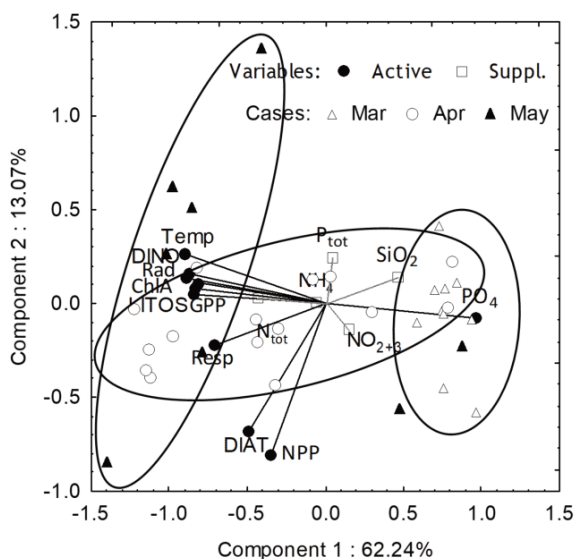
Fig. 3. Concentrations of nutrients in the Gulf of Riga during spring 2014 at three sampling sites. DIN, dissolved inorganic nutrient; TN, total nitrogen; DSi, dissolved inorganic silica; DIP, dissolved inorganic phosphorus; TP, total phosphorus.

not vary noticeably until May, when they started to increase rapidly, reaching the highest values.

Changes in water temperature showed dynamics of typical spring warming (Fig. 4) closely following increasing solar radiation (Fig. 2). Water temperature gradually rose from 1.6 to 14.6  $^{\circ}\text{C}$  during the study period (Fig. 4), reaching the point of maximal water density (salinity 5.5; 2.7  $^{\circ}\text{C}$ ) on April 2 and triggering the onset of thermal stratification at this point.

#### Phytoplankton biomass and production

Diatoms, dinoflagellates and autotrophic ciliate *Mesodinium rubrum* were analyzed during present study, as they are the dominating groups in the vernal phytoplankton community (Jurgensone et al. 2011). The succession of phytoplankton spring bloom in the Gulf of Riga followed the pattern for temperate coastal waters. The phytoplankton development was divided in three phases: pre-bloom period, growth-peak phase and declining phase. In the pre-bloom period, which lasted all March, diatoms comprised more than 80% of phytoplankton biomass (Fig. 4). Diatom *Thalassiosira baltica* dominated during this phase, and relatively small amount of biomass was composed by *Achnanthes taeniata*. The growth-peak phase started on April 2 and lasted until April 24, when increase of diatoms *Chaetoceros holsaticus* and *Chaetoceros wighamii* was detected. They gradually



**Fig. 6.** Results of PCA analysis. Plot for environmental variables of Principal component 1 and Principal component 2. Temp, temperature; Rad, Solar radiation, ChlA, chlorophyll *a*; DINO, dinoflagellates; LITOS, Litostomatea (*Mesodinium rubrum*); DIAT, diatoms; GPP, gross primary production; NPP, net primary production; Resp, community respiration; Nutrients (Ntot, Ptot, NH<sub>4</sub>, NO<sub>2+3</sub>, PO<sub>4</sub>, SiO<sub>2</sub>). Each ellipse compiles majority of cases from the same month (March, April, May), starting from the right side.

bloom termination and sedimentation of the biomass, within a period of approximately two weeks, accounts for half of the annual organic matter input to the benthos in the northern Baltic Sea. Moreover, it can account for 30 to 65% of annual primary production, depending on the dominant species (Heiskanen, Kononen 1994). Diatoms sink rapidly as intact cell aggregates, whereas the bulk of dinoflagellates disintegrate in the water column and sink slowly as refractory phytodetritus or in forms of resistant resting cysts (Heiskanen, Kononen 1994). Also zooplankters profit from spring bloom, as it is one of the triggers for their reproduction (Peinert et al. 1982).

The total value of spring GPP varied between stations from 116 to 127 g C m<sup>-2</sup> in the present study. However, the majority of GPP was produced in May (72 to 88 g C m<sup>-2</sup>) when dinoflagellates and autotrophic ciliate *Mesodinium rubrum* dominated. Consequently, only one third of spring production composed of diatoms could be effectively sedimented, while the remaining part of production regenerated in the water column, resulting in a considerably lower sedimentation rate and different nutritional value for benthic organisms.

There has been continuous discussions over increasing evidence of dinoflagellate dominance over diatoms. Kononen and Niemi (1984) suggested that diatom-dominated spring blooms tended to follow cold winters

with long-lasting ice cover, but mild ice-free winters or early ice breakup in spring favoured dominance of dinoflagellates during the spring bloom in the northern Baltic Sea. Klais et al. (2011) hypothesized that a specific sequence of seasonal events, involving wintertime mixing and resuspension of benthic cysts, followed by proliferation in stratified thin layers under melting ice, favoured successful seeding and accumulation of dense dinoflagellate populations over diatoms in coastal areas. In our study, the spring bloom began with the dominance of diatoms in the phytoplankton (Fig. 4), even after a mild winter. Thus, our study presents a possibility to test the classical theories of phytoplankton spring bloom development. The sampling of phytoplankton started at the beginning of March after a week of sunny weather (Fig. 2), when the early bloom of diatoms could be expected. The biomass of phytoplankton was low and 80% was composed by diatoms; however dinoflagellates and *M. rubrum* were already present in small quantities. At later sampling time environmental conditions favoured to growth of diatoms.

The mixing of water masses was promoted by stormy weather prevailing in March (Fig. 2). Vertical mixing of the water column is tolerated mainly by diatoms (Cushing 1989), mostly heavy diatom cells like *Thalassiosira baltica* and chain-forming *Achnantes taeniata*. Although turbulence decreases average light intensity, it favours non-motile cells by retaining them in euphotic layer (Kjørboe 1993). However, the biomass and gross primary production was low (Fig. 3, 4) during the turbulent period, as the phytoplankton cells, presumably, were often forced out of the shallow euphotic layer. However, community respiration of plankton was also low compared to that in later phases of the bloom. PCA suggested relation of lower respiration rate and relatively higher NPP values to diatom dominance (Fig. 6). According to Falkowski and Owens (1978) and Spilling and Markager (2008), diatoms have a lower respiration rate and higher growth rates under light limitation than dinoflagellates.

The onset of stratification might have triggered the spring diatom bloom. Unfortunately, no vertical profiles were examined during the present study, yet in theory during low winter temperatures (e.g. 1.6 °C in the present study) the seasonal warming is causing vertical mixing of water column until the maximum density point is reached (2.7 °C at the salinity of 5.5 prevailing in the Gulf of Riga). After that thermal stratification begins. In present study the maximum density point was reached on April 2. but the following increasing temperatures, presumably, stabilized the stratification. Constant increase of phytoplankton biomass was observed after the maximum density point was reached and thus we argue that onset of stratification triggered the diatom spring bloom. Although the conventional peak of diatoms was not observed, a rise in *Chaetoceros* spp. biomass was detected. *Chaetoceros* spp. is characteristic during the peak of the spring bloom in the

Gulf of Riga (Yurkovskis 1999; Jurgensone et al. 2011). During the growth-peak phase, increased consumption of nutrients from the upper water layer, which previously was hindered by vertical mixing, began.

While the initial spring nutrient concentrations were relatively low (Fig. 3) and the DIN:DIP ratio indicated nitrogen limitation according to the conventional Redfield ratio (Redfield 1963), PCA analysis recognized only DIP as a significant factor (Fig. 6) for phytoplankton development in the Gulf of Riga during spring 2014. Riverine runoff caused by precipitation played a major role supplementing fresh DIN (mainly in the form of  $\text{NO}_3$ ) to surface waters, but did not contribute to concentration of DIP. Accordingly, throughout the study period the DIN:DIP ratio increased from 14 up to more than 70, when the exhaustion of phosphates occurred due to biological uptake processes. Arrigo (2005) attributed diatoms to “bloomers” that invest energy in reproduction under low DIN:DIP ratios, as more phosphorus is required for synthesis of ribosomal RNA used for cell divisions, allowing diatoms to grow exponentially. Indeed, an increasing trend of diatom biomass was observed until April 24, when DIP decreased to its lowest values, resulting in an increased DIN:DIP ratio, despite a concurrent slight reduction in DIN.

The biomass of dinoflagellates *Peridiniella catenata* and autotrophic ciliates *Mesodinium rubrum* increased as the bloom of diatoms gradually ceased at the end of April. However the diatoms never completely disappeared from the phytoplankton community, even if the composition of species showed decline of the bloom. As the DIN and DIP concentrations were depleted after the end of the diatom bloom, particularly, in stations distant from riverine influence, the production of dinoflagellates and autotrophic ciliates could be based only on regenerated nutrients. In station 101A, fresh nitrate supply was observed, especially after rainy periods (May 22), obviously explaining the persistent existence of diatoms in this station. This indicates ability of more efficient assimilation of nitrates for *T. baltica* and *Chaetoceros* spp. than other spring phytoplankton taxa. Consequently, we deduce that spring diatoms contributed to the “new production”, whereas dinoflagellates *P. catenata* and autotrophic ciliates *M. rubrum* reincorporated the excreted nutrients and contributed mainly to “regenerated production”. Furthermore, the diatom associated nitrate uptake in the Gulf of Riga has been proved also experimentally (Berg et al, 2003).

Lower growth rates, as well as higher photosynthetic capacity at higher irradiance and higher respiration rates (Spilling, Markager 2008) allows dinoflagellates to thrive at lower nutrient concentrations, effectively using regenerated nutrients. Also, dinoflagellates, in contrast to diatoms, prefer high water column stability and are negatively affected by turbulence and mixing (Margalef 1978; Klais et al. 2013). At the declining phase of diatom bloom, an increase in biomass of all phytoplankton groups was observed, resulting in the highest values of total phytoplankton biomass at the end

of April and beginning of May. With the development of dinoflagellates and ciliates, also an increase in GPP was observed; however, 78 to 90% of the GPP were lost via community respiration (Fig. 5). The increased NPP at the end of May could be attributed to a secondary increase of diatoms in the near-shore stations (101A and 119), presumably supported by rise of river-originated DIN.

## Conclusions

The present study described untypical spring conditions after mild winter. However with climate changes, mild winters might become typical, as there is evidence that winter conditions in the study area have become milder over the last twenty years, and duration as well as coverage of ice in the Gulf of Riga has declined. The present study showed that the most important factors triggering the spring diatom bloom were onset of stratification, high concentrations of nitrates and also phosphates, as increased uptake of DIP was associated with diatoms. The diatoms are more rapidly growing and produce the larger part of NPP, which potentially sediments delivering high quality food for benthic organisms.

The nutrients remaining in the water column after the spring diatom bloom are regenerated by dinoflagellates and autotrophic ciliates *Mesodinium rubrum*, which produce the bulk of vernal GPP in the Gulf of Riga. However, most of the fixed carbon associated with regenerated production is lost via planktonic metabolic processes and only a minor part of it might reach the sediments, thus increasing the pelagic production in general. Lack of snow and spring flooding retains a lot of terrestrial nutrients in the catchment area, strengthening the importance of spring precipitation that creates pulse-like inflows of nutrients in the Gulf of Riga. As the diatom bloom is triggered by environmental conditions, which are not favourable for other phytoplankton groups, the bloom intensity will depend on the concentration of inorganic nutrients in the area. It might be speculated that in the future, small scale diatom blooms will be observed in the vicinity of river mouths over prolonged time periods and that the majority of phytoplankton assemblage in the spring might be composed of dinoflagellates and ciliates.

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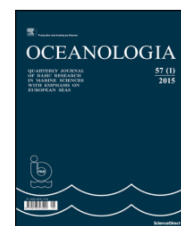




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ORIGINAL RESEARCH ARTICLE

# Primary productivity in the Gulf of Riga (Baltic Sea) in relation to phytoplankton species and nutrient variability

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Primary production;  
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*Aphanizomenon flosaquae*;  
Gulf of Riga;  
Baltic Sea

**Summary** The seasonal patterns of primary production, phytoplankton biomass, chlorophyll *a*, and nutrients were investigated in the central part of the Gulf of Riga (Baltic Sea) during 2011 and 2012. Annual primary productivity in the gulf was in the range of 353.4–376.2 gC m<sup>-2</sup>. Maximum carbon fixation rates occurred during the phytoplankton spring bloom from April to May when the winter nutrient pool was rapidly exhausted, suggesting the use of regenerated nutrients already in spring. The new production calculated on the draw-down of nitrates amounted to 51.80% of spring net community production. The production rates during summer were considerably lower owing to the availability of only regenerated nutrients and limited nitrogen fixation. Autumn was established as the least productive season. In autumn despite the elevated nutrient concentrations, the increasingly limited light hindered photosynthetic activity. Species governing the nutrient fluxes and the productivity of the Gulf of Riga are the diatom species responsible for new production in spring. The photosynthetic ciliate *Mesodinium rubrum* ((Lohmann) Hamburger & Buddenbrock 1911) prevailed in all seasons and significantly correlated with elevated productivity, while diazotrophic cyanobacteria *Aphanizomenon flosaquae* (Ralfs ex Bornet & Flahault 1886) contributed to new production in the summer nutrient regenerating system.

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## 1. Introduction

Primary production in an aquatic ecosystem depends on the photosynthetic process carried out by autotrophic organisms such as phytoplankton, phytobenthos, and macroalgae, where phytoplankton is the main primary producer. The taxonomical composition of the microalgae, the physiological and ecological characteristics of individual species, and the availability and optimal use of essential resources such as light and nutrients are the major factors controlling growth processes and phytoplankton production (Smayda and Reynolds, 2001).

The primary production in the Gulf of Riga, considered one of the most eutrophic areas of the Baltic Sea, thus far has only been measured from 1989 to 1997. Earlier researchers used the  $C^{14}$  method (Andrushaitis et al., 1992) but later, during the project investigating the Gulf of Riga ecosystem from 1993–1995, the oxygen method was used (Olesen et al., 1999; Wassman and Tamminen, 1999). Andrushaitis et al. (1992) calculated an annual production of  $250 \text{ gC m}^{-2}$ , while Olesen et al. (1999) suggested that annual production could exceed  $350 \text{ gC m}^{-2}$ . The discrepancy was attributed to an underestimation of the gross primary production by the  $C^{14}$  method in systems with high growth rates and respiratory losses (Olesen et al., 1999). Simultaneously, during a comparative assessment of the coastal and open areas of the south-eastern Baltic Sea from 1993 to 1997, Wasmund et al. (2001) concluded that annual primary productivity in the Gulf of Riga reaches  $250\text{--}255 \text{ gC m}^{-2}$ , attributing the eutrophic status to the Gulf of Riga. Boreal environment determines the scenario of phytoplankton development in the gulf (Jurgensone et al., 2011; Olli and Heiskanen, 1999; Yurkovskis et al., 1999). Diatoms *Pauliella taeniata* ((Grunow) F.E. Round & P. W. Basson 1997) and *Thalassiosira baltica* ((Grunow) Ostensfeld 1901) dominate the spring blooms after ice melt from April to May. *Chaetoceros* spp. becomes dominant towards the end of the bloom accompanied by dinoflagellates *Peridiniella catenata* ((Levander) Balech 1977), and ciliate *Mesodinium rubrum*. With the development of thermal stratification and the depletion of nutrients, the spring bloom phytoplankton species are sedimenting. Blooms of cyanobacteria can be observed in July–August with dominating species *Aphanizomenon flosaquae*. Chlorophytes and cryptophytes are often accompanying cyanobacterial blooms. The second bloom of diatoms terminates the phytoplankton succession in September–October after the disruption of the thermocline and convective mixing of water column (Jurgensone et al., 2011; Yurkovskis et al., 1999 and the references therein). In autumn and winter, wind-induced mixing of the water column brings up nutrients from the bottom to the surface, supporting the growth of phytoplankton (Rydberg et al., 1990). But the significant shortening of daylight and low light intensity (Vihma and Haapala, 2009) hinders photosynthetic activity.

Although the phytoplankton and nutrient dynamic is well described in the Gulf of Riga, the linkage between phytoplankton production and nutrients to date is insufficiently described. This, in turn, does not allow proper characterization of the eutrophication process in the Gulf of Riga. Even less attention was paid to phytoplankton species involved in the primary production. Therefore the aim of this study was to examine the seasonal variation of primary production with

a focus on the controlling nutrient fluxes as well as the species composition involved in the production.

## 2. Material and method

### 2.1. Study area

The Gulf of Riga (Baltic Sea) is a shallow water body with an average depth of 26.2 m. Its surface area is  $16,330 \text{ km}^2$ , however, its drainage area covers  $135,700 \text{ km}^2$ . The south-eastern part of the gulf receives 86.0% of the total river runoff from the main rivers, the Daugava, Lielupe, Gauja, and Salaca (Yurkovskis et al., 1993). The salinity is low (5–7 PSU) due to weak water exchange with the Baltic Sea and the large freshwater impact (Berzinsh, 1995). During winter, the water column of the gulf is well-mixed to the bottom due to convective and wind-induced mixing. During the productive season the stratification restricts vertical water exchange and promotes nutrient accumulation in the bottom layer (Yurkovskis, 2004).

Nutrient limitation is the most important factor governing the phytoplankton community. Most of the earlier nutrient limitation studies in the Gulf of Riga showed that the spring phytoplankton blooms are mainly nitrogen-limited in the central part while the coastal areas can be phosphate-limited (Tamminen and Seppälä, 1999). In contrast, recent studies of long-term phytoplankton data indicated that the spring blooms are mainly phosphorus limited, but could shift to nitrogen or silicate limitation for diatoms in the later stages of the bloom due to the faster regeneration of phosphate relative to the other nutrients (Jurgensone et al., 2011). During the summer period, the system is nitrogen limited in terms of phytoplankton (Balode et al., 1998; Pöder et al., 2003; Tamminen and Seppälä, 1999) when both DIN and DIP are depleted in the upper mixed layer, whereas silicates are always present excluding silica limitation as a structuring factor for the summer community.

### 2.2. Sampling

Sampling was performed by ships A-90 “Varonis” and r/v “Salme”. Samples were collected at 5 regular monitoring stations in the central part of the Gulf of Riga (Fig. 1), 15 times each, over a period from April 2011 to October 2012 (Table 1) covering the full seasonal spectrum. Samples for the physical and chemical variables were taken simultaneously with biological variables. Water temperature and salinity was measured using a water probe (SBE 19plus Sea-Cat, USA). Water transparency was measured with Secchi disc. The water for physicochemical variables, phytoplankton, chlorophyll *a* concentrations, and primary production was sampled as an integrated sample from the euphotic upper layer (0–10 m).

### 2.3. Analytic analysis

Nutrient concentrations were determined according to Grasshoff et al. (1983), e.g. ammonium ( $\text{NH}_4^+$ ) and phosphate ( $\text{PO}_4^{3-}$ ) were measured by the indophenol blue and molybdenum blue methods, respectively. The sum of nitrate and

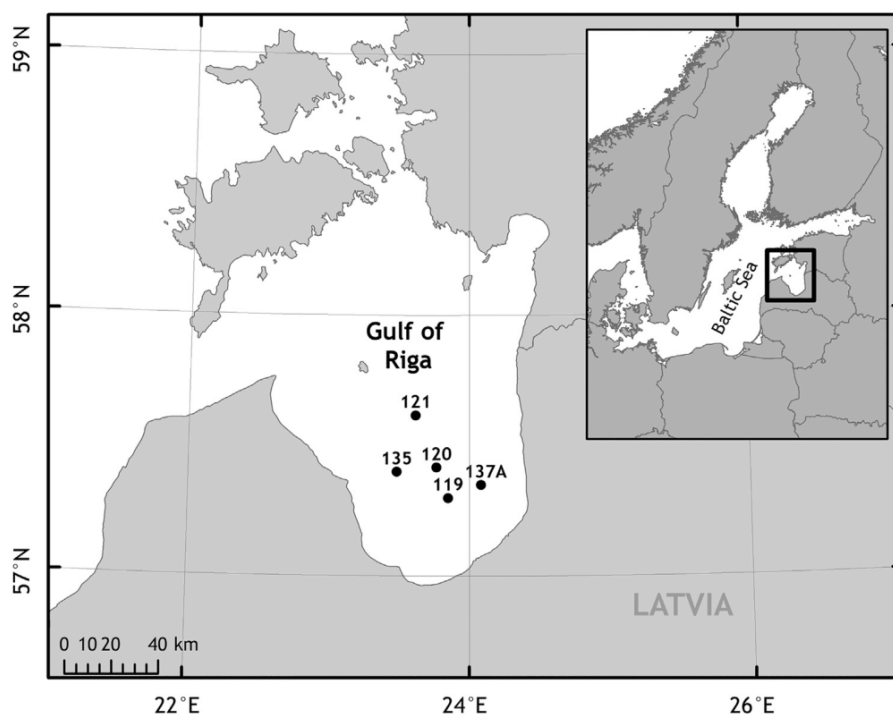


Figure 1 The location of the sampling stations in the Gulf of Riga.

**Table 1** Average temperature, Secchi depth, hydrological and hydrochemical data in the central part of the Gulf of Riga during 2011 and 2012.

		Temp. [°C]	PAR [mol photons m <sup>-2</sup> d <sup>-1</sup> ]	Secchi depth [m]	Photic zone depth [m]	PO <sub>4</sub> <sup>-3</sup> [μmol l <sup>-1</sup> ]	P <sub>tot</sub> [μmol l <sup>-1</sup> ]	SiO <sub>4</sub> [μmol l <sup>-1</sup> ]	NO <sub>2+3</sub> <sup>-</sup> [μmol l <sup>-1</sup> ]	NH <sub>4</sub> <sup>+</sup> [μmol l <sup>-1</sup> ]	N <sub>tot</sub> [μmol l <sup>-1</sup> ]	DIN/DIP ratio
2011	Apr	2.27	50.29	2.3	6.10	0.28	1.25	25.83	20.05	0.47	53.38	76.26
	May	6.24	45.85	2.8	7.50	0.03	1.00	5.23	4.17	0.10	37.32	128.38
	Jun	17.99	56.37	2.5	6.80	0.08	0.95	2.75	0.22	0.00	39.50	2.97
	Aug	19.71	48.95	4.0	10.70	0.02	0.65	4.99	0.17	0.29	33.36	23.44
	Oct	14.30	9.39	5.1	13.80	0.04	0.53	10.04	1.22	1.31	31.80	68.63
2012	Nov	9.33	11.63	3.9	10.40	0.41	0.76	20.26	5.95	0.04	28.26	14.79
	Jan	3.57	15.54	3.0	8.10	1.10	1.56	31.34	12.77	0.09	36.91	11.69
	Mar	0.18	20.65	4.1	11.00	1.00	1.40	33.44	15.63	0.07	39.25	15.83
	Apr	1.00	39.92	3.5	9.40	0.97	1.46	30.51	15.30	0.07	39.16	16.32
	May	8.08	41.81	2.1	5.60	0.04	1.45	2.05	2.87	0.07	44.94	39.89
	Jun	10.61	51.69	3.2	8.50	0.04	0.76	2.72	0.69	0.41	31.72	27.08
	Jul	15.30	49.54	3.8	10.20	0.01	0.67	3.25	0.40	1.47	36.84	132.13
	Aug	19.02	58.44	3.3	8.80	0.04	0.66	6.10	0.09	0.64	33.15	22.60
Sep	16.30	34.57	4.5	12.30	0.10	0.58	8.61	0.40	1.24	31.90	17.44	
Oct	12.95	10.77	4.3	11.70	0.26	0.69	13.69	2.59	1.49	28.10	16.23	

nitrite (NO<sub>2+3</sub><sup>-</sup>) was determined by nitrite reaction with an azo dye after the reduction of nitrate to nitrite in a copper-coated cadmium column. The nitrite was determined by reaction with an azo dye and nitrate was determined as the difference between nitrite and the sum of nitrate and nitrite. Dissolved silicate (SiO<sub>4</sub>) was determined colorimetrically according to the procedure described by Grasshoff et al. (1983). The total nitrogen (N<sub>tot</sub>) and total phosphorus (P<sub>tot</sub>) were analyzed as nitrate and phosphate after wet digestion with persulfate. Dissolved inorganic nitrogen

(DIN) is the sum of NO<sub>2+3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>. All laboratory analyses were performed in an accredited laboratory (ISO/IEC 17025).

#### 2.4. Chlorophyll *a* and phytoplankton analysis

Concentrations of chlorophyll *a* (Chl *a*, mg m<sup>-3</sup>) were measured according to the standard method of the Manual for Marine Monitoring in the COMBINE Programme of HELCOM (HELCOM, 2006).

Phytoplankton samples (300 ml) were fixed with acid Lugol's solution. Subsamples of 10 and 25 ml of fixed samples were settled in a sedimentation chamber for 12 h and counted according to the Uthermöl technique with an inverted microscope at 200× and 400× magnification. The number of counted cells in all subsamples exceeded 500 (Edler, 1979; HELCOM, 2006; Olenina et al., 2006; Utermöhl, 1958). The biomass was expressed as mg m<sup>3</sup> of wet weight. *M. rubrum* was included in phytoplankton counts as the only representative of division Ciliophora.

## 2.5. Primary production measurements

The light and dark bottle oxygen technique (Olesen et al., 1999) was used in order to evaluate the productivity of the study area. Water was filled in 15 transparent, calibrated glass bottles for oxygen measurements. Bottles were divided into 5 groups (with 3 replicates in each group) to imitate the light conditions at specific depths of the euphotic layer: Start, 100%, 66%, 23% and 0% light transmittance. Specific light transmittance to each group was provided by wrapping the bottles in the plastic optical filters produced by GAM-PRODUCTS, Inc.: no filter for 100% transparency, 1514 GAM for 66% transparency, 1516 GAM for 23% transparency and aluminium foil for 0% transparency. Initial oxygen concentrations were fixed with Winkler reagents (1 ml manganese chloride and 1 ml alkaline iodide) before incubation. All vials were mounted on a rotating wheel and submerged in the onboard incubator with a continuous flow of outboard seawater to ensure ambient water temperature and *in situ* illumination during the 24 h incubation. Photosynthetically active radiation (PAR) was measured on board using the LI-1400 Data Logger and the LI-190 Quantum Sensor during experimental incubation. At the end of incubation, samples were fixed with Winkler reagents. Oxygen concentrations were determined by titration with sodium thiosulphate.

Oxygen consumption in the dark bottles was used as a proxy for community respiration (CR), while the other three groups were used to evaluate daily, water column, primary production rates. Measured oxygen concentrations were converted to carbon units according to the stoichiometry of photosynthesis equation. The approximate attenuation coefficient ( $k$ ) was calculated for each sampling from the simultaneously measured Secchi depth according to the equation  $k = 1.7/D_s$ , where  $D_s$  is Secchi depth. The depth of specific light conditions ( $z$ ) was calculated from  $z = -\ln I_z/I_o/k$ , where  $I_z$  is light intensity at a specific depth (66% or 23%) and  $I_o$  is the light intensity below the surface (100%). Daily water column net community production (NCP, gC m<sup>-2</sup> d<sup>-1</sup>) rates were estimated by trapezoidal integration of the data from various light conditions. Gross primary production (GPP, gC m<sup>-2</sup> d<sup>-1</sup>) was calculated summing up the NCP and CR. GPP, NCP, and CR values from 5 stations were averaged to get the monthly average. Annual primary production was calculated as the GPP monthly averages multiplied by the number of days and summed up for 365 days.

## 2.6. New production calculation from nutrient concentrations

Since primary production rates have low representativeness in time and space, there have been attempts to use other

parameters to calculate them, such as nutrient depletion (Rahm et al., 2000; Wasmund et al., 2005), increase in particulate organic carbon (Wasmund et al., 2005), and changes in CO<sub>2</sub> concentrations (Schneider et al., 2003). In our study, we applied the nutrient depletion method described in detail by Wasmund et al. (2005). We used data obtained during 2012, because the sampling frequency was higher this year, and calculated primary production, assuming that carbon, nitrogen, and phosphorus are assimilated in a stable molar ratio of 106:16:1 (Redfield et al., 1963). In April, what we consider the starting month of the spring bloom, the DIN:DIP ratio was 16.3 (Table 1), we assumed that the PO<sub>4</sub><sup>3-</sup> excess production as described by Rahm et al. (2000) was not relevant for our calculations. The nutrient concentration decrease pattern suggests that the new production period lasts until June. We used concentration change ( $\Delta$ DIN) in the upper mixed layer (0–20 m) to calculate new production for the period from April to May (1st period, 37 days) and for the period from May to June (2nd period, 23 days). We also considered air depositions of nitrogen in our calculations. Since there was no published information on air deposition for 2012, values calculated for 2010 were used instead. The air deposition over the whole surface area of the Gulf of Riga was 9973 t of nitrogen in 2010 (HELCOM, 2013). Averaging the deposited amount over the surface area of the Gulf of Riga (16,330 km<sup>2</sup>), we estimated that nitrogen air deposition is 0.12 mmol m<sup>-2</sup> day<sup>-1</sup>. Since the Gulf of Riga is much more significantly impacted by river runoff than air deposition, we also considered the amount of DIN delivered by the four largest rivers, the Daugava, Gauja, Lielupe, and Salaca, which constitute close to 90% of freshwater input to the Gulf of Riga (Yurkovskis et al., 1993), over the respective period. The monitoring frequency was not sufficient for our purposes, so we used the linear regression method (Hirsch et al., 2010) to estimate missing values. The method employs the use of weighted regressions of concentrations on time, discharge, and season. This weighting results in a set of weights on every observation in the dataset, based on the selected values of time and discharge. So, we used known values of specific time and discharge to estimate the expected value of concentration. Data from national monitoring (e.g., flowrate and nutrient concentrations), stored in the database of Latvian Environment, Geology and Meteorology Centre, were used as input data. Estimated nitrogen loads were averaged over the whole area of the Gulf of Riga. Furthermore, from April to May, depletion of DIN could be observed in water layer 20–30 m. We used this concentration change to calculate an additional primary production for the 1st period.

Diatom biomass production was estimated from the silicate consumption by using N:Si = 1.25 mol mol<sup>-1</sup> constant conversion factor (Sarthou et al., 2005). The nitrogen units thereafter were converted into carbon units by the Redfield ratio of C:N = 6.625 (Redfield et al., 1963).

## 3. Results

### 3.1. Environmental factors

The seasonal variation in water temperature, Secchi depth, PAR and nutrient concentrations are summarized in Table 1.

### 3.2. Phytoplankton and chlorophyll *a*

The highest phytoplankton biomass and Chl *a* were observed in spring – April 2011 and May 2012 (5715, 5411 mg m<sup>-3</sup> and 18.5, 29.1 mg m<sup>-3</sup>, respectively) (Fig. 2). However, the typical spring bloom of phytoplankton with high biomass and more than 90.0% dominance of diatoms, consisting mainly of *P. taeniata*, *Chaetoceros wighamii* (Brightwell 1856), and *T. baltica*, was observed only in April 2011. The succession of phytoplankton in May 2011 and 2012 was formed mainly from three taxonomical groups where single species composed up to 72.2–93.4% of the corresponding phytoplankton group – diatoms (*T. baltica*), dinoflagellates (*P. catenata*), and ciliophora (*M. rubrum*).

The summer (June–September) phytoplankton was characterized by relatively low Chl *a* and total phytoplankton biomass (Fig. 2). In this period, cyanobacteria (mostly N<sub>2</sub>-fixing *A. flosaquae*) in both years constituted 15.2–57.8% of total phytoplankton biomass with the highest value in July 2012. The dominance of photosynthetic ciliate *M. rubrum* (56.3% of total biomass) was recorded in June 2012 (Fig. 2).

The autumn (October, November) phytoplankton consisted of cyanobacteria, diatoms, and *M. rubrum* (28.2%, 18.4% and 19.8%, respectively) in 2011, whereas in 2012 autumn was dominated by diatoms (>50.3%). The Chl *a* values were slightly higher in 2011 than in 2012 (Fig. 2). In the winter, total phytoplankton biomass (143.6–268.5 mg m<sup>-3</sup>), as well as Chl *a* concentrations (1.62–1.71 mg m<sup>-3</sup>), were low. The relative abundance of *M. rubrum* in the phytoplankton community increased during winter, reaching 34.2% of the total biomass in January. The next two most abundant groups, cyanobacteria (mainly *A. flosaquae*) and diatoms, composed 23.3 and 17.4%, respectively. The beginning of the increase in phytoplankton biomass was detected in March when *M. rubrum* composed, on average, 48.4% of total biomass.

### 3.3. Primary production and respiration

The data of primary production obtained from 5 stations were averaged for each month due to low variability in hydrological and hydrochemical conditions at the individual stations. Therefore, the patchiness of biological communities is the main source of measurement uncertainties. On average, the

GPP was highest during the spring. Thereafter, it gradually decreased over summer and reached minimum values during the autumn–winter period (Fig. 3). Multiple regression analyses with dominant species as explanatory values showed the significant importance of *P. catenata* and *M. rubrum* in the formation of GPP in springtime in both years ( $r^2 = 0.59$ ,  $p = 0.009$ ,  $n = 9$ ). The carbon biomass variance of both species explains 59.2% of GPP variance in spring. However, during summer, when similar dominance of *M. rubrum* and *A. flosaquae* was observed, no significant correlation could be established.

Plankton CR varied between 0.01–3.12 gC m<sup>-2</sup> d<sup>-1</sup> (average 1.01 gC m<sup>-2</sup> d<sup>-1</sup> of both years), accounting to 40.4% of GPP in 2011 and 68.5% in 2012. The rate of respiration mostly followed the pattern of GPP (Fig. 3), except in April 2012 when respiration exceeded GPP. NCP is also highest during the spring bloom and decreased over summer, except for in April 2012, when negative values of NCP were observed.

### 3.4. New production calculation from nutrient concentrations

The change of DIN concentrations in water from 15.3 μmol l<sup>-1</sup> to 2.87 μmol l<sup>-1</sup> (ΔDIN = 12.4 μmol l<sup>-1</sup>) in the upper mixed layer (0–20 m) amounted to the new production of 1647 mmol C m<sup>-2</sup> for the period from April to May (1st period) and ΔDIN = 2.18 μmol l<sup>-1</sup> resulted in new production of 289 mmol C m<sup>-2</sup> for the period from May to June (2nd period). The depletion of DIN from April to May in water layer 20–30 m (ΔDIN = 5.04 μmol l<sup>-1</sup>) amounted to an additional primary production of 331 mmol C m<sup>-2</sup> in the 1st period. The new production, estimated from average nitrogen air deposition rate (0.12 mmol m<sup>-2</sup> day<sup>-1</sup>), was 29.0 and 18.0 mmol C m<sup>-2</sup> for the 1st and 2nd periods, respectively. The calculated average supply of riverine DIN was 289 t day<sup>-1</sup> in April, 87 t day<sup>-1</sup> in May and 38 t day<sup>-1</sup> in June. Averaging received nitrogen over the area of the Gulf of Riga, we estimated an additional supply of 31.2 and 6.45 mmol N m<sup>-2</sup> in the 1st and 2nd periods, respectively. This resulted in the new production of 205 and 40.1 mmol C m<sup>-2</sup> in the 1st and 2nd periods, respectively.

The total new production estimated from DIN consumption summed to 2212 and 347 mmol C m<sup>-2</sup> (26.6 and

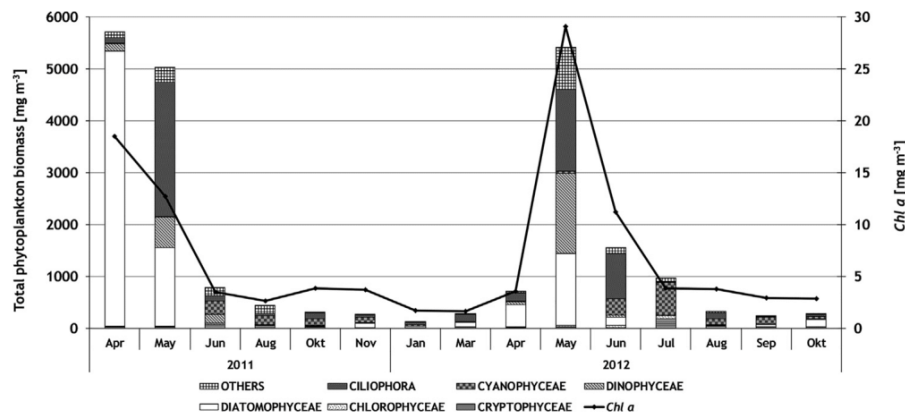


Figure 2 Total phytoplankton biomass and Chl *a* concentrations in the central part of the Gulf of Riga during 2011 and 2012.

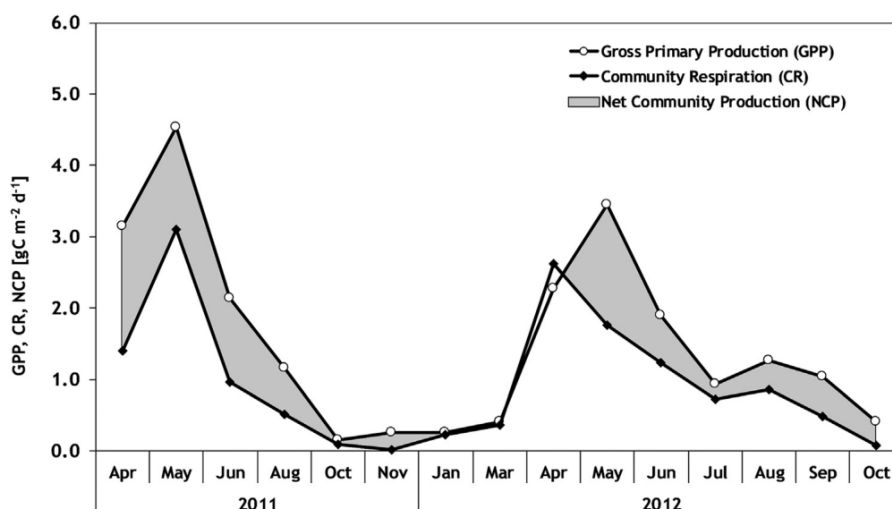


Figure 3 Average daily production (GPP and NCP) and CR in the central part of the Gulf of Riga during 2011 and 2012.

4.23 gC m<sup>-2</sup>) for the 1st and 2nd period, respectively. The average new primary production rates estimated from the available DIN pool in the upper mixed layer and loads from atmosphere and rivers are 0.72 and 0.17 gC m<sup>-2</sup> d<sup>-1</sup> in May and June, respectively.

The change of silicate concentration in the upper mixed layer from April to May 2012 (Table 1) gave an estimate of  $\Delta\text{Si} = 569 \text{ mmol m}^{-2}$  for the 1st period. The concentration changes from 30.5  $\mu\text{mol l}^{-1}$  to 15.3  $\mu\text{mol l}^{-1}$  in the 20–30 m water layer, which gives an additional 155 mmol m<sup>-2</sup> of silicate accessible for diatom growth. By applying conversion factors we estimated that new production of diatoms was 5997 mmol C m<sup>-2</sup> in the 1st period, corresponding to a new production rate of 1.95 gC m<sup>-2</sup> d<sup>-1</sup>.

## 4. Discussion

### 4.1. Annual and seasonal primary productivity

In the northern temperate and boreal seas, including the Baltic Sea, the spring bloom, sustained by the nutrient winter pool, lasts approximately one month, but typically dominates the annual phytoplankton productivity cycle, contributing 40.0–60.0% of the annual carbon fixation (Heiskanen, 1998). The autumn bloom, sustained by the delivery of nutrients from deeper water layers upon the breakdown of seasonal stratification, is considered second most important period for annual phytoplankton productivity cycle. The summer production and biomass are considered to be low in comparison to the spring bloom period, while winter production is usually neglected as important. This has been somewhat challenged in the past (Platt et al., 1989; Stigebrandt and Djurfeldt, 1996) especially in the case of summer productivity (Sahlsten et al., 1988). In our study, the spring bloom (April–May) comprised 46.3% while summer productivity made another 44.5% of annual productivity. The autumn-winter productivity (October–March) comprised the remaining 10.2% of the annual productivity. Moreover, in our study we established that the least productive period is during October–November

despite the water temperature still remaining rather high (13.4°C) and nutrient concentrations substantially increasing due to the breakdown of thermal stratification and resuspension of re-mineralized nutrients from deeper layers. The autumn is characterized by strong, westerly winds that bring mild and moist Atlantic air to northern Europe (Vihma and Haapala, 2009), resulting in dense cloud cover and frequent rain. At the same time the shortening of daylight hours (from 10 h 50 min in October to 6 h 40 min in December) can be observed. Therefore, we can hypothesize, that meteorological conditions in autumn lead to strong light limitation of GPP despite rather good water transparency conditions measured as Secchi depth (Table 1). Later, the onset of colder winter air temperatures results in clear skies and higher light intensity. As a result, relatively low but still comparable primary production was also measured during winter months that previously were considered unproductive.

The annual primary productivity in the Gulf of Riga reached values as high as 353–376 gC m<sup>-2</sup> in our study, while in the previous studies (Andrushaitis et al., 1992; Wasmund et al., 2001) the estimated annual primary productivity of the Gulf was only 250–255 gC m<sup>-2</sup> for the period of 1993–1995. However, this estimate was based on measurements that had not included the most productive period of phytoplankton succession from the end of March until the end of April when the biomass of spring diatoms can reach even 20.3 g m<sup>-3</sup> (Yurkovskis et al., 1999). This allowed the Olesen et al. (1999) to hypothesize that primary productivity in the Gulf of Riga can exceed 350 gC m<sup>-2</sup>. So, our values of annual production were similar to estimations of Olesen et al. (1999). At the same time, we cannot exclude the possibility that the productivity values in our study were still underestimated since sampling frequency was still too low to fully capture spring phytoplankton bloom development. For example, phytoplankton biomass and composition from April to May 2012 could not explain the depleted pool of SiO<sub>4</sub>, suggesting that diatom bloom between these sampling events was unregistered by our study at least at the level of that observed in April 2011.

#### 4.2. New production

The values of new production calculated from nutrient concentrations ( $0.70$  and  $0.17 \text{ gC m}^{-2} \text{ d}^{-1}$ , in May and June, respectively) were substantially lower than measured NCP rates, e.g.,  $1.68$  and  $0.72 \text{ gC m}^{-2} \text{ d}^{-1}$ , in May and June, respectively. The total new production, calculated from the nitrate consumption, was equivalent to 51.8% of spring NCP. Smetacek et al. (1984) divided the spring bloom into two stages. Stage 1 was characterized by a rapid bloom of diatoms exhausting the winter-accumulated nutrient pool where production is strictly “new” in the sense of Dugdale and Goering (1967). Stage 2 was characterized by the dominance of dinoflagellates and an increase of protozooplankton. Loss rates of this planktonic system were amongst the lowest of the year, indicating a great retention capacity (Smetacek et al., 1984). The new production during stage 1 was >75.0% of NCP, but during stage 2 it was approximately 50%. These data were consistent with our calculations where new production, based on nutrient consumption, composed 51.8% of NCP during both stages of spring bloom. At steady state, there should be a balance between the input of nitrogen and the export of carbon, implying that on a longer time scale, sedimentary loss from the pelagic system approaches new production (Eppley et al., 1983). This could be the case in the Gulf of Riga as the bloom of diatoms, that used most of nitrates and predominantly contributed to the new production and sedimentation fluxes, was largely unobserved in 2012. However, if new production is calculated from the consumption of  $\text{SiO}_4$  according to Wasmund et al. (2013), it alone gives an average estimate of  $1.95 \text{ gC m}^{-2} \text{ d}^{-1}$  for the first period. As no other algae, except diatoms, can use  $\text{SiO}_4$ , the bloom maxima of diatoms should be assumed between the sampling occasions in April and May 2012 followed by rapid sedimentation as diatoms composed only 25.2% of total phytoplankton biomass in May. The excess consumption of silica can be explained either by different silicification of diatom species (Olli et al., 2008) or by diatom resting spore formation as this process requires plenty of silicate. It has been reported that the resting spores generally have higher sinking rates than vegetative cells (Aldredge et al., 1995). The spore formation in the deeper water layers could explain the  $\text{SiO}_4$  consumption in the 20–30 m (data not shown) layer of the Gulf of Riga.

#### 4.3. Influence of dominant species on the nutrient fluxes and productivity

The species which exert a dominant role in the planktonic ecosystem are often those that govern the fluxes of organic matter and nutrients in the pelagic system (Heiskanen, 1998). To understand the functioning of the aquatic ecosystem it is necessary to understand the role, regulation, and species-specific properties of the “key” species (Verity and Smetacek, 1996).

The main “key” planktonic species dominating almost all seasons was photosynthetic ciliate *M. rubrum*. Leppänen and Bruun (1986) reported that *M. rubrum* contributed about 10.0% of the primary production during spring in the open northern Baltic. Similar values of 6.00–9.00% of phytoplankton biomass and production have also been shown for

*M. rubrum* in the Gdańsk Basin of the southern Baltic Sea (Witek, 1998). It appears that in the Gulf of Riga, *M. rubrum* plays an even more important role in the primary production than in other regions of the Baltic Sea, since its biomass composed 18.2–73.9% of the total phytoplankton biomass during the spring bloom period in May, 6.22–41.4% during summer, 14.9–22.2% during autumn, and 40.1–61.3% during winter. Significant positive correlation was detected between the biomass of *M. rubrum* and GPP ( $r = 0.650$ ,  $p > 0.001$ ,  $n = 42$ ). The importance of *M. rubrum* in the Gulf of Riga was observed during periods when nutrient recycling was the most important (spring–summer) as well as during periods when nutrients were freely available, but the light limited the phytoplankton development (autumn–winter). It has been observed that *M. rubrum* demonstrates an ability to accumulate near the sea surface and to photosynthesize at high light intensities (Esteban et al., 2010). At the same time, it has been noted that *M. rubrum* can also tolerate the low-light conditions, composing the main phytoplankton biomass also during winter period (Moeller et al., 2011). In addition, its rapid swimming behaviour appears to reduce its susceptibility to grazing (Jonsson and Tiselius, 1990) and may increase its ability to utilize nutrient micropatches (Stoecker et al., 1991). Our study confirms that the *M. rubrum* is a highly competitive and opportunistic species that substantially contributes to the productivity of the Gulf of Riga.

Another “key” species substantially contributing to productivity and internal nutrient fluxes of the Gulf of Riga is *A. flosaquae*. Filamentous,  $\text{N}_2$ -fixing cyanobacteria are well known for bloom formation during August–September in the Baltic Sea (Kahru et al., 1994). In our study, the relative dominance of *A. flosaquae* (18.7–37.4% of total phytoplankton biomass) begun in June when inorganic nutrients (both, N and P) were exhausted, reached a maximum in July (59.4–65.2%) and continued until September (21.3–42.1%). The blooms of *A. flosaquae* are usually associated with calm weather, high surface temperatures, availability of phosphates, and a low DIN:DIP ratio (Kononen et al., 1996). However, according to the results of this study, the phosphates were exhausted already during the spring bloom creating the apparent phosphorus limitation in May (Table 1). This suggested that  $\text{N}_2$  fixation was not likely to occur during the summer. Furthermore, the increase of *A. flosaquae* biomass from  $309 \text{ mg m}^{-3}$  (in June 2012) to  $543 \text{ mg m}^{-3}$  (in July 2012) was observed simultaneously with the fast increase of the DIN:DIP ratio as well as increase of total N (Table 1). The river runoff and atmospheric deposition was of the secondary importance, since both these nutrient pathways are relatively small during the summer and unlikely to sustain, or let alone increase, the observed population. At the same time, Ploug et al. (2010) showed that *A. flosaquae* was highly productive in the Baltic Sea with high rates of C and N assimilation and the capacity to release a large fraction (35.5%) of newly assimilated N to the surrounding water. This allowed us to create a hypothesis that the population of *A. flosaquae* sustains the observed population level by rapid recirculation of phosphorus upon the death of phytoplankton cells and the assimilation of nitrogen via  $\text{N}_2$  fixation to compensate nitrogen loss in the sedimentation pathway.

The importance of *M. rubrum* and *A. flosaquae* was also observed during the autumn and winter seasons. However, more observations were needed to understand their roles

during those seasons as well as regulatory factors of these species under conditions of limited light and replenished nutrients.

## 5. Conclusions

The annual primary productivity in the Gulf of Riga reached values 353–376 gC m<sup>-2</sup> in our study. It showed no significant increase of productivity since 1992–1998. Spring bloom (April–May) comprised 46.2% of annual production with maximal carbon fixation rates and draw-down of winter nutrient pool. The new production calculated from consumption of nitrates amounted to 51.8% of spring NCP. Detailed examination of phytoplankton species along with measurements of productivity revealed key species governing the nutrient fluxes and the productivity of the Gulf of Riga. The autotrophic ciliate *M. rubrum* prevailed in all seasons and significantly correlated with elevated productivity, while diazotrophic cyanobacteria *A. flosaquae* contributed to “new production” in the summer nutrient-regenerating system.

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# Stable Carbon and Nitrogen Isotope Composition in Suspended Particulate Matter Reflects Seasonal Dynamics of Phytoplankton Assemblages in the Gulf of Riga, Baltic Sea

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## Abstract

The ratio of stable carbon and nitrogen isotopes in the suspended particulate matter has been widely used to study processes occurring in the marine ecosystem. At the same time, the signals provided by isotope ratios in coastal ecosystems can be difficult to interpret, due to several, often contradictory processes taking place simultaneously. In this study, we hypothesized that the carbon and nitrogen isotopic variation is predominantly affected by seasonally occurring phytoplankton species succession in the Gulf of Riga, Baltic Sea. Cyclical seasonal patterns were observed for carbon and nitrogen isotopic compositions of both SPM and phytoplankton data. Enrichment of heavy isotopes in the Gulf of Riga took place during spring phytoplankton bloom (from on average between +7.1 and +8.8 ‰, and between –23.7 and –21.9 ‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively) and pooled at significantly lower values (from +3.1 to +5.1 ‰ and from –28.7 to –25.1 ‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively) for the rest of the year. At the same time, the spatial gradient of isotope ratios was sporadic and inconclusive. The results showed that terrestrial and anthropogenic input to particulate matter is negligible from spring to autumn. Multivariate analysis revealed that the observed seasonal variability was indeed driven by variation in phytoplankton species composition. The diatoms, dinoflagellates, and the ciliate *Mesodinium rubrum* facilitated enrichment of  $^{15}\text{N}$  and  $^{13}\text{C}$  in spring. In contrast, atmospheric nitrogen fixation by cyanobacteria and the assimilation of their released nutrients by other organisms resulted in lower  $\delta^{15}\text{N}$  values during summer. This variability requires careful considerations for conducting food web studies in temperate coastal and estuarine environments during high phytoplankton biomass periods.

**Keywords** Suspended particulate matter · Stable isotope analysis · Phytoplankton composition · Transitional waters · Seasonal changes

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## Introduction

Suspended particulate matter (SPM) is a dynamic pool of both living and non-living particles that can have a role in the functioning of food webs, nutrient and contaminant cycling, and system productivity, particularly in coastal and estuarine environments (Cresson et al. 2012; Golubkov et al. 2017; Jędruch et al. 2017; Xu et al. 2019). The amount and composition of SPM in estuarine and coastal systems are affected by various external sources like riverine inflows, coastal erosion, and atmospheric deposition, as well as internal processes like primary production and organic matter mineralization.

The opportunities presented by variable isotopic composition in different sources, like  $\delta^{13}\text{C}$  ratio of +10‰ in dissolved inorganic carbonates (Wimmer et al. 2013)

versus  $\delta^{13}\text{C}$  ratio of  $-28$  to  $-27\text{‰}$  in terrestrial SPM material (Marcelina et al. 2018; Winogradow et al. 2019), has been successfully explored to characterize extent of terrestrial influence in estuarine and continental shelf waters (McKinney et al. 2010; van de Merwe et al. 2016; Jędruch et al. 2017). Similarly, temporal  $^{15}\text{N}$  depletion of SPM due to fixation of atmospheric nitrogen by diazotrophs or seasonally observable enriched SPM  $\delta$  values has been used to characterize impact of internal processes (Rolff 2000; Montoya et al. 2002; Landrum et al. 2011; Marcelina et al. 2018; Winogradow et al. 2019).

At the same time, the limited number of seasonal studies of carbon and nitrogen isotope variation presents somewhat controversial evidence demonstrating from none or very limited seasonal change of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Harmelin-Vivien et al. 2008; van de Merwe et al. 2016) to substantial seasonal fluctuations (Remeikaite-Nikiene et al. 2017; Marcelina et al. 2018). Therefore, it can be assumed that in some areas, the change in isotope ratio, caused by external sources, is offset by changes created by seasonally occurring internal processes or vice versa. However, there are only a few seasonal studies, like Remeikaite-Nikiene et al. (2017), that attempts to link seasonal changes of external sources, e.g., river runoff, with internal processes, e.g., algae blooms, to substantiate such an assumption. Therefore, the present study aims to evaluate factors and processes that control spatial and temporal changes of stable isotope ratios in the SPM pool in an estuarine like gulf, i.e., the Gulf of Riga, Baltic Sea.

In the Baltic Sea surface, SPM isotopic content is largely controlled by the presence or absence of phytoplankton that incorporates dissolved nutrients into SPM (Winogradow et al. 2019). As dissolved nutrient concentrations in the water column decrease, phytoplankton exhibits less discrimination to absorbing isotopically enriched and energetically more consuming dissolved carbon species, e.g., bicarbonate or atmospheric  $\text{CO}_2$  (Golubkov et al. 2017).

In the case of nitrogen, the dissolved nitrogen in spring is rapidly incorporated into particulate phase by phytoplankton growth. This reveals how isotope kinetics influence the bulk particulate  $\delta^{15}\text{N}$  value—as the dissolved nitrogen pool is depleted, fractionation of isotopes decreases as well and the particulate  $\delta^{15}\text{N}$  values increase over spring bloom (Savoye et al. 2003). When nitrogen limiting conditions occur in the summer, diazotrophic cyanobacteria (most commonly *Aphanizomenon* sp., *Nodularia spumigena*, and *Dolichospermum* sp.) become an important dissolved nitrogen source in the surface layers of the Baltic Proper (Karlson et al. 2015). These photosynthetic bacteria can cause a significant drop in  $\delta^{15}\text{N}$  value by fixating isotopically depleted atmospheric nitrogen ( $\delta^{15}\text{N}=0\text{‰}$ ). Furthermore, other organisms can incorporate the dissolved ammonia and organic nitrogen

freshly generated by cyanobacteria into SPM (Ploug et al. 2010).

This let us hypothesize that the seasonal variation of SPM carbon and nitrogen isotope ratios in the study area is predominantly affected by the seasonal succession and the biomass of phytoplankton species. We also hypothesized that the effect of freshwater runoff on isotopic composition of SPM is greatest in the area directly receiving it with fading signal strength further away in transitional waters. To test these hypotheses, we conducted a temporary and spatially resolved study of carbon and nitrogen stable isotope composition of SPM together with phytoplankton species composition, and concentrations of chlorophyll *a*.

## Materials and Methods

### Study Area

The Gulf of Riga is a relatively shallow, semi-enclosed sub-basin of the Baltic Sea with the average depth of 26.2 m, water volume of 424 km<sup>3</sup>, and water residence time of 2 to 4 years (Yurkovskis et al. 1999; Purina et al. 2018). It is strongly influenced by freshwater runoff, since its drainage area (135,700 km<sup>2</sup>) significantly exceeds the Gulf of Riga surface area (16,330 km<sup>2</sup>). The average annual freshwater discharge (31 km<sup>3</sup> year<sup>-1</sup>) comprises around 7% of the volume (424 km<sup>3</sup>) of the Gulf (Berzinsh 1995). The freshwater runoff is distributed unevenly with 86% of it being discharged in the south-eastern part of the Gulf of Riga, mainly from its largest tributaries, the rivers Daugava (20.4 km<sup>3</sup> year<sup>-1</sup>), Lielupe (3.54 km<sup>3</sup> year<sup>-1</sup>), and Gauja (2.33 km<sup>3</sup> year<sup>-1</sup>) (Berzinsh 1995; FAO 2016). This creates an estuary-like transitional water area of up to 15 km radius from the Daugava river mouth. Water exchange with the Baltic Proper is facilitated through the Irbe Strait in the north-west and the Suur Strait in the north. That in combination with river discharge forms a pronounced latitudinal salinity gradient, most evident in spring, when salinities of 2 g kg<sup>-1</sup> and below can be observed in south-eastern part of the Gulf and 6–7 g kg<sup>-1</sup> in the Irbe Strait (Stipa et al. 1999; Skudra and Lips 2017). Furthermore, the southern part of the Gulf of Riga receives municipal waste water from the Riga city wastewater treatment plant. Approximately 350,000 m<sup>3</sup> of treated waste water is discharged one kilometer from the shore daily (Aigars et al. 2017).

### Sampling

Sampling addressing interannual and spatial variability was done from May 2015 to May 2019 from the research vessel “Salme,” the LR navy hydrological vessel

“Varonis,” and a multiple purpose ship “Mare.” Each year samples were collected in spring, summer, and autumn at 28 stations located in the Gulf of Riga and in the Daugava, Lielupe, and Gauja rivers’ delta areas (Fig. 1). Seasonal variation was assessed during 2017, when sampling at stations 101A, D, G, and L (Fig. 1) was done 1–3 times per month from March to November (except August, when sampling was done only at station 101A). SPM, primary production measurement, and phytoplankton samples in the Gulf of Riga were collected as an integrated (0–10 m) sample by a 10-m plastic hose ( $\varnothing$  5 cm, collected sample volume 5 L) from the euphotic layer on the board of the research vessel. The method in detail, including quality assurance procedures, is described in HELCOM Guidelines for marine monitoring (HELCOM 2017). Water samples for nutrient concentrations were taken by Niskin type bathymeters at 0, 5, and 10 m depth horizons.

Surface layer SPM in the rivers was collected from the riverbank with a bucket attached to a telescopic shaft and filled in 5-L plastic cans, which were prewashed with 0.1 N HCl and rinsed with MilliQ grade deionized water, for short-term storage and transportation.

Water temperature and salinity vertical profiles were measured using CTD (conductivity, temperature, and depth) water probe (SBE 19plus Sea-Cat, Sea-Bird Scientific, USA) with vertical resolution of 0.5 m. Water transparency was measured with a Secchi disc.

### Analytical Procedures

The concentration of chlorophyll *a* (Chl *a*) was measured according to HELCOM protocols (HELCOM 2017). Chl *a* was collected as SPM on glass microfiber filters (Whatman GF/C, equivalent to 1.2  $\mu$ m pore size), and afterwards

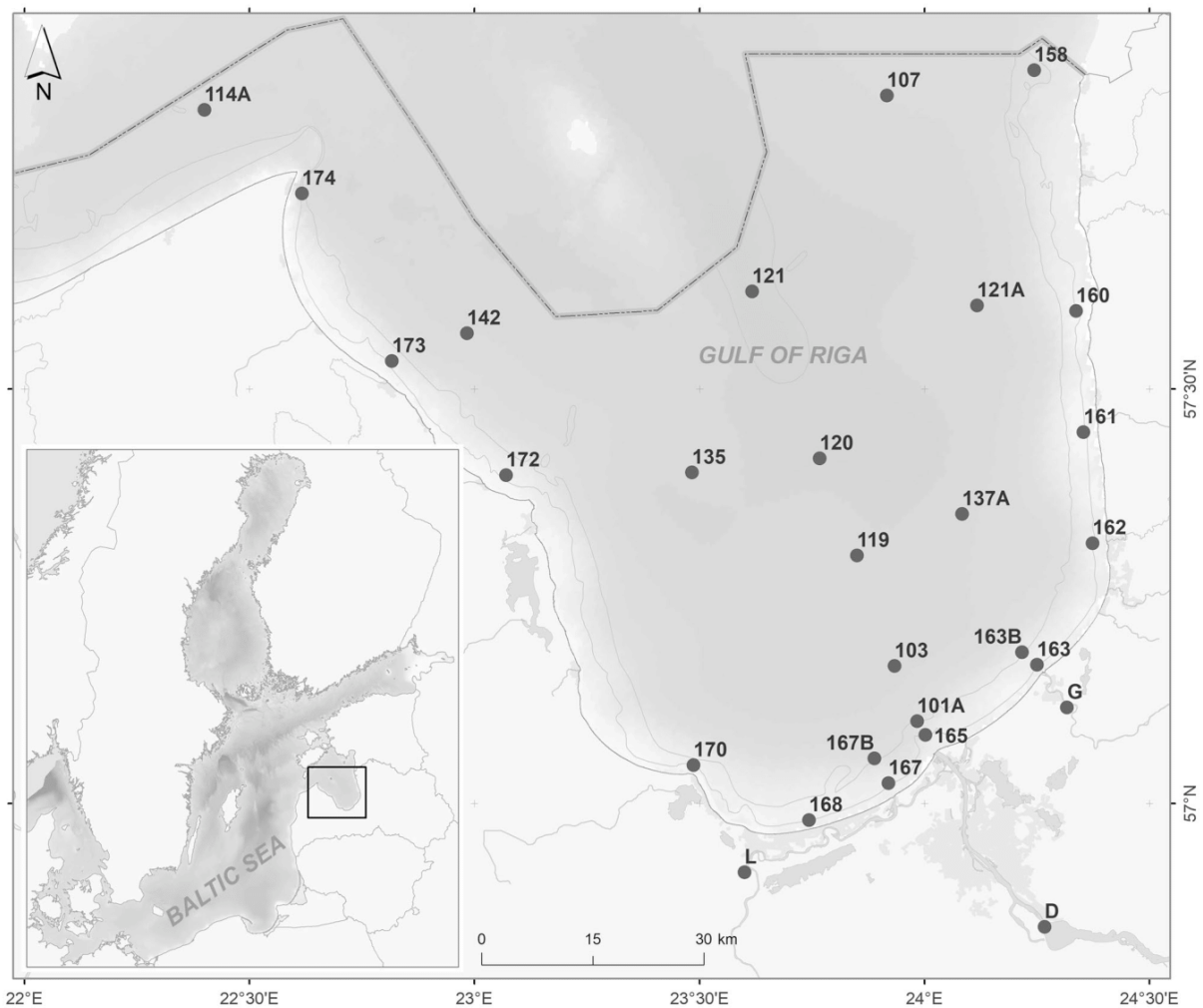


Fig. 1 Sampling stations in the Gulf of Riga and Large Rivers

extracted in 96% ethanol for 24 h and further analyzed using a spectrophotometer (Cary 100 Conc UV–Visible Spectrophotometer, Varian, Australia).

Phytoplankton samples (300 mL) were fixed with acid Lugol's solution. Subsamples of 10 and 25 mL of fixed samples were settled in a sedimentation chamber for 12 h and counted according to the Uthermöl technique using an inverted microscope (Leica DMI 3000, Leica Microsystems GmbH, Germany) at  $\times 200$  and  $\times 400$  magnification. The number of counted cells in all subsamples exceeded 500 (Utermöhl 1958; Olenina et al. 2006; HELCOM 2017). The carbon content ( $\mu\text{g C m}^{-3}$ ) was calculated according to Menden-Deuer and Lessard (2000).

Nutrient concentrations were determined according to Grasshoff et al. (1983). The concentrations of ammonium ( $\text{NH}_4^+$ ) and phosphate ( $\text{PO}_4^{3-}$ ) were measured employing the indophenol blue and molybdenum blue methods, respectively. Nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), after reduction to nitrite in a copper coated cadmium column, were determined by nitrite reaction with an azo dye. Dissolved inorganic oxidized nitrogen ( $\text{NO}_{2+3}$ ) is the sum of nitrite and nitrate. The total nitrogen ( $\text{N}_{\text{tot}}$ ) and total phosphorus ( $\text{P}_{\text{tot}}$ ) were analyzed as nitrate and phosphate after wet digestion with persulfate. The dissolved silicates (DSi) were determined photometrically after silicate reaction with ammonium molybdate. Quality of the analyses was checked by inter-laboratory comparison exercises in the Quality Assurance of Information for Marine Environmental Monitoring in Europe Programme (QUASIMEME), from which our laboratory took part, with Z-scores in the range of  $[-2; 2]$ .

### Stable Isotope Analysis

For stable isotope analysis, the water sub-samples were vacuum filtered for at least 30 min on pre-combusted (at  $450^\circ\text{C}$  for 2 h) 24-mm diameter glass microfiber filters (Whatman GF/F, equivalent to  $0.7\ \mu\text{m}$  pore size) to collect sufficient amount of material (160–2540 mL of sampled water until the filtering rate was  $1\ \text{mL s}^{-1}$ ). Once the samples were filtered, 5 mL of MilliQ grade water was added to wash off residual soluble salts. All filters were air dried at room temperature and thereafter lightly folded in aluminum foil cups for storage in desiccator until further preparation and analysis.

For gravimetric SPM mass determination, pre-weighted nitrocellulose membrane filters (Millipore, 45 mm diameter,  $0.45\ \mu\text{m}$  pore size) were used. After filtration (330–2080 mL of sampled water until the filtering rate was  $1\ \text{mL s}^{-1}$ ) and drying, the nitrocellulose filter weights were measured and mass concentration  $\gamma_f$  ( $\text{mg L}^{-1}$ ) and consequently mass of SPM on GF/F filter was calculated as follows:

$$\gamma_f = \frac{m_{f+s} - m_f}{V_f},$$

where  $m_{f+s}$  is filter + SPM mass,  $m_f$  filter mass, and  $V_f$  filtered water volume through nitrocellulose filter. Due to restricted sampling (1 sample per station, no replicates) and non-homogenous distribution of SPM on GF/F filters (e.g., randomly distributed visible cyanobacteria filaments), acidification to remove inorganic carbonates was not performed as it has been reported to affect  $\delta^{15}\text{N}$  values (Kolasinski et al. 2008; Brodie et al. 2011). The carbonates in the Gulf of Riga form a very minor proportion of total carbon as demonstrated by Carman et al. (1996); therefore, it has been expected that carbonates would not have detectable impact on carbon isotopic ratio.

Prior to the stable isotope analyses, dried GF/F filters holding SPM were cut with a cross-section into 4 equal pieces (pseudo-subsamples). Thereafter, each pseudo-subsample was wrapped in a separate tin cup and analyzed in the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of Latvia, using an elemental analyzer (EuroEA-3024, EuroVector S.p.A, Italy) coupled with a continuous flow stable isotope ratio mass spectrometer (Nu-HORIZON, Nu Instruments Ltd., UK). Isotope ratios were reported relative to Vienna Pee Dee Belemnite with a lithium carbonate anchor (VPDB-LSVEC) for  $\delta^{13}\text{C}$  and to atmospheric nitrogen (AIR) for  $\delta^{15}\text{N}$  as parts per thousands (‰):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$ ,  ${}^{15}\text{N}/{}^{14}\text{N}$ . The average value of the 4 pseudo-subsamples was used to represent sample isotopic ratio. For stable isotope ratio measurement quality control, an internal standard sample of glutamic acid and international reference material L-glutamic acid USGS-40 (Reston Stable Isotope Laboratory of the US Geological Survey, Reston, Virginia, NIST@RM 8573) were used. Repeated measurements of 121 internal standard exhibited reproducibility of  $0.14\text{‰}$  for  $\delta^{13}\text{C}$  and  $0.21\text{‰}$  for  $\delta^{15}\text{N}$ . Reference material USG-40, where stable carbon isotopic and nitrogen isotopic compositions with combined uncertainties are  $\delta^{13}\text{C}_{\text{VPDB-LSVEC}} = -26.39 \pm 0.04\text{‰}$  and  $\delta^{15}\text{N}_{\text{AIR}} = -4.52 \pm 0.06\text{‰}$  (Qi et al. 2003), was used to check accuracy of the stable isotope ratio determination. Our results for reference standard USGS-40 were  $\delta^{13}\text{C} = -26.38$  ( $\text{SD} = \pm 0.03\text{‰}$ ,  $n = 20$ ) and  $\delta^{15}\text{N} = -4.54$  ( $\text{SD} = \pm 0.08\text{‰}$ ,  $n = 20$ ).

### Primary Production Measurements

In order to evaluate the study area productivity, the light and dark bottle oxygen technique was used (Olesen et al. 1999).

Water samples were filled in 18 transparent, calibrated glass bottles for oxygen measurements. Bottles were divided into 6 groups and wrapped in the plastic optical filters (GAM-PRODUCTS) with 3 replicates to imitate the light conditions at specific depth of euphotic layer: 100%, 66%, 53%, 23%, 13%, and aluminum foil for 0% light transmittance. Initial oxygen concentrations were fixed with Winkler reagents (1 mL manganese chloride and 1 mL alkaline iodide) before incubation. The remaining samples were placed on a rotating wheel in the incubator with an electric thermometer that controls the water temperature close to natural conditions. At the end of incubation, samples were fixed with Winkler reagents. Oxygen concentrations were determined using the iodometric method—titration with standardized sodium thiosulfate in acid solution according to ISO 5813:1983. Oxygen consumption in the dark bottles (0% light transmittance) was used as a proxy of the phytoplankton community respiration, while the other five groups were used to evaluate the daily primary production rates of the water column.

Oxygen concentrations were converted to carbon units according to stoichiometry of the photosynthesis equation. Water elimination coefficient ( $k$ ) was calculated for each sample from measured Secchi depth as follows:

$$k = \frac{1.7}{D_s},$$

where  $D_s$  is Secchi depth (m). The depth of specific light conditions ( $z$ ; units expressed as meters below water surface) was calculated from:

$$z = -\frac{\ln\left(\frac{I_z}{I_0}\right)}{k},$$

where  $I_z$  is light intensity at specific depth (66%, 53%, 23%, 13%) and  $I_0$  the light intensity below surface (100%). Daily water column net primary production (NPP,  $\text{g C m}^{-2} \text{ day}^{-1}$ ) rates were estimated by trapezoidal integration of the data from the various light conditions. Gross primary production (GPP,  $\text{g C m}^{-2} \text{ day}^{-1}$ ) was calculated by summing the NPP and the community respiration (R).

### Statistical Analysis

The relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the distance from the mouth of the river Daugava (a proxy of runoff impacts) as well as salinity were analyzed by Pearson's correlations. The analysis was done per sampling period (months) when data from at least 10 different stations were obtained.

$K$ -means clustering algorithm was used to identify seasonal differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Hartigan and Wong 1979). The  $k$ -means algorithm establishes  $k$  centroids

and clusters points by assigning them to the nearest centroid so that the total intra-cluster variation is minimized. The value of  $k$  was prespecified based on an exploratory analysis and, ultimately, it was set to 2.

Principal component analysis (PCA) was performed to classify the main sources of data spatial variability. Each cluster, defined by  $k$ -means algorithm, was analyzed independently to identify environmental pressures specific to the season. PCA was conducted on the correlation between the isotope values and environmental parameters (temperature, salinity, Chl  $a$ , concentration of nutrients ammonium, phosphate, nitrite + nitrate) and also abundant phytoplankton groups, separately. Statistical analysis and visualization of the results were done using software R 3.6.1 (Wickham 2009; R Core Team 2019).

## Results

### Physicochemical Parameters

The dynamics of temperature and nutrient concentrations in the Gulf of Riga (Online Resource 1) followed a typical seasonal pattern for temperate coastal waters. Low temperatures and high nutrient concentrations were observed during the autumn and winter months, whereas the summer months were characterized by high temperature and low nutrient concentrations. Salinity also followed a seasonal pattern with a slight decrease during the winter. Moreover, it significantly correlated (Table 1) with the distance from the Daugava river mouth in all studied periods ( $r=0.61$ – $0.87$ ;  $p<0.01$ ), expressing persistent spatial gradient within the Gulf of Riga.

### Spatial and Temporal Variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in SPM

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values varied from  $-30.6$  to  $-18.5\text{‰}$  and from  $-0.6$  to  $+12.9\text{‰}$ , respectively, over the entire 2015–2019 study period in the Gulf of Riga.  $\delta^{13}\text{C}$  showed sporadic correlation with both salinity and the distance from the region of joint inflow of the main rivers (Table 1).  $\delta^{15}\text{N}$  demonstrated consistently negative correlation to the distance and salinity, though with varying significance levels (Table 1).

The carbon isotopes in SPM at station 101A appeared to reach an equilibrium with river SPM only during the convective water mixing periods from autumn to early spring coinciding with larger river runoffs and low phytoplankton GPP (Fig. 2; Fig. 3). In correspondence with the increase of phytoplankton biomass (Fig. 2), the values of  $\delta^{13}\text{C}$  in the SPM of the Gulf of Riga rapidly increased during April 2017 reaching maximum by April 25 (Fig. 3). Thereafter,

**Table 1** Pearson's correlation coefficients ( $r$ ) for the relations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and spatial gradients—surface salinity (Sal) and distance from the mouth of the river Daugava (Dist)

	May 2015			May 2017			Aug 2017			Nov 2016		
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sal	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sal	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sal	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sal
$\delta^{13}\text{C}$			0.20			0.44*			-0.01			-0.22
$\delta^{15}\text{N}$	0.35		-0.55*	-0.15		-0.59**	-0.36		-0.47*	0.47*		0.07
Dist	0.07	-0.46*	0.68**	0.37	-0.42	0.87***	0.23	-0.53*	0.61**	-0.49*	-0.06	0.85***

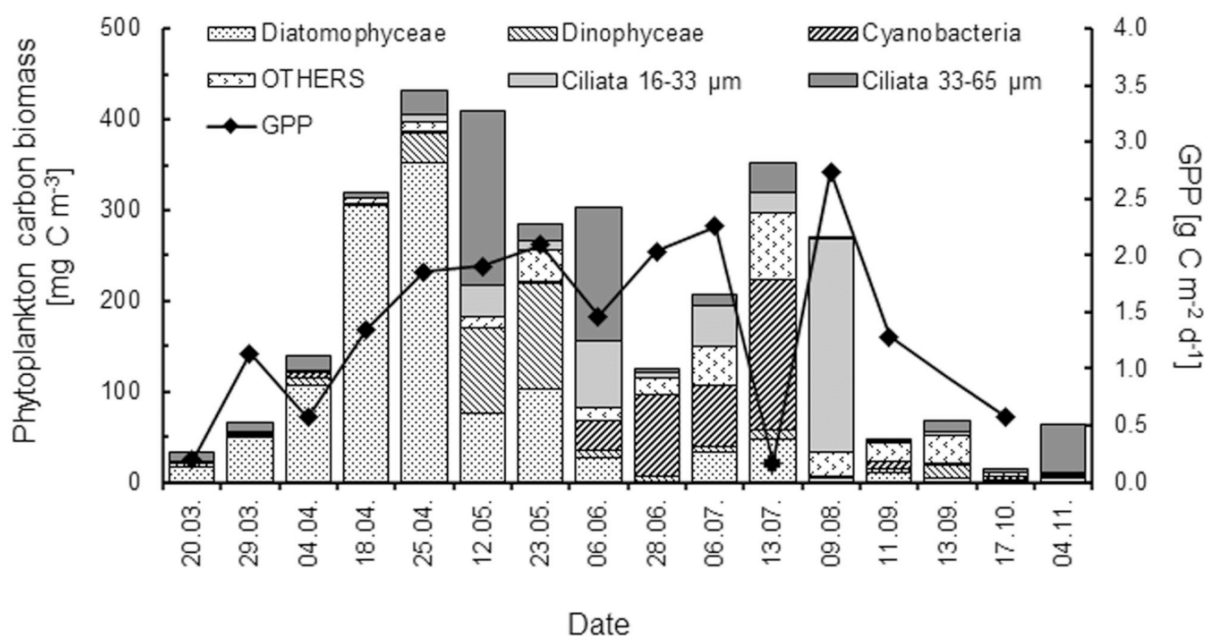
\* $0.01 \leq p \leq 0.05$ ; \*\* $0.001 \leq p < 0.01$ ; \*\*\* $p < 0.001$

the slow decline of  $\delta^{13}\text{C}$  was observed throughout May and the summer months. The  $\delta^{13}\text{C}$  values in riverine SPM followed an opposite pattern that coincided with runoff intensity by demonstrating decrease of values during spring and summer.

$\delta^{15}\text{N}$  values of SPM in the Gulf of Riga exhibited a bimodal distribution with two maxima in spring and autumn. The spring (Fig. 3) maxima was reached on May 12 after a rapid  $^{15}\text{N}$  enrichment in April. Thereafter, unlike the  $\delta^{13}\text{C}$ , the  $\delta^{15}\text{N}$  values quickly decreased, reaching a summer minimum on June 28. The broader second  $\delta^{15}\text{N}$  maxima was observed during August and September (Fig. 3). Although the  $\delta^{15}\text{N}$  in riverine SPM exhibited general increase during spring and summer, the seasonal dynamic substantially differed from that observed in the Gulf of Riga. Nitrogen isotope ratio in 101A appeared to even out with river  $\delta^{15}\text{N}$

values over winter; however, equilibrium was reached a month later than for  $\delta^{13}\text{C}$ .

The  $k$ -means clustering algorithm revealed two seasonally distinct groups of SPM isotope ratios in the Gulf of Riga samples. Cluster I (Fig. 4) represents April and May (i.e., vernal period), when both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  had more enriched values (from +3.6 to +11.1‰, mean +7.6 ± 1.8‰, and from -27.3 to -18.5‰, mean -22.6 ± 2.1‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively). During the rest of the year (cluster II; Fig. 4) isotope ratios were pooling at lower values (from 0 to +6.5‰, mean +4.0 ± 2.1‰, and from -30.6 to -25.4‰, mean -27.5 ± 1.2‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively) that are closer to relative isotope ratios of freshwater SPM observed in the river stations (from -0.7 to +8.5‰, mean +2.9 ± 2.8‰ and from -33.0 to -27.5‰, mean -30.0 ± 1.8‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively).



**Fig. 2** Temporal changes of phytoplankton carbon biomass by taxonomic classes and gross primary production (GPP) in the Gulf of Riga (station 101A) from March 2017 to November 2017. Group

OTHERS includes classes *Chlorophyceae*, *Cryptophyceae*, *Euglenophyceae*, *Ebriophyceae*, *Prasinophyceae*, *Prymnesiophyceae*, and *Incertae Sedis*

The results of PCA showed that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were affected by different abiotic parameters and gradients (Fig. 5). Salinity and nitrates + nitrites were the main environmental drivers (PC1; 34.4%) on overall data variability during the spring season (cluster I; Fig. 5; Online resource 2). Chl *a*,  $\delta^{13}\text{C}$ , and phosphates together contributed considerably to PC2 (cluster I; Fig. 5) implying  $\delta^{13}\text{C}$  relation to spring phytoplankton dynamics. The impact of seasonal changes on  $\delta^{13}\text{C}$  became more pronounced within cluster II data. They revealed temperature, phosphates, and  $\delta^{13}\text{C}$  as the main contributors to PC1 during the summer–winter period (cluster II; Fig. 5).  $\delta^{15}\text{N}$  had no influence on cluster I data variability, but together with the spatial (salinity) and temporal (chl *a* dynamics, nitrates + nitrites, ammonium) environmental gradients contributed to PC2 during the summer–winter period (cluster II; Fig. 5; Online resource 2).

### Impact of Seasonal Succession of Phytoplankton on Isotopic Composition of SPM

The spring bloom in 2017 had started by late March–early April reaching maximum biomass in late April (Fig. 2; Online Resource 3). The early spring bloom (March and April) was dominated by arctic and arctic-boreal diatoms (mainly *Pauliella taeniata*, *Thalassiosira baltica*, and *Chaetoceros wighamii*) while the late spring bloom (May, early June) was dominated by dinoflagellate *Peridiniella catenata* and mixotrophic ciliate *Mesodinium rubrum*. The gross primary production (GPP) (Fig. 2; Fig. S2 in Online Resource 3), mainly manifested as planktonic community respiration, started to increase with the development of phytoplankton spring bloom (April–May) reaching the first maximum in late May–early June.

The second maximum of GPP was observed in July–August, indicating intensive development of summer species. Summer phytoplankton from late June to September was characterized by a mix of different functional groups—cyanobacterium *Aphanizomenon flosaquae*, ciliates, chlorophytes, and cryptophytes (Fig. 2; Online Resource 3). During the autumn after convective mixing in October–November, diatoms became more abundant again, though decreasing GPP values indicated low activity of the phytoplankton community.

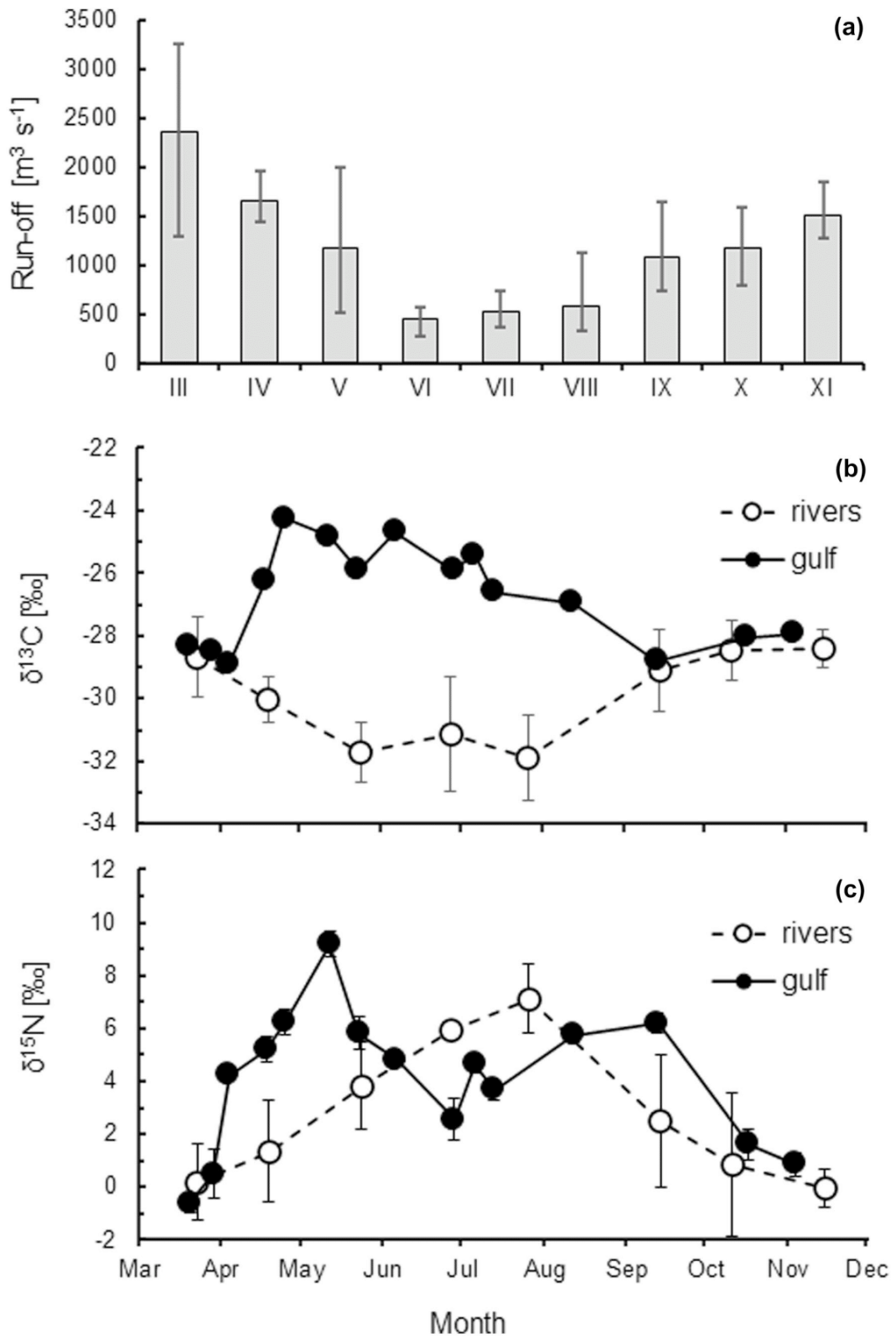
PCA conducted to identify impacts of biotic (phytoplankton succession) parameters on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  affirms their relation (Fig. 6; Online resource 2). The  $\delta^{13}\text{C}$ , as one of the main contributors to PC1 in both seasonally distinct data pools (cluster I; cluster II), evidently is directly linked to processes in the phytoplankton community. This demonstrates a clear negative correlation with diatom biomass in the spring (cluster I) and shows a direct link to carbon mass of dinoflagellates and small-sized *M. rubrum* (cluster II). Although to a lesser degree, the  $\delta^{15}\text{N}$  shows clear correlation

to specific taxa as well, contributing to PC2 for both data pools, diatoms during spring (cluster I), and large-sized *M. rubrum* and cyanobacteria during the summer–winter period (cluster II).

## Discussion

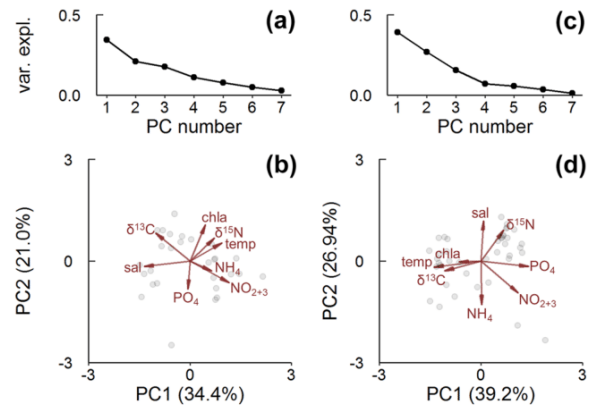
The spatial gradients of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  have been previously documented in estuarine and continental shelf waters (McKinney et al. 2010, van de Merwe et al. 2016, Jędruch et al. 2017). Furthermore, it has been argued that observed seasonal variability of  $\delta^{13}\text{C}$  values of SPM in coastal lagoons is driven by the seasonal dynamic of riverine inputs (Remeikaite-Nikiene et al. 2017; Marcelina et al. 2018). However, in contrast with findings in these studies, we did not establish a clear spatial gradient of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the Gulf of Riga. The results strongly indicate that the cause of the variability in C and N isotope fractionation are biological processes. The co-variation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with seasonally changing abiotic factors, like temperature and nutrient concentrations, establishes the seasonal nature of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as observed by Savoye et al. (2003), reflecting the seasonal succession of phytoplankton species.

The dynamic of phytoplankton biomass and species succession in the Gulf of Riga followed the general pattern for cold temperate coastal waters (Olli and Heiskanen 1999; Yurkovskis et al. 1999; Jurgensone et al. 2011; Gustafsson et al. 2013; Purina et al. 2018). The pronounced phytoplankton bloom resulted in enrichment of  $^{13}\text{C}$  in SPM, similarly to that observed by Remeikaite-Nikiene et al. (2017), as well as in enrichment of  $^{15}\text{N}$ . The  $\delta^{15}\text{N}$  curve observed in the river mouth stations (Fig. 3c) supports this statement as the phytoplankton biomass (expressed as chl *a*) in the river mouth reaches maximal values during summer months, as demonstrated by earlier study of Aigars et al. (2014), whereas the chl *a* values in the open part of the Gulf of Riga peak during the spring. The decrease of  $\delta^{13}\text{C}$  values in the riverine water during the summer were in clear contrast to the increase of  $\delta^{13}\text{C}$  values observed in the Gulf of Riga. This demonstrates the insignificance of allochthonous terrestrial material to phytoplankton in transitional waters of the Gulf during summer in contrast to other studies conducted in estuaries or river mouths of the Baltic Sea (Golubkov et al. 2017; Remeikaite-Nikiene et al. 2017; Marcelina et al. 2018). On the other hand, an increased proportion of allochthonous SPM in transitional waters was indicated during periods with low phytoplankton activity by analogous  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in pooled river SPM and transitional water SPM values in station 101A during winter (Fig. 3), and elevated C:N ratios before the spring bloom and in late autumn (Table S2 in Online Resource 1).



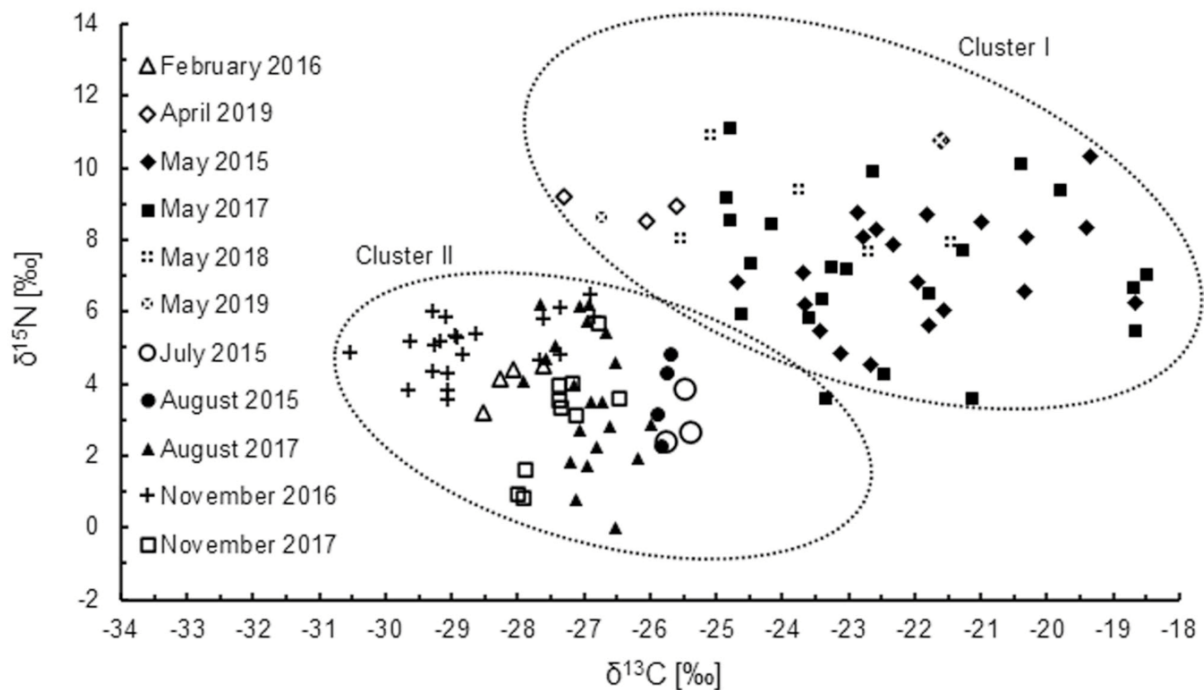
**Fig. 3** Average monthly river runoffs (a) and temporal changes of  $\delta^{13}\text{C}$  (b) and  $\delta^{15}\text{N}$  (c) in SPM of the joint river input and the Gulf of Riga (station 101A) from March 2017 to November 2017

The PCA clearly indicates that capacity to affect seasonal fractionation of  $^{13}\text{C}$  varies among the phytoplankton groups during active biomass growth periods. Variation in  $\delta^{13}\text{C}$  values is mostly driven by diatoms in early spring and dinoflagellates and 33–65  $\mu\text{m}$  sized *M. rubrum* in late spring, while other phytoplankton groups have minor or no effect.  $\delta^{13}\text{C}$  values in non-vernal periods are driven by dinoflagellate and 16–32  $\mu\text{m}$  sized *M. rubrum* blooms. The relationship between  $\delta^{15}\text{N}$  and phytoplankton species is more complex than that of  $\delta^{13}\text{C}$ . The rapid increase in  $\delta^{15}\text{N}$  values in March–April can be explained by the development of the early spring bloom. The dominating phytoplankton species during this period were diatoms, known to prefer the more  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$  rather than  $\text{NH}_4^+$  for nitrogen uptake during active growth phase (Domingues et al. 2011). After exhaustion of DSi pool (Table S2 in Online resource 1), diatoms were replaced by dinoflagellates and large-sized *M. rubrum* in late spring (May) further depleting the inorganic nitrogen winter pool by transferring the remainder of it from dissolved phase (majority of it in  $\text{NO}_3^-$  form) to particles (Savoye et al. 2003). The relations between  $\delta^{15}\text{N}$ , chl *a*, and salinity in the spring indicate enhanced growth in



**Fig. 5** The results of principal component analysis for cluster I (a, b) and cluster II (c, d) in combination with environmental parameters: temperature (temp), salinity (sal), concentration of chlorophyll *a* (chl*a*), concentration of nutrients ammonium ( $\text{NH}_4$ ), phosphate ( $\text{PO}_4$ ), nitrite + nitrate ( $\text{NO}_{2+3}$ ). Variation explained (var.expl.) represented by eigenvalues of the axes (a, c) and biplot (b, d) of individual data scores—points—and loadings of explanatory variables—arrows

transitional waters. This may be due to increased agricultural runoffs being rapidly taken up by the fast-growing phytoplankton species before their dispersion across the Gulf of Riga.



**Fig. 4** Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the Gulf of Riga during years 2015–2019. Observations were grouped within clusters using the *k*-means algorithm (dotted lines)

The observed decrease of  $\delta^{15}\text{N}$  values in the summer was most likely caused by the development of the  $\text{N}_2$ -fixing cyanobacterium *A. flosaquae*. In the Baltic Sea, this species has more  $\text{N}_2$ -fixing heterocysts in June than in late summer (Klawonn et al. 2016). The observed difference in the number of heterocysts can explain  $^{15}\text{N}$  enrichment in SPM after June, while *A. flosaquae* still maintains a significant proportion in phytoplankton biomass.

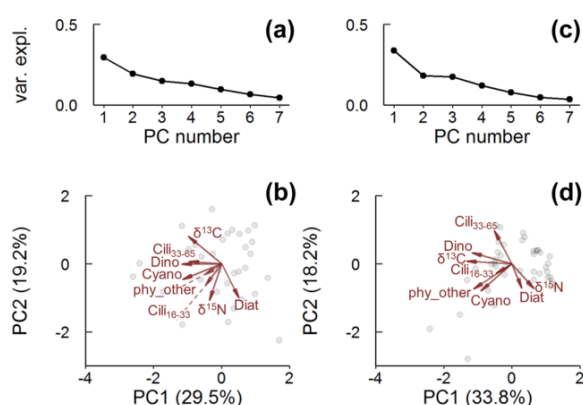
However, the negative correlation between cyanobacteria biomass and  $\delta^{15}\text{N}$  values is not obvious in summer according to PCA (Fig. 6, cluster II) most likely because the cyanobacteria were never the overwhelmingly dominating taxa in the non-vernal phytoplankton community during our study.  $\delta^{15}\text{N}$  values by the end of spring bloom in April only slightly exceeded the range of values that are typical for deep ocean inorganic nitrogen (Jędruch et al. 2017; Pantoja et al. 2002) or Baltic Proper surface SPM  $\delta^{15}\text{N}$  values (Winogradow et al. 2019) suggesting very limited impact of the river runoff, which delivers inorganic nitrogen of terrestrial and anthropogenic origin (see isoscapes of DIN, total nitrogen, and  $\delta^{15}\text{N}$  over the study period in Online resource 4, visualized with Ocean Data View v5.5.1. (Schlitzer 2021)). This is in line with the budget calculations presented by Müller-Karulis and Aigars (2011), which demonstrate that on average, 81% of annual nitrogen input was denitrified and 3% was buried. Therefore, only a minor fraction of annual input with clear anthropogenic  $\delta^{15}\text{N}$  signal is being retained in the water column.

Consequently, it could be expected that in the absence of a significant external nitrogen pool by the end of spring bloom, the  $\delta^{15}\text{N}$  value in SPM would be determined by the recycling of an already assimilated nitrogen pool. Contrary to this assumption, the highest  $\delta^{15}\text{N}$  value was observed in

May, when no further phytoplankton carbon biomass increase could be detected (due to lack of dissolved phosphates and silicates; Table S2 in Online Resource 1). The most plausible explanation is the shift in phytoplankton species composition, e.g., successive diatom replacement by ciliates *M. rubrum* in May. It is highly possible that the fast vertical migration ability of this ciliate manifests  $\delta^{15}\text{N}$  value increase from utilizing the bottom layer nitrogen pool (Lips and Lips 2017), as can be observed in the  $\delta^{15}\text{N}$  values transitioning from April 25 to May 23. Furthermore, in early June, *M. rubrum* did not increase the bulk  $\delta^{15}\text{N}$  values despite its large abundance in the phytoplankton biomass, likely due to depletion of deeper dissolved nitrogen pools. Additionally, it is possible that unfiltered water, as sampled in our study, contains other taxa, e.g., small-sized zooplankton that could increase the overall  $\delta^{15}\text{N}$  values of bulk SPM as shown by Rolff (2000).

## Conclusions

The Gulf of Riga receives substantial riverine discharges, which are enriched by nitrogen originating from agricultural lands and municipal wastewater plants located in the drainage basin, as well as direct inputs from municipal wastewaters from Riga and Jurmala cities. The combined discharges form a strong and geographically distinctly located source of nitrogen and carbon of anthropogenic and terrestrial origin. Nevertheless, the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values of combined river SPM input signal can only be detected in the stations under direct impact of the river inflow over winter. Results of the present study demonstrate that neither terrestrial or anthropogenic sources or even a combination of them has sufficient strength of its  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  signal to create distinct spatial gradient of  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values of SPM in the Gulf of Riga during the productive period (spring–autumn). We established that the main driver for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  variability in SPM during this period is the succession of phytoplankton species. It evidently defines carbon and nitrogen isotopic ratios of SPM during the spring bloom and substantially affects isotope ratios until mid–autumn. Diatoms, dinoflagellates, and *M. rubrum* show the strongest positive relation to isotopic changes in the Gulf of Riga, whereas the diazotrophic cyanobacteria have an observable but a statistically insignificant negative effect on  $\delta^{15}\text{N}$  values. The results of this study emphasize the need to consider seasonal and spatial variations of the phytoplankton community, while assessing the spatial or seasonal variability of carbon and nitrogen isotope ratios of the SPM. In addition, the indirect evidence provided by this study let us hypothesize that the ciliate *M. rubrum* facilitates  $\delta^{15}\text{N}$  increase by incorporating near-bottom nitrogen before vertical migration to euphotic zone.



**Fig. 6** The results of principal component analysis for cluster I (a, b) and cluster II (c, d) in combination with carbon mass of abundant phytoplankton groups: diatoms (Diat), dinoflagellates (Dino), ciliates *Mesodinium rubrum* with cell size between 33 and 65  $\mu\text{m}$  (Cili\_33-65) and cell size between 16 and 33  $\mu\text{m}$  (Cili\_16-33), cyanobacteria (Cyano), and other taxa (other). Variation explained (var.expl.) represented by eigenvalues of the axes (a, c) and biplot (b, d) of individual data scores—points—and loadings of explanatory variables—arrows

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s12237-022-01071-z>.

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## ORIGINAL RESEARCH ARTICLE

# Seasonal variation in size structure and production of autotrophic plankton community in eutrophied, low-light environment: A focus on *Mesodinium rubrum*

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**Abstract** Temporal variations in the primary production of the size-fractionated autotrophic plankton community were studied in coastal-estuarine waters of the eutrophic Gulf of Riga, Baltic Sea. The community was net-autotrophic during spring and summer and net-heterotrophic during autumn. The results of the present study clearly demonstrate strong covariation between net primary production (NPP) and  $<56 \mu\text{m}$  fractionated community biomass, particularly small-sized ( $16\text{--}33 \mu\text{m}$ ) *Mesodinium rubrum*, implying that the majority of NPP stems from the lower end of the size spectrum. A pronounced size distribution shift was observed within the *M. rubrum* population. Large-sized (length  $\geq 34 \mu\text{m}$ ) *M. rubrum* was the most abundant in the first half of the productive season (until week 24), whereas small-sized *M. rubrum* dominated during the stratified period.

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## 1. Introduction

*Mesodinium rubrum* is a mixotrophic Litostomatea ciliate that possesses plastids preying upon cryptophyte algae (Johnson et al., 2016; Hansen and Fenchel, 2006). It is highly productive in turbid waters and at low light irradiance (Crawford, 1989; Herfort et al., 2012; Johnson and Stoecker, 2005; Moeller et al., 2011). *Mesodinium rubrum* is often abundant in estuarine-coastal waters (Cloern et al., 1994; Leles et al., 2017; Sanders, 1995), including the

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brackish Baltic Sea (Lips and Lips, 2017; Purina et al., 2018; Rychert, 2004). Additionally, under certain conditions, it forms blooms (red tides) (Taylor et al., 1971), yet *M. rubrum*-induced red tides are not reported from the temperate Baltic Sea region – the research area of the present study.

The main environmental factors promoting the growth and development of *M. rubrum* in temperate zones are season-specific (Johnson et al., 2013; Lips and Lips, 2017; Montagnes et al., 2008). Due to its motile abilities, *M. rubrum* benefits from strengthened stratification and the associated depletion of dissolved inorganic nutrients in the surface layers (Lips and Lips, 2017; Nishitani and Yamaguchi, 2018). Additionally, an elevated CO<sub>2</sub> concentration is not a hindrance to the successful development of *M. rubrum*; in contrast, Baltic *M. rubrum* increases its photosynthetic rate and forms a higher abundance under high-CO<sub>2</sub> treatment (Lischka et al., 2017). Thus, projected future climate change for the Baltic Sea region, (i.e., an increase in temperature, runoff and pH) and a decrease in salinity (BACC II, 2015; Havenhand, 2012; Omstedt et al., 2012), might expand the spatial and temporal space suitable for *M. rubrum* (Mitra et al., 2014). Considering the regional importance of *M. rubrum* in the Baltic Sea (Lips and Lips, 2017; Rychert, 2004), as well as its potential for expanded range and density in the future, it is essential to improve the knowledge base of *M. rubrum* ecology to better comprehend potential shifts in the functioning of the future Baltic Sea food web.

*Mesodinium rubrum* displays a wide size distribution (from 15 to 70 µm); therefore, size distinction is introduced in numerous studies on *M. rubrum* (e.g., Johansson, 2004; Johnson et al., 2016; Montagnes et al., 2008), revealing different ecological responses to environmental changes between size classes. Notably, *M. rubrum* has recently been recognised as a species complex consisting of at least two described and accepted species – *M. rubrum* and *Mesodinium major* (cf. Garcia-Cuetos et al., 2012; Johnson et al., 2016). Both have a similar morphology and possess plastids of the same origin (red plastid cryptophytes), but they show differences in cell length and ecology. *Mesodinium rubrum* cell length ranges between 16–35 µm, whereas longer cells are associated with *M. major*. In Danish waters of the Baltic Sea, *M. rubrum* is abundant during summer and early autumn, but *M. major* occurs mainly in the winter and early spring (Garcia-Cuetos et al., 2012). Recently, another *M. rubrum*-like species was identified in a Japanese brackish lake (Nishitani and Yamaguchi, 2018). It is seemingly morphologically identical to the *M. rubrum/major* complex but shows contrasting behaviour; it naturally preys upon green plastid cryptophytes. However, as molecular methods were not utilised in the present study, we refer to all *M. rubrum*-like ciliates as *M. rubrum*.

The estimates of planktonic primary production and identification of its main contributors are crucial to the comprehension of carbon flow dynamics and ecosystem functioning in general. The size and structure of the composition greatly influences the functioning of the pelagic food web (Lancelot and Muyllaert, 2011; Tremblay and Legendre, 1994). Moreover, the recently proposed mixotrophic-centric paradigm for marine ecology highlights the need for

detailed characterisation of mixotrophic functional groups (Mitra et al., 2014, 2016) to provide sufficient data for their inclusion within regional food web models. In the present study, we evaluated the contribution of *M. rubrum* to primary production in the eutrophic Gulf of Riga. To achieve this, we assessed the production of size-fractionated autotrophic communities, including small-sized (length of 16–33 µm) and large-sized *M. rubrum* (length ≥ 34 µm), and explored potential associations between production and seasonally changing environmental factors.

## 2. Material and methods

### 2.1. Study area

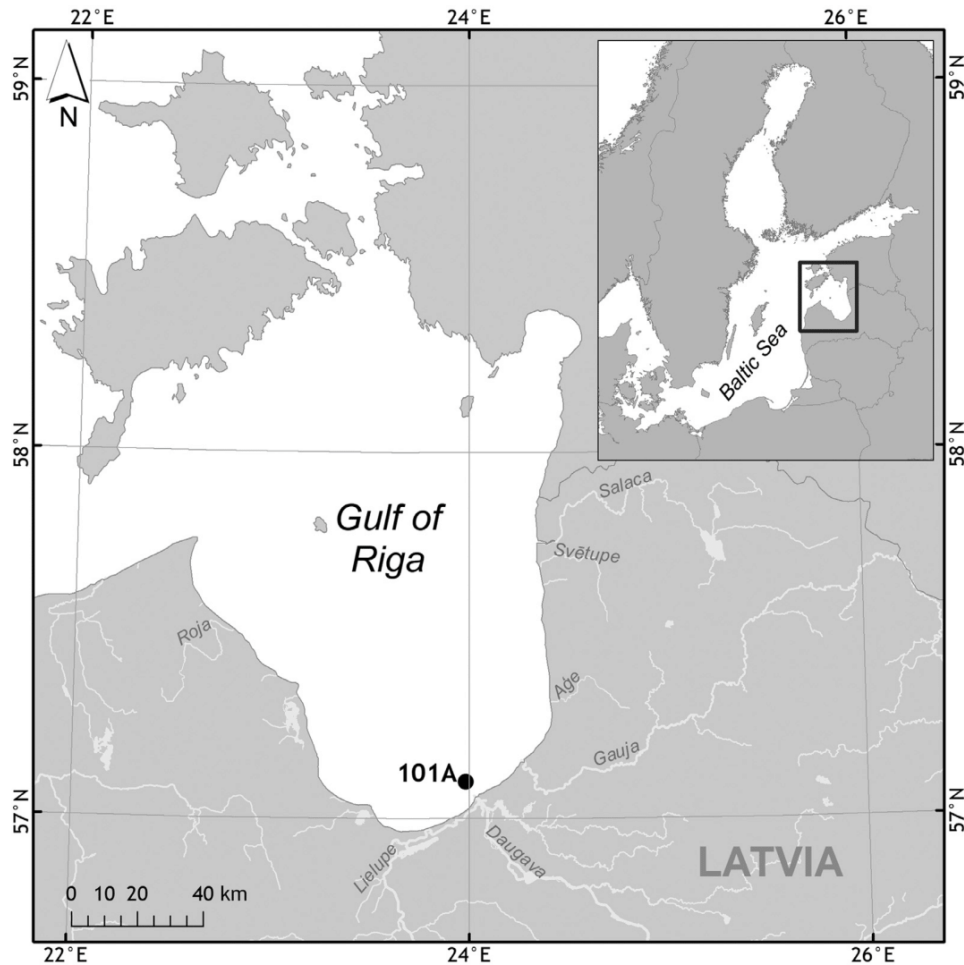
The Gulf of Riga (GoR) is a brackish semi-isolated area (16 330 km<sup>2</sup>, 424 km<sup>3</sup>; Berzinsh, 1995) of the Baltic Sea. It is connected to the Baltic Proper by narrow straits that confine water exchange. The shallowness of the GoR (average depth of 26 metres; Kotta et al., 2008) ensures the development of pronounced seasonal thermal stratification during the summer (Kotta et al., 2008). The mean salinity of the GoR ranges between 5 and 7 PSU, but it has a decreasing southward salinity gradient as the majority (approximately 86%) of the riverine runoff inflows into the southern part of the GoR (Berzinsh, 1995). Therefore, the southern GoR is characterised by higher water turbulence and increased values of riverine export (e.g., nutrients, suspended and dissolved organic matter, and chromophoric dissolved organic matter) that result in decreased water transparency. The study site (Figure 1) was located in the southern GoR to explore the habitat most suitable to *M. rubrum* within the GoR and thus evaluate the maximal potential effect *M. rubrum* may have on local primary production.

### 2.2. Sampling

Sampling was conducted at a national monitoring station (101A; Figure 1) located approximately 5 km from the mouth of the Daugava River. Samples were collected from 20 March 2017 to 24 October 2017 (Table 1) between 8 am and 10 am.

Water temperature and salinity were measured using a water probe (SBE 19plus Sea-Cat, USA). Photosynthetically active radiation (PAR) was measured using a LI-COR Data Logger equipped with an LI-190R Quantum Sensor. Water transparency was measured with a Secchi disc. Samples for estimating the concentration of nutrients (dissolved inorganic nitrogen (DIN): ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>); dissolved inorganic phosphorus (DIP): phosphate (PO<sub>4</sub><sup>3-</sup>); dissolved silica (DSi): silicate (SiO<sub>4</sub><sup>-</sup>)), phytoplankton community parameters (abundance, biomass) and primary production were obtained from the upper layer (0–10 m) using an integrated hose (inner diameter 25 mm) following HELCOM COMBINE Monitoring Guidelines (HELCOM, 2017). Nutrient concentrations were determined according to Grasshoff et al. (1983) (for a detailed description, see Purina et al. (2018)). All laboratory analyses were performed in an accredited laboratory (ISO/IEC 17205).

Integrated samples for phytoplankton analysis and estimation of primary production were divided into two types



**Figure 1** The location of sampling Station 101A (coordinates 57.10°N 23.98°E; depth approx. 22 m) in the Gulf of Riga.

(unfractionated and fractionated  $<56 \mu\text{m}$ ) immediately after sampling. A size-fractionated approach has been widely used to extend the understanding of phytoplankton dynamics, as cell size directly affects responses to environmental variations (Malone and Chervin, 1979; Sin, 2000; Soria-Piriz et al., 2017) and associated impacts on the food web, e.g., via production (Cotti-Rausch et al., 2020; Mousseau et al., 1998; Probyn, 1990). The phytoplankton samples were divided by reverse fractionation: passed through a sieve with a mesh size of  $56 \mu\text{m}$  (henceforth:  $<56$ -fractionated). The  $56 \mu\text{m}$  sieve was chosen for fractionation based on the observed distribution of *M. rubrum* size classes in the long-term data collected at Station 101A (see database <https://latmare.lhei.lv/>).

Phytoplankton samples (300 ml) were fixed with acid Lugol's solution (final conc. 0.5%). Subsamples of 10 and 25 ml of fixed samples were settled in a sedimentation chamber for 8 and 18 hours, respectively, and counted according to the Uthermöl technique with an inverted microscope (Leica DMI 3000, Leica Microsystems GmbH, Germany) at 200x and 400x magnification. The number of counted cells in all subsamples exceeded 500 (HELCOM 2017; Olenina et al., 2006; Utermöhl, 1958). Both groups of samples (unfractionated and  $<56$ -fractionated) were handled identically during the analysis.

Phytoplankton organisms were identified to the lowest possible taxonomic rank. Their names and classification complied with the accepted binomial nomenclature of the World Register of Marine Species (version 2021). Classification of *M. rubrum* into size classes  $>34 \mu\text{m}$  and  $16\text{--}33 \mu\text{m}$  was based on the maximum cell dimension (HELCOM PEG biovolume file at <https://helcom.fi/helcom-at-work/projects/peg/>) and considering thresholds applied in other studies conducted in the Baltic Sea region ( $33 \mu\text{m}$ ; Johansson, 2004). The biomass was expressed as  $\text{gC m}^{-2}$ . The carbon content was calculated according to Menden-Deuer and Lessard (2000).

### 2.3. Primary production measurements

Primary production rates were determined for each type of phytoplankton sample (unfractionated and  $<56$ -fractionated) separately. The light and dark bottle oxygen technique was used (Bender et al., 1987; Olesen et al., 1999) to evaluate the primary production of the study site. Fifteen transparent, calibrated (approximately 100 ml) glass bottles were filled with water for oxygen measure-

**Table 1** Measurements of environmental variables in the upper 10 m layer at the sampling location (Station 101A) in the Gulf of Riga during 2017. PO<sub>4</sub><sup>3-</sup> – dissolved inorganic phosphate, μmol l<sup>-1</sup>; SiO<sub>4</sub><sup>-</sup> – dissolved silicate, μmol l<sup>-1</sup>; NO<sub>2</sub><sup>-</sup> – nitrite, μmol l<sup>-1</sup>; NO<sub>3</sub><sup>-</sup> – nitrate, μmol l<sup>-1</sup>; NH<sub>4</sub><sup>+</sup> – ammonium, μmol l<sup>-1</sup>; Secchi – water transparency depth, m; Temp – temperature, °C; Sal – salinity, PSU; PAR – photosynthetically active radiation, mol photons m<sup>-2</sup> d<sup>-1</sup>. \* – observations not included in the PLSR.

	Week	PO <sub>4</sub> <sup>3-</sup>	SiO <sub>4</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	Secchi*	Temp	Sal	PAR
20-Mar	12	1.40	66.2	0.49	64.51	3.6	1.5	2.3	4.5	44
29-Mar	13	1.04	54.1	0.40	58.40	1.2	1	4.0	4.0	62
04-Apr	14	0.93	55.8	0.46	49.74	0.6	1.1	5.6	4.4	207
18-Apr	16	0.49	18.3	0.50	27.20	0.6	1.4	5.9	5.3	132
24-Apr	17	0.26	4.5	0.43	19.57	0.1	1.5	6.7	5.1	39
23-May	21	0.05	6.2	0.17	6.03	0.5	1.8	15.0	5.4	163
06-Jun	23	0.09	3.3	0.04	1.84	0.3	1.5	16.0	5.4	337
15-Jun	24	0.09	2.7	0.04	0.39	0.26	1.5	16.6	4.6	347
28-Jun	26	0.15	2.9	0.02	0.34	1.5	1.8	18.0	4.7	305
06-Jul	27	0.07	7.5	0.08	1.02	0.4	1.5	17.2	4.8	254
13-Jul	28	0.09	2.7	0.08	1.02	0.2	1.5	17.2	4.8	76
19-Jul	29	0.11	5.1	0.02	0.18	1.2	2.5	18.5	4.8	152
09-Aug	32	0.20	12.0	0.12	3.43	0.4	2.5	19.3	4.4	266
21-Aug	34	0.14	10.8	0.06	1.81	0.4	2.5	18.9	4.6	142
11-Sep	37	0.57	25.7	0.16	7.63	0.4	1.6	15.6	4.7	86
20-Sep	38	0.59	26.8	0.14	6.74	0.9	1.2	13.5	4.1	150
28-Sep	39	0.68	33.6	0.23	9.77	1.4	1.2	13.6	4.7	139
17-Oct	42*	NA	NA	NA	NA	2.2	1.9	10.6	4.1	29
25-Oct	43*	NA	NA	NA	NA	0.9	2.0	6.8	4.6	82

ments for both unfractionated and <56-fractionated samples (30 bottles in total). Bottles were divided into 5 groups with 3 replicates in each group for both sample types. One group of samples was set as an initial state, and the oxygen concentration was fixed with Winkler reagents (1 ml manganese chloride and 1 ml alkaline iodide) prior to incubation. The other four groups were incubated for 24 hours under conditions imitating light transmittance at specific depths of the euphotic layer: 100%, 66%, 23% and 0% light transmittance. To achieve these conditions, we wrapped the bottles in plastic optical filters (GAMPRODUCTS, Inc.) accordingly: no filter for 100% transparency, 1514 GAM for 66% transparency, 1516 GAM for 23% transparency and aluminium foil for 0% transparency. All vials used in the incubation were mounted on a rotating wheel and submerged in the onboard incubator with a continuous flow of seawater to ensure the ambient water temperature and *in situ* illumination during the 24-hour incubation. Every incubation was started approximately at the same time - between 11 am and 1 pm. After 24 hours, the samples were fixed with Winkler reagents. Oxygen concentrations were determined by titration with sodium thiosulfate according to ISO 5813:1983.

Oxygen consumption in the dark bottles (0% light transmittance) was used as a proxy of community respiration, while the other three groups (100%, 66%, and 23% light transmittance) were used to evaluate daily net primary production rates in the water column. The measured oxygen concentrations were converted to carbon units according to the stoichiometry of the photosynthesis equation (the conversion factor from ml O<sub>2</sub>/l to gC/m<sup>3</sup> was 0.5357) (Bender et al., 1987; Van Niel, 1949). The approximate attenuation coefficient (*k*) was calculated for each sampling

from Secchi depth (*D<sub>s</sub>*) as:

$$k = 1.7/D_s.$$

The depth of specific light conditions (*z*) was calculated from:

$$z = -\frac{\ln\left(\frac{I_z}{I_0}\right)}{k}$$

where *I<sub>z</sub>* is the light intensity at a specific depth (66% or 23%) and *I<sub>0</sub>* is the light intensity below the surface (100%). The daily net primary production rate (NPP, gC m<sup>-2</sup> d<sup>-1</sup>) was estimated by trapezoidal integration of the data from various light conditions. Gross primary production (GPP, gC m<sup>-2</sup> d<sup>-1</sup>) was calculated by summing NPP and respiration (gC m<sup>-2</sup> d<sup>-1</sup>). Annual gross primary production was calculated as the GPP monthly averages multiplied by the number of days and summed up for 365 days, assuming that production occurs only during the productive season, neglecting the period from November to February.

## 2.4. Statistical analysis

Data visualisation and analysis were performed using R software v.3.6.1 (R Core Team 2019; Wickham, 2009). A comparison of NPP between the unfractionated community and <56-fractionated community was conducted by the Wilcoxon signed-rank test.

The correlation between the primary production rates (NPP, GPP) and environmental variables was obtained by applying a partial least squares regression (PLSR). PLSR was performed using the functionality of the 'pls' package (Liland et al. 2022; Mevik and Wehrens, 2015) setting method to SIMPLS (De Jong, 1993). PLSR is a regression-like

technique which can handle the multi-collinearity issue and variables that are not normally distributed.

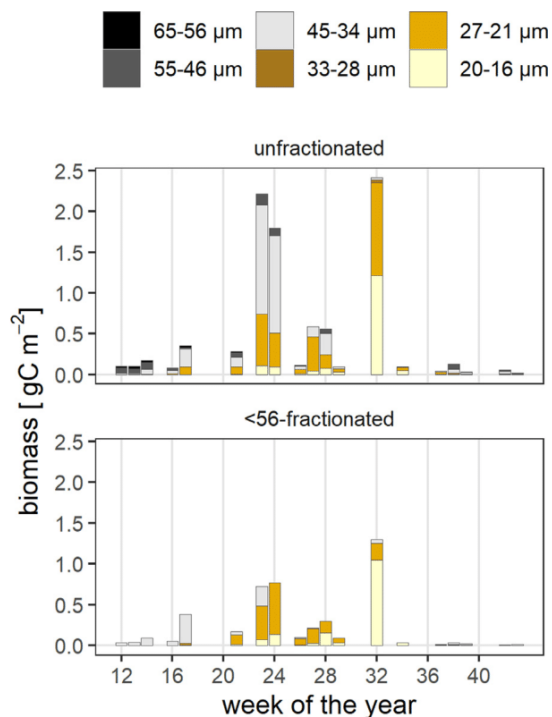
The importance of each explanatory variable in the PLSR model was estimated by the procedure of the Variable Importance for the Projection. Variable importance (expressed in percentage) was calculated based on weighted sums of the absolute regression coefficients by applying *varImp* function from the 'caret' package (Kuhn, 2021). The weights were calculated separately for each outcome as a function of the reduction of the sums of squares across the number of PLSR components. Prior to PLSR analysis, carbon masses of phytoplankton groups were Hellinger-transformed and all variables were centred and scaled to unit variance.

Unfractionated and <56-fractionated communities were analysed separately. The last two observations (weeks 42 and 43) were not included in the PLSR analysis due to a lack of nutrient data.

### 3. Results

#### 3.1. Size range of *Mesodinium rubrum*

A wide size range (16–65  $\mu\text{m}$  in length; Figure 2) was observed in the *M. rubrum* population. The population was dominated by large ( $\geq 34 \mu\text{m}$ ) specimens during the spring and by smaller *M. rubrum* in the following period. Average-sized cells (33–28  $\mu\text{m}$ ) were less prevalent; therefore, we established division into two groups – large-sized and small-sized. Cells 34  $\mu\text{m}$  or longer were designated as large, while those under 34  $\mu\text{m}$  were designated as small.



**Figure 2** Dynamics of size classes within the *Mesodinium rubrum* population in an unfractionated and <56-fractionated phytoplankton community of the Gulf of Riga.

#### 3.2. Environmental conditions: abiotic drivers

The temporal dynamics of the measured environmental parameters are shown in Table 1. Overall, environmental variables changed seasonally, with the highest temperature and lowest nutrient values during the summer. Salinity and water transparency varied marginally, whereas PAR intensity was mainly dependent on the season and cloud cover and varied considerably during the studied period.

#### 3.3. Environmental conditions: phytoplankton population

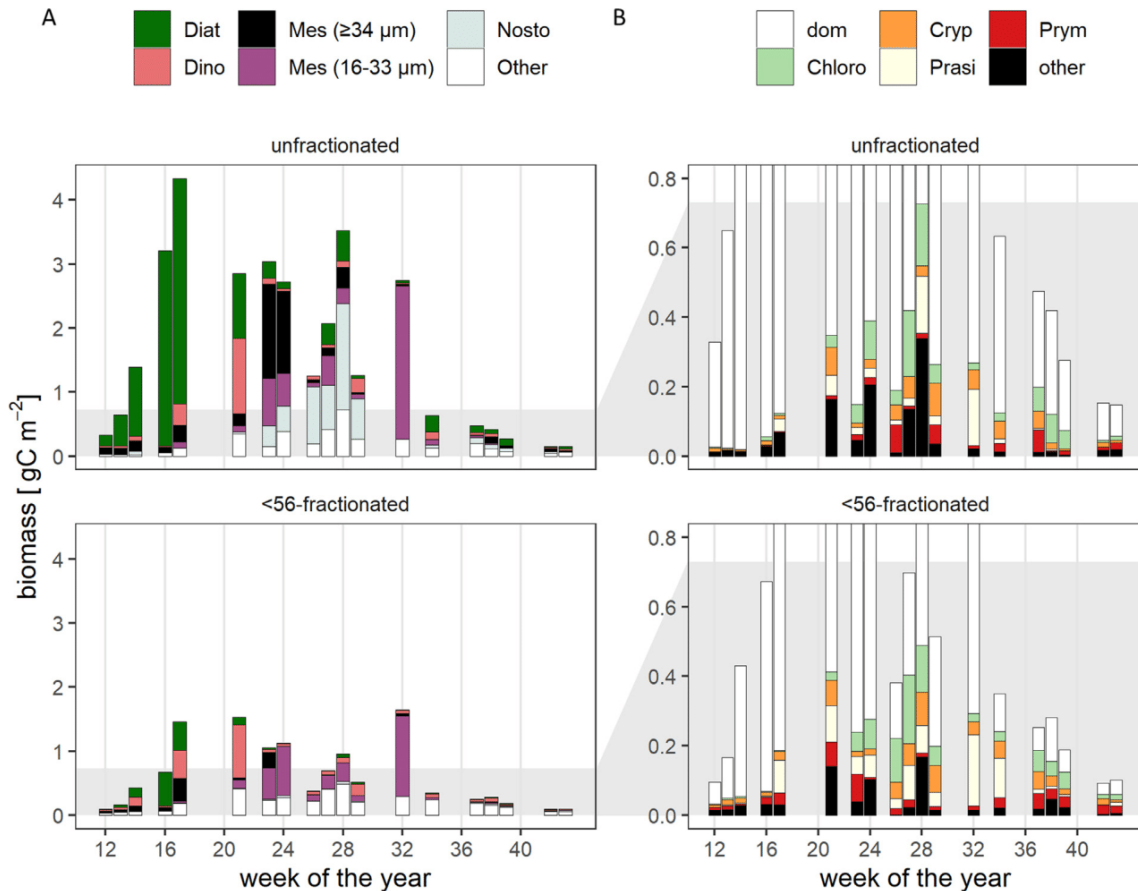
The phytoplankton community showed a typical succession for temperate coastal waters during the study period (Figure 3). The highest total carbon mass (3.2–4.3  $\text{gC m}^{-2}$ ) within the unfractionated autotrophic community was observed in spring (weeks 16–17), when diatoms, mainly *Chaetoceros wighamii* and *Thalassiosira baltica*, prevailed. Diatoms were successively replaced by motile taxa, (i.e., the dinoflagellate *Peridiniella catenata* and the large-sized ( $\geq 34 \mu\text{m}$ ) ciliate *M. rubrum*), which dominated the phytoplankton community until mid-June (week 24). *M. rubrum* contributed approximately 70% to the carbon mass of both the unfractionated and <56-fractionated communities during weeks 23–24.

Cyanobacteria and small-sized (16–33  $\mu\text{m}$ ) *M. rubrum* co-dominated during the summer period (weeks 26–32). The highest carbon mass of the cyanobacterium *Aphanizomenon flos-aquae* (1.66  $\text{gC m}^{-2}$ ) and small-sized *M. rubrum* (2.39  $\text{gC m}^{-2}$ ; 95% of total carbon mass) was observed in mid-July (week 28) and early August (week 32), respectively. Chlorophyceae (*Dictyosphaerium ehrenbergianum*, *Oocystis* spp., and *Raphidocelis sigmoidea*), Cryptophyceae (*Plagioselmis prolunga* and *Teleaulax* spp.), Prasinophyceae (*Pyramimonas* spp.) and Prymnesiophyceae (*Chrysochromulina* spp.) increased in carbon mass during the summer period (weeks 23–32), showing similar dynamics as cyanobacteria and small-sized *M. rubrum* (Figure 3).

The phytoplankton community in <56-fractionated samples showed an analogous succession pattern, except for large diatoms and filamentous cyanobacterium *A. flos-aquae*, which were filtered out during fractionation (Figure 3). Dinoflagellates (*Heterocapsa rotundata* and *P. catenata*) together with small-sized *M. rubrum* dominated the <56-fractionated community during spring when *M. rubrum* contributed up to 40% of the total carbon mass. A slight increase in Chlorophyceae (*D. ehrenbergianum* and *R. sigmoidea*), Cryptophyceae (*P. prolunga* and *Teleaulax* spp.) and Prasinophyceae (*Pyramimonas* spp.) was observed during cyanobacteria dominance (weeks 26–29). Notably, small-sized *M. rubrum* reached its maximum carbon mass soon afterward and compiled approximately 80% of the total carbon mass (week 32).

#### 3.4. Primary production

The GPP and NPP of both the unfractionated and <56-fractionated autotrophic communities varied seasonally (Figure 4). Overall, the planktonic community was net autotrophic during spring and summer and net heterotrophic



**Figure 3** Temporal dynamics of A) dominant phytoplankton taxonomic groups and B) other phytoplankton taxonomic groups in unfractinated (upper) and 56-fractionated samples (lower) from the top 10 m layer at the sampling location (Station 101A) in the Gulf of Riga. Diat – Diatomophyceae; Dino – Dinophyceae; Mes ( $\geq 34 \mu\text{m}$ ) – large-sized *Mesodinium rubrum*; Mes (16–33  $\mu\text{m}$ ) – small-sized *Mesodinium rubrum*; Nosto – Nostocophyceae/cyanobacteria; dom – dominant groups – shown in graph A; Chloro – Chlorophyceae; Cryp – Cryptophyceae; Prasi – Prasinophyceae; Prym – Prymnesiophyceae. Mind the differences in scales of Y-values, follow the grey area for rescaling.

during autumn (Figure 4A). The amount of both NPP and GPP of the unfractinated community was not significantly different from the amount produced by the <56-fractionated community (for NPP  $V_{\text{Wilcoxon}} = 205.00$ ,  $p = 0.488$ ,  $n = 19$ ; for GPP  $V_{\text{Wilcoxon}} = 194.00$ ,  $p = 0.708$ ,  $n = 19$ ). The only evident disparity was during the spring bloom (weeks 12 to 16), when the NPP of the <56-fractionated community was almost twice as low as the NPP of the unfractinated community. From week 17 to week 23, NPP increased from 0.04 to 0.53  $\text{gC m}^{-2} \text{d}^{-1}$  and from 0.02 to 0.28  $\text{gC m}^{-2} \text{d}^{-1}$  within the unfractinated and <56-fractionated communities, respectively (Figure 4A). Afterwards, a decrease in NPP was observed, dropping below 0  $\text{gC m}^{-2} \text{d}^{-1}$  by week 29. The sampling events conducted in weeks 28 and 29 were characterised by low PAR values (Table 1), potentially causing a decrease in NPP values (Figure 4A).

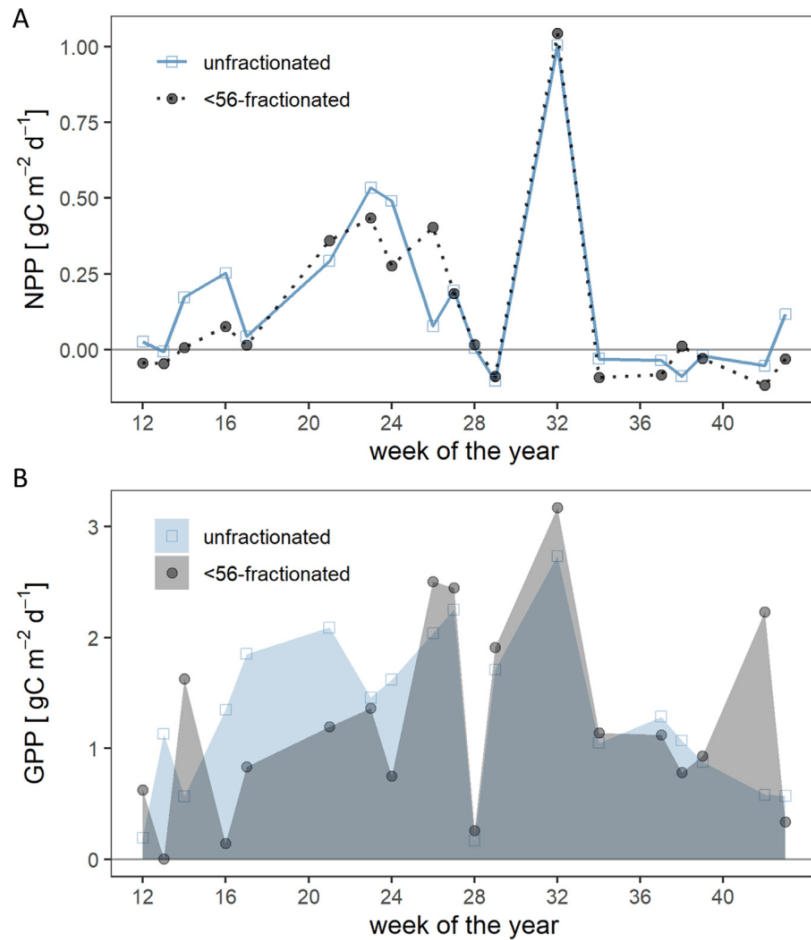
### 3.5. Environmental conditions influencing primary production

The first two components of PLSR model explained 59.7% and 54.4% of the primary production (NPP, GPP) variance

for unfractinated and <56-fractionated communities, respectively (Table 2, Figure 5). The results showed that NPP was influenced by fewer environmental factors than GPP (Figure 6). The analysis identified small-sized *M. rubrum* and PAR as the most important influencing parameters for NPP in both unfractinated and <56-fractionated communities (Figure 6A, C), whereas for GPP, besides small-sized *M. rubrum* and PAR, also nutrients, temperature, and carbon mass of diatoms were identified as important factors (Figure 6B, D).

## 4. Discussion

Surveys of primary production in the GoR have been sporadic in the last decades (Olesen et al., 1999; Wassmann and Tamminen, 1999; Wasmund et al., 2001), mainly covering the period from 1993 to 1997. More recently, the dynamics of primary production in the GoR were analysed in relation to seasonal changes in the phytoplankton community and nutrient concentrations (Labucis et al., 2017; Purina et al., 2018). However, the abovementioned studies focused on



**Figure 4** Average daily A) net primary production (NPP) and B) gross primary production (GPP) of unfractionated and <56-fractionated autotrophic communities from the top 10 m layer at the sampling location (Station 101A) in the Gulf of Riga.

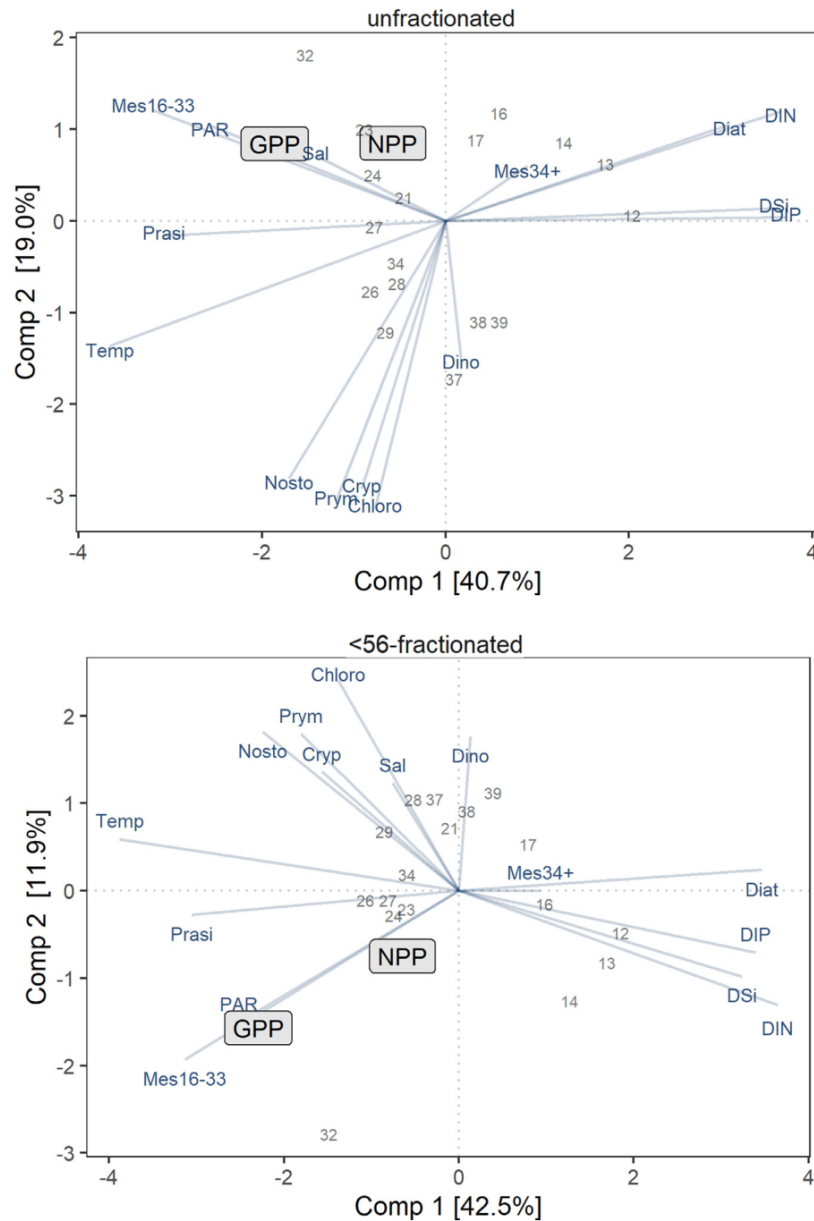
**Table 2** Results of partial least squares regression (PLSR). Comp – components; SS – the sum of squared loadings.

	Comp1	Comp2	Comp3	Comp4	Comp5
<b>unfractionated</b>					
SS loadings	3.79	1.42	0.993	0.57	0.50
Explained variance [%]	40.70	19.00	10.10	8.40	6.50
Cumulative Proportion [%]	40.70	59.70	69.80	78.20	84.70
<b>&lt;56-fractionated</b>					
SS loadings	5.67	3.03	1.32	0.76	0.36
Explained variance [%]	42.50	11.90	13.40	7.60	9.00
Cumulative Proportion [%]	42.50	54.40	67.80	75.40	84.40

primary production by the unfractionated community. The present study is the first to analyse the production of the fractionated autotrophic community of the GoR.

Size fractionation is often used to separate phytoplankton communities into picoplankton, nanoplankton and microplankton (Fenchel, 1988); however, it is also a useful approach to investigating other size fractions of interest. The results of the present study clearly demonstrate strong

covariation between NPP and <56-fractionated community taxa (Figure 4), particularly the small-sized (16–33  $\mu\text{m}$ ) *M. rubrum* (Figures 5–6). This implies that the majority of NPP stems from the lower end of the size spectrum. Size fractionation eases the pressure of light, space and nutrient limitation on smaller organisms, thus not only revealing their growth and production potential under experimental conditions (Sommer et al., 2016) but also confirming their impor-

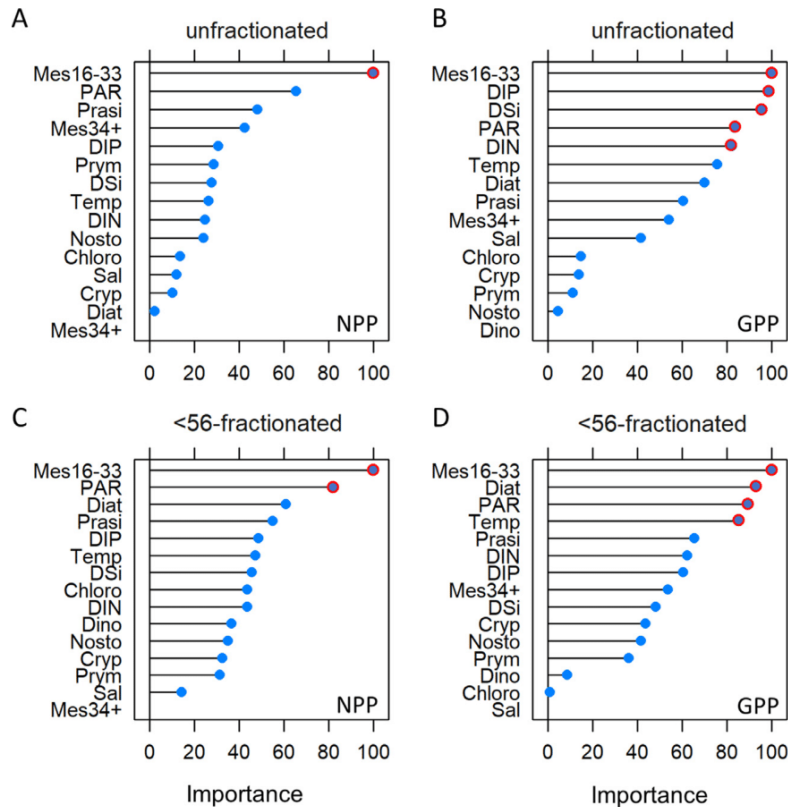


**Figure 5** Partial least squares regression (PLSR) triplots associated to the first two components. PLSR performed between the primary production rates (NPP – net primary production; GPP – gross primary production) and environmental variables (DIN – dissolved inorganic nitrogen; DIP – dissolved inorganic phosphorus; DSI – dissolved silicate; PAR – photosynthetically active radiation; Sal – salinity; Temp – temperature; carbon mass of phytoplankton groups: Diat – Diatomophyceae; Dino – Dinophyceae; Mes34+ – large-sized ( $\geq 34 \mu\text{m}$ ) *Mesodinium rubrum*; Mes16–33 – small-sized (16–33  $\mu\text{m}$ ) *Mesodinium rubrum*; Nosto – Nostocophyceae/cyanobacteria; Chloro – Chlorophyceae; Cryp – Cryptophyceae; Prasi – Prasinophyceae; Prym – Prymnesiophyceae. Primary production rates are represented as labels on a grey background; environmental variables are represented as solid navy-blue lines; sampling week (sites) are shown as grey numbers.

tance in the production of unfractionated samples. Small cells of primary producers have an advantage due to their larger surface-to-volume ratio that facilitates nutrient uptake compared to larger cells (Falkowski and Oltiver, 2007).

Fractionation exposed unfractionated community as inefficient net-producers at the study site due to their high respiration rates (Figure 4). Notably, the respiration of the

unfractionated community was high because it also included heterotrophic protozoans (e.g., tintinnids) and metazoans (e.g., rotifers and Copepoda nauplii). In general, respiration of microzooplankton is estimated to reach 35–43% on average of daily primary production (Calbet and Landry, 2004). Due to the size overlap, it was not possible to filter out zooplankton prior to incubation without also removing diatoms,



**Figure 6** Variable importance for the projection (VIPs) for explanatory variables of partial least squares regression (PLSR) model. A) VIPs of NPP of unfractionated community, B) VIPs of GPP of unfractionated community, C) VIPs of NPP of <56-fractionated community, and D) VIPs of GPP of <56-fractionated community. The most influential variables (VIPs > 80%) are marked with red circles.

filaments of cyanobacteria, and dinoflagellates. Hence, the results of respiration (and GPP) should be interpreted with caution if compared to estimates obtained by a different method (i.e., other than the light-dark bottle oxygen technique).

The annual GPP of the unfractionated community in the present study was 8.5–14.1% lower (i.e., 323 gC m<sup>-2</sup>) than that estimated for the central part of the GoR (Purina et al., 2018; GPP estimated using the same approach as in the present study). This is most likely a result of lowered water transparency due to coastal water turbulence and a direct impact of opaque freshwater discharge at the present study site. Indeed, Secchi depth varied between 2.3 and 5.1 m in the central part of the GoR (Purina et al., 2018), whereas it did not exceed 2.5 m in this study (southern coastal area). However, *M. rubrum* showed significant covariation to primary production rates in both areas (Purina et al., 2018; Figure 5) despite the different underwater light conditions expressing its flexible nature. Additionally, in several other Baltic subbasins, *M. rubrum* has been stated as a significant contributor to primary production (Höglander et al., 2004; Johansson, 2004; Lips and Lips, 2017; Nielsen and Kjørboe, 1994), implying its essential role as one of the main producers in the Baltic Sea.

*Mesodinium rubrum* is known to have migration behaviour based on the response of phototaxis (Crawford and Lindholm, 1997) and has wide temperature, salinity and

light tolerances (Lindholm and Mörk, 1990; Olli et al., 1996). It can migrate vertically over tens of metres per day (Hajdu et al., 2007), exploiting the nutrient-rich lower layers. *Mesodinium rubrum* benefits from this behaviour under stratified conditions in the shallow Baltic Sea (Lips and Lips, 2017). Due to the focus on the surface layer in the present study, subsurface accumulations of motile phytoplankton are most likely missed, potentially resulting in an underestimate of the overall abundances and biomass of *M. rubrum*. However, the efficient production of *M. rubrum* is attributed to mixotrophy and photosynthetic machinery of cryptophyte-originated chloroplasts that are well adapted to dim light (Daneri et al., 1992; Herfort et al., 2012) rather than the ability of vertical migration. The photosynthetic activity of *M. rubrum* increases with the availability of cryptophytes, although with a 7-day lag (Gustafson et al., 2000). In general, mixotrophy is an advantage under nutrient-limited conditions (Mittra et al., 2014), and it is an important feeding strategy during the decline of spring blooms and during summer or other periods when the system shifts from net autotrophy to net heterotrophy (Haraguchi et al., 2018). However, the ecological flexibility of *M. rubrum* and its implications for its phototrophic production remain poorly understood and require further in-depth research.

Cryptophytes are common in the GoR during summer (Figure 3). Hence, the acquisition of chloroplasts does not limit the growth of *M. rubrum*, allowing it to reach the

highest efficiency in photosynthetic activity without notable limitations. Nevertheless, cryptophytes affect various aspects of the performance of *M. rubrum*. In addition to the aforementioned physiological components, the availability of *Teleaulax* cryptophytes reduces the average size and volume of *M. rubrum* cells, as the high prevalence of cryptophytes promotes cell division (Gustafson et al., 2000). A potential explanation for a shift to small-sized *M. rubrum* during summer in the GoR is that cryptophytes were the most abundant in the period between weeks 26 and 32. A shift from larger to smaller *M. rubrum* during summer has also been observed in other Baltic Sea regions. This is explained by increased grazing pressure (Johansson, 2004; Rychert, 2004; Witek, 1998), higher temperature (García-Cuetos et al., 2012; Haraguchi et al., 2018), and low DIN values (Haraguchi et al., 2018) during the summer.

In line with future global projections, climate change scenarios for the GoR region foresee a continuation of already occurring air temperature and precipitation increases. A consequent drop in frost and ice days will follow (BACC II, 2015). Overall, the winters will become milder, but the summers will become more pronounced. The changes will promote stratification and inorganic nutrient (especially nitrogen) limitation in the surface layer, as well as potentially decrease the light availability even further (Skudra and Lips, 2017; Sommer et al., 2012; Winder and Schindler, 2004). Organisms with coping mechanisms to nutrient deficiency in the euphotic layer (e.g., motile *M. rubrum*, diazotrophic cyanobacteria) will outperform others under such conditions (Griffiths et al., 2016; Spilling and Markager, 2008; Wasmund and Uhlig, 2003). Moreover, future climate conditions appear non-detrimental to cryptophytes (Gaillard et al., 2020), thus predicting the continuous availability of cryptophyte-originated chloroplasts ensuring autotrophy of *M. rubrum*. Several studies have revealed that *M. rubrum* is able to remain photosynthetic and survive for months at low irradiance (Johnson and Stoecker, 2005) considering its efficient inorganic nutrient uptake rates (Stoecker et al., 1991; Tong et al., 2015; Wilkerson and Grunseich, 1990). Therefore, an increase in the prevalence of *M. rubrum*, along with a consequent rise in primary production, can be expected in the region, especially during the summer period.

Last, the present study demonstrated a close link between *M. rubrum* (especially small-sized *M. rubrum*) and NPP in coastal waters of the GoR (Figure 6). However, large-sized *M. rubrum* was coabundant with small-sized *M. rubrum* for a short period of time, soon after the spring bloom (Figure 2), limiting direct comparison between them. It is highly likely that the unequal amount of available PAR in spring and summer had a role in the identified different contributions to NPP between the size classes of *M. rubrum*.

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## Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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