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Stable C and N Isotope Composition of Suspended Particulate Organic Matter in the Neva Estuary: The Role of Abiotic Factors, Productivity, and Phytoplankton Taxonomic Composition

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Abstract: Knowledge of carbon and nitrogen isotopic ratios in organic matter and their changes is important when studying nutrient cycles in aquatic ecosystems. Relationships between $\delta^{13}C$ and $\delta^{15}N$ values of suspended particulate organic matter (POM), water temperature, salinity, pH, redox potential, chlorophyll *a* concentration, primary production, and biomasses of different taxonomic groups of phytoplankton in the Neva Estuary were statistically analyzed. We tested the hypothesis that the studied physicochemical and biogeochemical characteristics, as well as the species composition of phytoplankton and its productivity, can be significant predictors of changes in the isotopic ratios of suspended particulate organic matter in estuaries. In the Neva Estuary, $\delta^{13}C_{POM}$ (−16.8–−27.6‰) and $\delta^{15}N_{POM}$ (2.3–7.3‰) changed synchronously. Statistical analysis showed that for both isotopes, the photosynthetic activity and taxonomic composition of phytoplankton are important. For $^{13}C_{POM}$, the second most important factor was water salinity, which was apparently associated with the transition of algae from CO_2 to HCO_3 consumption during photosynthesis in estuarine waters. For $^{15}N_{POM}$ changes, the most important abiotic factor was pH. The study showed that the dependences of POM isotopic ratios on environmental variables obtained for continental and oceanic waters are also valid in transitional zones such as the Neva Estuary.

Keywords: stable isotopes; carbon; nitrogen; eutrophication; salinity; Gulf of Finland; Baltic Sea

1. Introduction

Stable isotope analysis is a standard technique for tracking organic matter transport and trophic interactions in both terrestrial and aquatic food webs [1]. The study of the isotopic composition of aquatic primary producers and its variability depending on abiotic environmental factors and taxonomic affiliation of primary producers in aquatic ecosystems is the key to understanding the cycle of autochthonous organic matter in water bodies [2]. A number of studies are devoted to the difference in the isotopic composition of phytoplankton, macroalgae, mangrove forests, and coastal swamps [2–9]. The important role of phytoplankton in the cycles of stable nitrogen isotopes in subtropical freshwater reservoirs and carbon in subtropical estuaries has been shown [10,11].

It is known that the carbon isotope ratios $^{13}C/^{12}C$ or "isotopic signature" expressed using the delta notation ($\delta^{13}C$) of organic matter of continental and marine origin is different. The largest active pool in the global carbon cycle is dissolved inorganic carbon ($\sum$ DIC) in the oceans. This pool is mainly composed of HCO_3^- (≈95% carbon in $\sum$ DIC), but also includes dissolved CO_2 (<1% of carbon) and $CO_3^{=}$ (≈5% of carbon). The $^{13}C/^{12}C$ isotope ratio in the ∑ DIC ocean pool depends on the atmospheric CO_2 input and the concentration of marine carbonates. Atmospheric CO_2 has an intermediate concentration of ^{13}C and averages $\delta^{13}C$ = −7.8‰ in areas without large settlements and industrial production and −9.9‰ in urbanized areas [12]. In continental waters, the concentration of ^{13}C in DIC depends on the ratio of dissolved atmospheric CO_2, emission of carbon dioxide during the dissolution of rock carbonates, and CO_2 release during the mineralization of soil organic matter coming from the catchment [12]. The isotopic ratio of rock carbonates, depending on the composition, varies from +2‰ in carbonate-rich to −12‰ in carbonate-poor soils.

The carbon isotope signature ($\delta^{13}C$) of organic matter coming from the catchment strongly depends on the prevailing type of photosynthesis in its vegetation [13]. The organic matter of plants with the C_3 pathway of photosynthesis has an average $\delta^{13}C$ value of about −27‰. Values for C_4 plants range from −17 to −9‰, with a mean of −13‰, and plants with crassulacean acid metabolism (CAM) (mainly succulents of desert areas) on average have −19‰ [12]. The photosynthesis of phytoplankton occurs along the C_3 pathway. However, the $\delta^{13}C$ values of photosynthetic organisms in the ocean do not always resemble the $\delta^{13}C$ values of terrestrial C_3 plants, since marine phytoplankton can use bicarbonate as a carbon source for photosynthesis, the concentration of ^{13}C in which is much higher than in the atmospheric CO_2 ≈ 0‰ [12]. The mean $\delta^{13}C$ values of marine, riverine, and lacustrine phytoplankton are close to −22‰, −27.5‰, and −34‰, respectively [12].

The factors that determine the $\delta^{15}N$ and $\delta^{13}C$ values of many phytoplankton species have been studied using pure algal cultures in the laboratory [4,14–16]. Laboratory culture experiments have shown wide fluctuations in the values of nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) in various algal species and in their isotope fractionation factors. They can be used as indicators of ^{15}N-enrichment and the assimilation of dissolved inorganic nitrogen by phytoplankton and the usage of carbon and nitrogen of different origin and in aquatic food webs. Although these studies have reported that the isotope ratios of suspended particulate organic matter (POM) can vary greatly over production cycles, little is known about the factors that determine its $\delta^{15}N$ and $\delta^{13}C$ values in natural systems.

It is assumed that in eutrophic waters, when intense photosynthesis leads to a lack of CO_2, algae begin to consume carbon from the air, where the ^{13}C content is higher than in water. As a result, the concentration of ^{13}C in suspended particulate organic matter increases [17]. In addition, the taxonomic composition of phytoplankton influences the fractionation of $^{13}C/^{12}C$ [4,18,19]. For example, it was shown that a high proportion of dinoflagellates in marine phytoplankton near the coast of Portugal was accompanied by an increase in $\delta^{13}C$ values [20]. On the contrary, an increase in the proportion of diatoms in the phytoplankton of the Mediterranean Sea led to a decrease in the ^{13}C concentration in plankton [21].

The concentration of ^{15}N isotope in aquatic ecosystems is closely related to the nitrogen cycle. The isotopic signature of phytoplankton nitrogen ($\delta^{15}N$) makes it possible to trace the nitrogen cycle in an aquatic ecosystem in order to determine the source and the predominant form of nitrogen compounds that phytoplankton consume [22,23]. The main nitrogen pool consists of nitrogen of organic matter and three mineral forms: ammonia form of nitrogen, nitrate form, and gaseous nitrogen [1]. In aquatic ecosystems, the processes that control the ratio of these forms are closely related to the microbial processes of nitrification and denitrification as well as nitrogen fixation [24]. Therefore, there is a clear need for further investigation of the factors that determine the $\delta^{15}N$ and $\delta^{13}C$ values of phytoplankton and suspended particulate organic matter in natural systems.

The Neva Estuary is the largest in the Baltic region, and the Neva River is the major contributor of freshwater to the sea [25]. The influx of nutrients into the estuary is significant due to the huge catchment area of the river and the location on the estuary coast of a large metropolis of St. Petersburg (5 million inhabitants), which discharges treated and untreated wastewaters into the estuary [26]. It currently receives 4630 tons of phosphorus and 57,000 tons of nitrogen annually, which is 8% of the nutrient load in the Baltic Sea from river runoff [27]. As a result, the primary productivity and biomass of planktonic autotrophic organisms in the estuary are among the highest in the Baltic Sea [8,28]. Recently, eutrophication has been increasing due to unfavorable weather conditions leading to an increase in the production of autochthonous organic matter in the estuary, which is not completely mineralized in the estuary waters and is carried further into the central part of the Gulf of Finland [29]. The phytoplankton of the estuary includes marine and freshwater species, the composition of which differs depending on environmental factors [30]. The isotopic ratios of suspended particulate organic matter, depending on the taxonomic composition of phytoplankton in the estuary, has not been studied previously, although this may be important for understanding the cycle of autochthonous and allochthonous organic matter not only in the Neva Estuary, but also in other estuaries with significant river runoff.

The study aims to determine carbon and nitrogen isotope ratios in suspended particulate organic matter depending on the abiotic and biotic characteristics of the aquatic environment. We tested the hypothesis that the physicochemical and biogeochemical characteristics of waters, as well as the species composition of phytoplankton and its productivity, can be significant predictors of changes in the isotopic ratios of suspended particulate organic matter in estuaries.

2. Materials and Methods

The Neva Estuary receives water from the Neva River, which is a relatively short canal (74 km) between Lake Ladoga and the Gulf of Finland, whose catchment area exceeds 280,000 km^2, and the water discharge averages 2490 m^3 s^{-1} (78.6 km^3 yr^{-1}), which is about one-fifth of the total river discharge into the Baltic Sea. The type of climate of the Neva Estuary region, according to Köppen–Geiger [31] climate classification, is Dfc (Snow climate, fully humid, cool summer). Soils on the estuary watershed according to Digital Soil Map of the World [32] are different types of Podzols in the northern part and different types of Podzoluvisol in the southern part, which are characterized by low pH and a low concentration or absence of carbonates. The Neva Estuary is brackish-water, non-tidal, with horizontal and vertical gradients of salinity and dominance of eurytopic species. The upper part of the estuary was separated from its lower part by the Flood Protective Facility (Dam). It consists of eleven dams separated by broad water passages and ship gates in its southern and northern parts (Figure 1). Due to the low transparency of the water, most of the estuary is free of bottom vegetation. Dense reeds of 300–600 m width belt only its shallow coastal zone. A more detailed description of the estuary was given in previous publications [8,29].

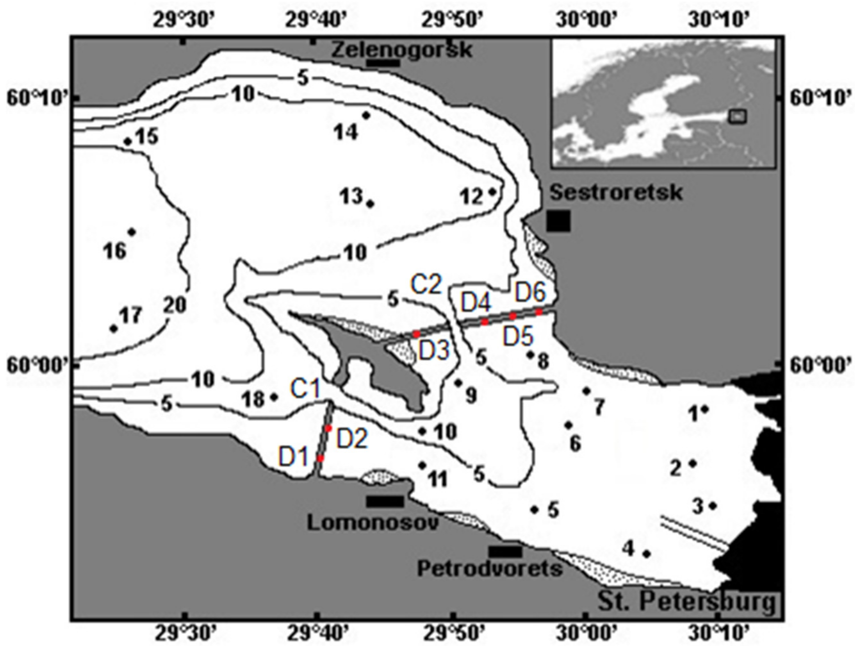

Figure 1. The Neva Estuary with an indication of the sampling stations. Black lines show 5, 10, and 20 m isobaths. Areas with dots indicate dense reeds. C1, C2—gates for vessels; D1–D6—waters gates in the St. Petersburg Flood Protection Facility.

2.1. Sampling

Eighteen stations in the Neva Estuary (Figure 1) were sampled twice: from 20th of July to 5th of August in 2018 and 2019. The temperature, salinity, and redox potential (ORP) were determined at each station using a CTD90m probe (Sea&Sun Tech., Trappenkamp, Germany) every 20 cm from the surface to the bottom. Taking into account that according to these measurements, the whole water column in the shallow upper part was mixed, we collected five water samples (2 L each): from the surface, half a meter from the bottom, and from three equal depths between them. Samples from different depths were taken in order to avoid errors associated with the vertical distribution of different phytoplankton species in the water column. These samples were mixed to make up a composite sample (10 L). Samples (three replicates of water collection) of chlorophyll *a*, suspended particulate organic matter, and stable isotope analysis (SIA) were taken from these composite samples.

At the stations 12–18, where temperature stratification was observed, composite water samples were taken from the layer above (UL) the thermocline. Five water samples (2 L each) were taken from the UL: from the surface, the thermocline, and from three equal depths between them. These samples were mixed to create a composite sample (10 L). The samples of chlorophyll *a*, suspended particulate organic matter, and samples for stable isotope analysis (three replicates of water collection) were taken from these composite samples.

2.2. Laboratory Analysis

Three hundred milliliters of water were filtered through 0.85 μm membrane filters (Millipore AAWP, Burlington, MA, USA) to determine the chlorophyll *a* (Chl *a*) concentration, which was followed by 90% acetone extraction and spectrophotometric determination [33]. Suspended particulate organic matter (POM) was determined after filtration (Whatman GF/F filters, Maidstone, UK) with the dichromate acid oxidation [33].

The primary production of plankton (PP) in the water column were measured by the oxygen method of light and dark bottles [34,35]. A more detailed description of the method and experimental design is given in Golubkov et al. [8].

For SIA, one liter of composite water sample passed through a 50 μm mesh to separate zooplankton and coarse particles were filtered through pre-combusted Whatmann GF/F 0.7 μm glass fiber filters to collect POM, which consisted mainly of estuarine phytoplankton, with an admixture of riverine phytoplankton, detritus, and terrigenous (with wastewater) POM. POM samples for SIA were frozen at −20 °C until analysis of stable isotope ratios. Before analysis, filters with POM were dried at 60 °C for 48 h. After drying, samples were homogenized in an agate mortar to make a composite sample. Analyses were performed on POM homogenized composite samples desiccated for 24 h in a desiccator. Triplicate POM composite samples (1.0–1.3 mg) were put into small tin capsules and weighed using a Mettler Toledo MX 5 balance with an accuracy of ±1 μg. The SIA was performed according to standard methods [36] using a Thermo Delta V Plus isotope mass spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with an element analyzer at the Joint Usage Center «Instrumental methods in ecology» of A.N. Severtsov Institute of Ecology and Evolution of RAS (Moscow, Russia, Russian Federation). An isotopic composition of C and N in organic matter was expressed in δ-notation relative to international standard (vPDB for carbon and the atmospheric N_2 for nitrogen) δ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where R= $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Samples were analyzed with reference gas calibrated against IAEA (Vienna, Austria) reference materials USGS 40 and USGS 41. The drift was corrected using an internal laboratory standard (casein). The standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values in the laboratory standard (n = 8) was <0.2‰.

2.3. Phytoplankton Assemblages

Phytoplankton (volume 0.3 L) was taken in one replicate from a composite sample and fixed with acid Lugol's solution. The phytoplankton taxa were identified and counted in sedimentation chambers (10–25 mL) with an inverted Hydro-Bios microscope. Phytoplankton biomass was calculated in total volume of algal cells according to Olenina et al. [37]. The biomasses for each species from the same taxonomic group were summarized and used in statistical analysis.

2.4. Statistical Analysis

Statistical analyses were performed using R software (version 3.6.0) [38]. The Pearson correlation coefficients matrix was produced using the "table.correlation" function in the PerformanceAnalytics R package [39]. Stepwise multiple linear regressions were performed to determine the most influential factors affecting $\delta^{13}C$ and $\delta^{15}N$ in POM. We have built "model.null" separately for $\delta^{13}C$ and $\delta^{15}N$ and "model.full" with all environmental factors, the taxonomic composition of phytoplankton and separately for $\delta^{13}C$ and $\delta^{15}N$ in POM. As a result, two models (null and full) were done for $\delta^{13}C$ and two were done for $\delta^{15}N$. Then, the "step" function (direction = "both") was used to add and remove factors from the model and to find the model with the lowest Akaike Information Criterion (AIC). AIC was used as an estimator of out-of-sample prediction error and thereby the relative quality of statistical models for a given set of data. The lower the value, the better for the AIC [40]. When the model with the lowest AIC was found, it was called the final.model. This procedure was done separately for $\delta^{13}C$ and $\delta^{15}N$ in POM, and two final.models for $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ were chosen and combinations of the most influential factors were found separately for $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$. Afterwards, we did an analysis of variance for individual factors included in the final models by the function "Anova" in the car R package [41] to determine which one is most important for the prediction of $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ in each final model. The residuals plots were checked for two final models, and it showed that residuals were unbiased and homoscedastic. A stepwise multiple linear regressions analysis is detailed in Mangiafico [40].

3. Results

3.1. $^{13}C_{POM}$ and $^{15}N_{POM}$ Ratios and Environmental Variables

The water temperature in the Neva Estuary during the sampling period varied from 18 to 24 °C. Data on water temperature in this range were evenly distributed over the entire set of values, since the mean and median practically coincided (Table 1). Salinity ranged from 0.08 to 2.81, with an average of 0.71 PSU, but a median of 0.14 PSU. This means that most of the water sampling sites were in slightly saline waters. The pH values were biased to the alkaline range from 7.8 to 10 and were almost evenly distributed across all stations. The ORP values varied in the range 148–312 mV, showing the presence of free electrons in the solution and the ability of the latter to take or donate electrons to chemical compounds contained in it.

The concentration of suspended particulate organic matter ranged from 0.9 to 4.9 g m^{-3} and chlorophyll *a* ranged from 5.6 to 98.1 mg m^{-3} (Table 1). The primary production of plankton changed during the study period by a factor of ten, depending on the station (from 0.31 to 3.96 gC m^{-2} d^{-1}). $\delta^{13}C_{POM}$ in the estuary varied from −16.8 to −27.6‰ (averaging about −25‰), $\delta^{15}N_{POM}$ varied from 2.3 to 7.3‰ (averaging about 4.8‰) (Table 1). Average values of $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ in the Neva River waters were −26.8‰ and 0.9‰, consequently [26].

Table 1. Number of samples (no.), maximum, minimum, median, interquartile range (IQR), average and standard deviation (SD) of environmental variables and $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ values in the Neva Estuary during the study period. S-water salinity; T-water temperature; ORP-RedOx potential; POM—concentration of suspended particulate organic matter; Chl *a*—concentration of chlorophyll *a*; PP—primary production of plankton.

Indexes	No.	Maximum	Minimum	Median	IQR	Average	SD
T, °C	36	24.48	18.43	20.57	2.28	21.01	1.54
S, PSU	36	2.81	0.08	0.14	0.91	0.71	0.88
pH	36	10.02	7.84	8.22	0.45	8.39	0.48
ORP, mV	36	312.84	148.34	255.72	47.10	250.02	42.77
POM, g m^{-3}	36	4.90	0.91	1.89	1.00	1.96	0.88
Chl *a*, mg m^{-3}	36	98.08	5.60	18.45	13.93	22.72	17.59
PP, gC m^{-2} d^{-1}	36	3.96	0.31	1.15	1.02	1.33	0.78
$\delta^{13}C_{POM}$, ‰	36	−16.82	−27.61	−25.22	3.75	−24.47	2.84
$\delta^{15}N_{POM}$, ‰	36	7.26	2.32	5.07	2.25	4.82	1.42
Neva River $\delta^{13}C_{POM}$, ‰ *	-	-	-	-	-	−26.8	0.1 **
Neva River $\delta^{15}N_{POM}$, ‰ *	-	-	-	-	-	0.9	0.1 **

* According to Golubkov et al. [26]; ** standard error.

3.2. Taxonomic Composition of Phytoplankton

Eighty-nine phytoplankton species belonging to seven taxonomic groups were found in the investigated part of the estuary during the study period (Table 2). Most of the species (36) belonged to green algae, while in second place in terms of species richness were cyanobacteria (19 species), and in third place were diatoms (16 species). The other four taxonomic groups included only from three to six species (Table 2). Nevertheless, the algae of these species-poor groups often had the highest biomasses. For example, the highest biomass recorded at one sampling station belonged to cryptophytic algae, with dinoflagellates in second place. However, if cryptophytic algae had both the highest median and mean biomasses for all stations, then this was not the case for dinoflagellates (Table 2). Green algae and diatoms were the first in frequency of occurrence; Euglenophyceae were the rarest of the seven taxonomic groups (Table 2).

Table 2. Number of species and occurrence, maximum, minimum, median, and mean biomass (wet weight mg m^{-3}), interquartile range (IQR), and standard deviation (SD) of each taxonomic group in phytoplankton assemblages in the Neva Estuary in midsummer 2018–2019.

Taxonomic Group	Number of Species	Number of Occurrence	Max	Min	Median	IQR	Mean	SD
Chlorophyceae	36	36	988.5	1.7	72.1	163.8	140.2	189.2
Cyanophyceae	19	35	1413.4	0	51.3	440.9	288.2	423.8
Bacillariophyceae	16	36	2234.9	9.9	133.1	368.2	364.0	493.7
Cryptophyceae	5	35	3240.9	0	296.1	436.5	427.0	548.3
Chrysophyceae	6	26	341.4	0	33.9	95.0	58.0	74.9
Dynophyceae	4	30	3200.0	0	98.7	162.0	203.5	529.6
Euglenophyceae	3	23	2059.0	0	52.3	457.2	273.5	440.0

Cryptophyceae accounted for the bulk (24%) and Bacillariophyceae played a significant role in the total biomass of phytoplankton (Figure 2). These two groups together accounted for 45% of the phytoplankton biomass. Cyanophyceae and Euglenophyceae were next in biomass, and Chrysophyceae had the lowest biomass (Figure 2).

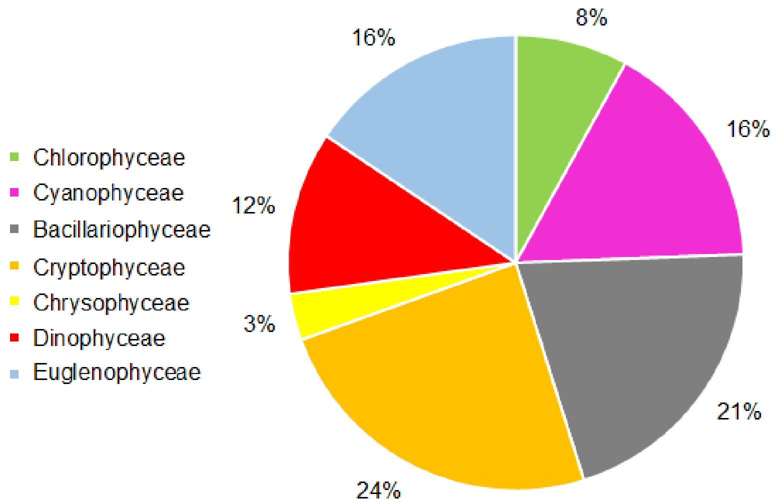

Figure 2. The contribution of various taxonomic groups to the total biomass of phytoplankton in the Neva Estuary in midsummer 2018–2019.

3.3. Relationship between $^{13}C_{POM}$ and $^{15}N_{POM}$ Ratios, Environmental Variables, and Taxonomic Composition of Phytoplankton

The analysis of the data, carried out by the Pearson's pair correlation method, showed that all the studied parameters are quite closely interrelated with each other. Values of $\delta^{13}C_{POM}$ significantly correlated with all the studied environmental variables (Figure 3). However, the strength of the statistical relationship and the level of confidence were different. The highest and most reliable positive correlation coefficients were obtained between $\delta^{13}C$ values and phytoplankton productivity indicators: chlorophyll *a* concentration and primary production of plankton. Slightly weaker statistical relationships were obtained between this isotope ratio, POM and pH. This was most likely due to the correlations of these variables with chlorophyll *a* and primary production (Figure 3). The close correlation of the POM concentration with the Chl *a* concentration suggests that phytoplankton constituted the bulk of suspended particulate organic matter, and its active photosynthesis led to an increase in pH values and a shift of this indicator to a more alkaline range.

Water salinity positively correlated with $\delta^{13}C_{POM}$ values and did not correlate with the chlorophyll *a* concentration. $\delta^{13}C_{POM}$ values also weakly negatively correlated with water temperature and ORP (Figure 3)

In general, $\delta^{15}N_{POM}$ and $\delta^{13}C_{POM}$ values were positively significantly correlated with each other (Figure 4). However, the $\delta^{15}N_{POM}$ values correlated somewhat differently with the other studied variables. Of the environmental variables studied, $\delta^{15}N_{POM}$ values most closely correlated with pH. This isotope ratio also showed a significant positive relationship with biotic environmental factors, but the relationship was weaker compared to $\delta^{13}C_{POM}$. At the same time, in contrast to $\delta^{13}C_{POM}$, the $\delta^{15}N_{POM}$ values did not show any relationships with water temperature, salinity, and ORP values (Figure 3).

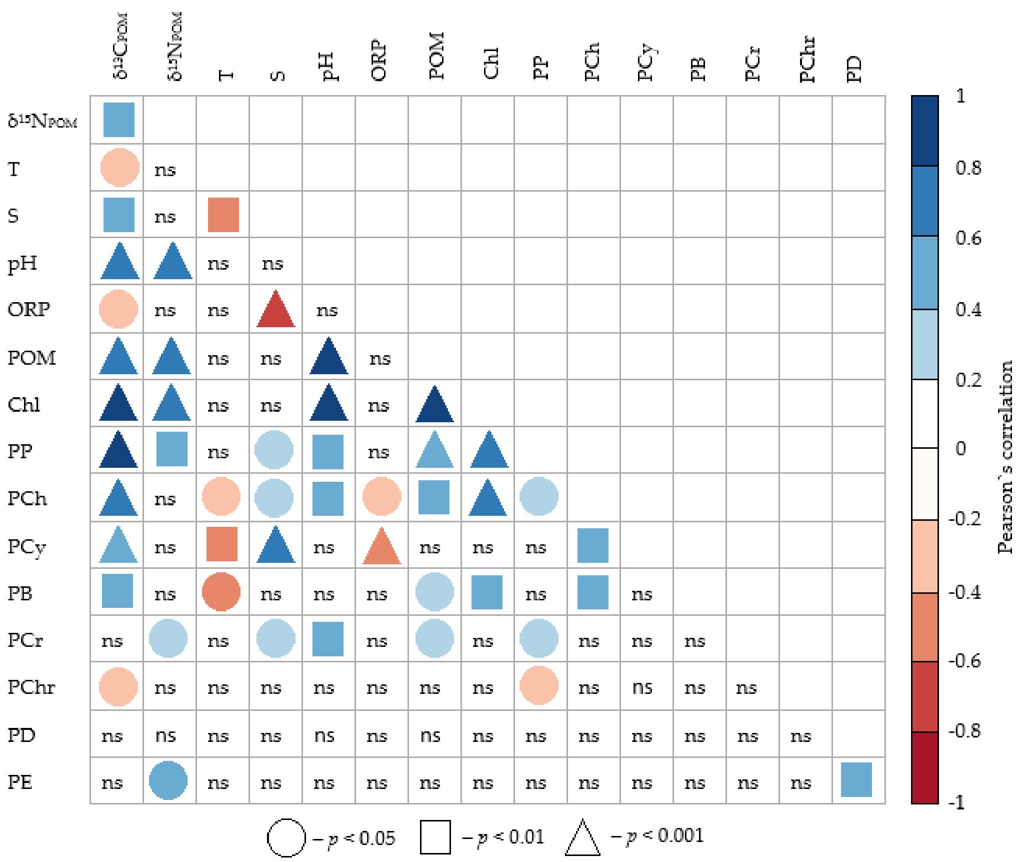

Figure 3. Pearson's correlation coefficients between $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ values of suspended particulate organic matter, environmental variables, biomasses of Chlorophyceae (PCh), Cyanophyceae (PCy), Bacillariophyceae (PB), Cryptophyceae (PCr), Chrysophyceae (PChr), Dynophyceae (PD) and Euglenophyceae (PE) in the Neva Estuary in midsummer 2018–2019. Abbreviations of environmental variables are the same as in Table 1.

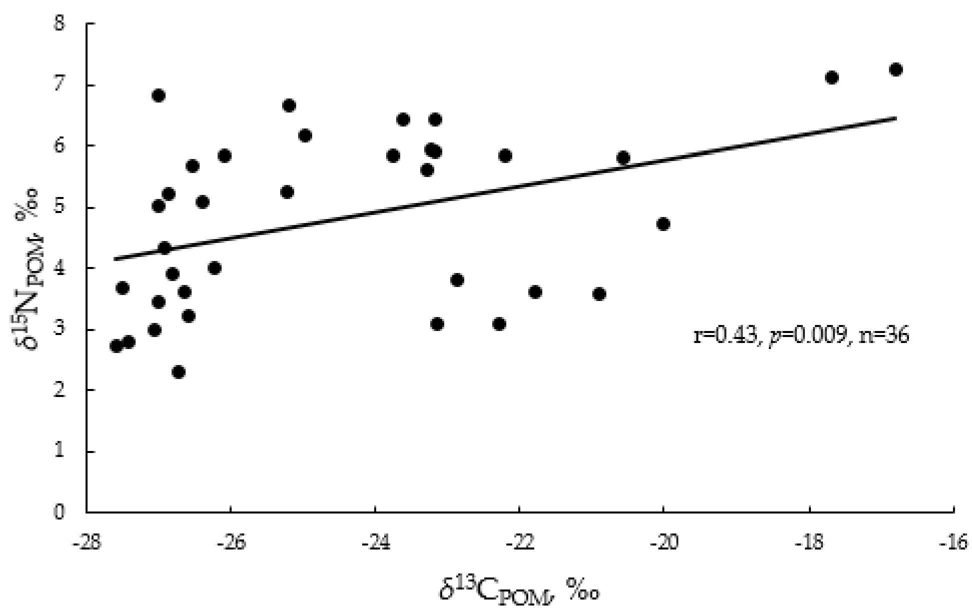

Figure 4. Linear regression between $\delta^{13}C$ and $\delta^{15}N$ in suspended particulate organic matter (POM) in the Neva Estuary in midsummer 2018–2019.

The pairwise correlation method showed that the carbon isotope ratio in suspended particulate organic matter significantly positively correlated with the biomasses of three groups of phytoplankton and negatively correlated with the biomass of one group. The $\delta^{13}C_{POM}$ values were most significantly correlated with the biomasses of Chlorophyceae and Cyanophyceae, and the correlation coefficient with the first group was higher (Figure 3). The positive relationship between $\delta^{13}C_{POM}$ values and Bacillariophyceae biomass was slightly weaker. In total, these three groups of phytoplankton accounted for 45% of the total phytoplankton biomass. Moreover, these groups had the highest biomasses at the same stations, as evidenced by the positive significant correlations between their biomasses. The $\delta^{13}C_{POM}$ values negatively correlated with the biomass of Chrysophyceae, which had the smallest proportion in the phytoplankton biomass. No significant relationship was found between the carbon isotope ratio and biomasses of the other three groups of algae (Figure 3).

The $\delta^{15}N_{POM}$ values practically did not correlate with the biomasses of various groups of algae. Only two groups of algae showed a weak positive relationship with $\delta^{15}N_{POM}$ values (Figure 3). We also tried to carry out a correlation analysis between $\delta^{15}N_{POM}$ and $\delta^{13}C_{POM}$ values and the biomass of certain phytoplankton species, but we did not get any significant correlations.

3.4. Stepwise Multiple Regression Analyses between Stable Isotope Ratios, Environmental Variables, and Phytoplankton Taxonomy Groups

Analysis by the method of pairwise correlations showed that the carbon and nitrogen isotope ratios correlate with the studied abiotic environmental factors and the biomasses of some taxonomic groups of phytoplankton. However, in nature, environmental factors often do not act separately from each other, but are intertwined in a complex system of interrelationships. To find out how these factors together can be associated with changes in the concentrations of carbon and nitrogen isotopes in suspended particulate organic matter, a stepwise multiple regression analysis was carried out. We conducted simulations and obtained two models, separately for $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ values, which best described the changes in these parameters in the estuary POM, taking into account the studied factors. According to plots of residuals vs. predicted values for $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ models (Figure 5), both models are unbiased and homoscedastic.

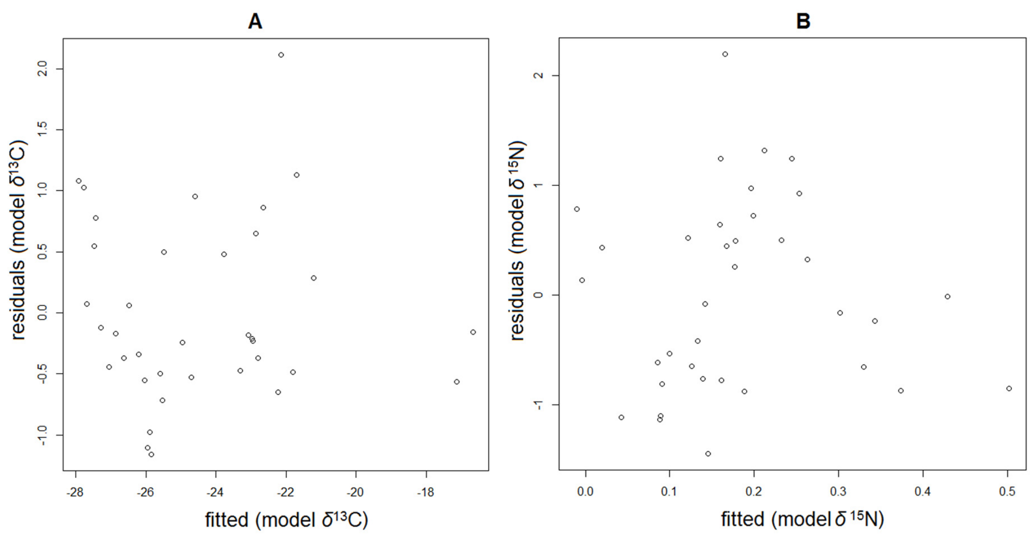

Figure 5. Plots of residuals vs. predicted values for $\delta^{13}C_{POM}$ (**A**) and $\delta^{15}N_{POM}$ (**B**) models.

For the carbon isotope model, six of the fourteen indicators turned out to be predictors: chlorophyll *a* concentration, water salinity, plankton primary production, as well as biomasses of Cryptophyceae, Euglenophyceae, and Chlorophyceae. The coefficient of linear determination of the model was high (R^2 = 0.93). However, in order to avoid the error of its overestimation, which occurs as a result of adding each additional predictor to the model, we calculated the adjusted R^2 (Adj R^2), and it also showed a high value of 0.92. This means that the resulting regression model was in good agreement with the observed values. This is also evidenced by the high level of significance (*p*-value) of the model—7.56×10^{-16}, i.e., the chances of obtaining a reliable result with this model were very high.

Three predictors that were obtained had a positive relationship with the $\delta^{13}C$ values of POM, and the relationship of the other three was negative. The combined increase in salinity, chlorophyll *a*, and primary production of plankton led to an increase in the concentration of the carbon isotope. On the contrary, an increase in the biomasses of Cryptophyceae, Euglenophyceae, and Chlorophyceae led to its decrease, i.e., they acted as negative predictors.

The resulting regression equation describing the change in $\delta^{13}C_{POM}$ values in the Neva Estuary depending on significant environmental factors is given in Table 3. To find out the significance of the predictors and which of them most strongly influenced the ^{13}C concentration in POM, we carried out the t-test and F-test and found that the most significant predictors in the $\delta^{13}C_{POM}$ models were the chlorophyll *a* concentration and water salinity. Their t-value and F-value were the highest. Consequently, it was the changes in chlorophyll *a* concentration and water salinity that most strongly influenced the changes in $\delta^{13}C_{POM}$ values. Chlorophyll *a* concentration and water salinity variables did not correlate with each other (Figure 3), so there was no multicollinearity problem in the model. For the $\delta^{13}C_{POM}$ value to increase by 1‰, the salinity of the water had to increase by 1.56 PSU, the chlorophyll *a* concentration had to increase by 0.14 mg m^{-3}, and plankton primary production had to increase by 0.49 gC $m^{-2}day^{-1}$. Although the values of plankton primary production had a positive effect on the increase in $\delta^{13}C$ values in the model, they did not play a major role in the increase in the isotope concentration, as evidenced by the low t-value and F-value, as well as the *p*-value. They were significant only in combination with other predictors but not by themselves.

The algal biomass was found to be a negative predictor of the regression model. The most significant factor was the biomass of Euglenophyceae, followed by the biomass of Chlorophyceae. Although the biomass of Cryptophyceae improved the model, it itself had little effect on the final result, i.e., as well as primary production, it was important only together with other factors. A decrease in the biomass of Euglenophyceae by 0.10, Chlorophyceae by 0.28, and Cryptophyceae by 0.42 g m^{-3} wet weight led to an increase in the $\delta^{13}C$ value of suspended particulate organic matter by 1‰ (Table 3).

Table 3. Results of the stepwise multiple regression analyses between $\delta^{13}C$ values of suspended particulate organic matter (Y variable), environmental variables, and biomasses of various phytoplankton groups (X variables). Abbreviations of environmental variables and phytoplankton groups are the same as in Tables 1 and 4.

Indexes						**Values**
Number of observations						36
F < 6, 29>						69.06
Residual stand error						0.80
R-squared						0.93
Adj R-squared						0.92
***p*-value**						7.56×10^{-16} ***
Y variable	X variables	tvalue	$\Pr(> \lvert t \rvert)$	F-value	$\Pr(> F)$	Regression equation
$\delta^{13}C_{POM}$	Chl	7.58	2.37×10^{-8} ***	57.40	2.40×10^{-8} ***	
	S	6.78	1.90×10^{-7} ***	46.03	1.90×10^{-7} ***	$\delta^{13}C_{POM}$ =
	PE	−3.07	4.58×10^{-3} **	9.44	4.58×10^{-3} **	0.14Chl+1.65S
	PP	1.55	0.13	2.40	0.13	−0.10PE+0.49PP
	PCh	−2.06	0.05 *	4.23	0.05	−0.28PCh
	PCr	−1.59	0.12	2.53	0.12	−0.04PCr−28.69

Asterisks indicate the significance of the correlation (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Table 4. Results of the stepwise multiple regression analyses between $\delta^{15}N$ values of particulate organic matter (POM) (Y variable), environmental variables, and biomasses of various phytoplankton groups (X variables). Abbreviations of environmental variables and phytoplankton groups are the same as in Tables 1 and 4.

Indexes						Values
Number of observations						36
F < 5, 30 >						10.3
Residual stand error						0.93
R-squared						0.63
Adj R-squared						0.57
***p*-value**						8.08×10^{-6}
Y variable	X variables	t value	Pr(>|t|)	F-value	Pr(>F)	Regression equation
$\delta^{15}N_{POM}$	pH	3.43	1.78×10^{-3} **	11.76	1.78×10^{-3} **	$\delta^{15}N_{POM}$ = 1.37pH−0.11PCy +0.44PP−0.49PChr +0.05PE−6.83
	PCy	−2.80	8.84×10^{-3} **	7.84	8.36×10^{-3} **	
	PP	1.71	0.10	2.92	0.10	
	PChr	−2.06	0.05 *	4.24	0.05 *	
	PE	1.37	0.18	1.88	0.18	

Asterisks indicate the significance of the correlation (* $P < 0.05$; ** $P < 0.01$).

For the $\delta^{15}N$ model of suspended particulate organic matter, five of the fourteen studied indicators were found to be significant predictors. Of these, the values of pH, plankton primary production, and the biomass of Euglenophyceae turned out to be positive predictors, and the biomasses of Cyanophyceae and Chrysophyceae were negative predictors (Table 4).

Although the model for nitrogen was statistically significant, the coefficients of linear determination (R^2) and adjusted R^2 (Adj R^2) were lower than for carbon, 0.63 and 0.57, respectively. The level of significance (*p*-value) of the model for the $\delta^{15}N$ values of suspended particulate organic matter was also lower than for the $\delta^{13}C_{POM}$ values, but it was still quite high (*p*-value = 8.08×10^{-6}). The most significant predictors of the $\delta^{15}N_{POM}$ model were the pH and biomass of Cyanophyceae; they had the highest t-value and F-value. However, an increase in pH had a positive effect on the $\delta^{15}N_{POM}$ values, while the biomass of Cyanophyceae had a negative effect (Table 4). A decrease in the biomass of Chrysophyceae also had a significant negative effect on the $\delta^{15}N_{POM}$. The primary production and biomass of Euglenophyceae separately did not significantly affect the $\delta^{15}N_{POM}$ values, but their addition to the regression model had a positive effect on the overall accuracy of the model. The obtained regression equation shows that to increase $\delta^{15}N$ values of POM by 1‰, it is necessary for the pH to increase by 1.37, for the primary production to by 0.44 $gCm^{-2}day^{-1}$, and for the biomass of Euglenophyceae to increase by 0.05 g m^{-3}. At the same time, the biomass of Cyanophyceae should decrease by 0.11 g m^{-3}, and that of Chrysophyceae should decrease by 0.49 g m^{-3}.

4. Discussion

4.1. Carbon Isotope Ratio in Suspended Particulate Organic Matter

According the results of this study, physicochemical and biogeochemical characteristics of waters, as well as the species composition of phytoplankton and its productivity, can be significant predictors of changes in the isotopic ratios of suspended particulate organic matter in estuaries. Analysis of pairwise correlations showed that its values had high significant positive correlations with the level of plankton primary production, chlorophyll *a* concentration, and water salinity.

It is well known that isotopic signature of organic matter of continental and marine origin is different [12]. In our study, the mean $\delta^{13}C$ values of POM were about 25‰ (Table 2), which is in the middle between the values for marine and river phytoplankton. In the estuaries of North America with significant river runoff, the $^{13}C_{POM}$ concentration increased with increasing water salinity, and this effect was observed in all seasons of the year [42]. In the Neva Estuary, we observed a similar trend. However, the carbon isotope signature in the Neva Estuary was also strongly correlated with plankton productivity (Figure 3). Moreover, multi-regression analysis of our data showed that for an increase in the concentration of stable carbon isotope in the suspended particulate organic matter of the Neva Estuary, a necessary condition was a simultaneous increase in the concentration of chlorophyll *a* and water salinity. This means that with active photosynthesis, as the salinity of water increased, plankton switch to the use of bicarbonates instead of CO_2 as carbon sources, which occurs when CO_2 dissolved in the water is exhausted during the mass development of algae, as was shown previously [4,43]. Our data show that at high levels of primary production in eutrophic waters, even a slight increase in salinity per 1 PSU leads to an increase in ^{13}C concentration.

The water temperature showed a pairwise correlation with the $\delta^{13}C$ values of suspended particulate organic matter (Figure 3), but it was not included in the number of significant environmental variables in the multi-regression model (Table 3). As was previously shown, the $\delta^{13}C$ values of phytoplankton in the Atlantic Ocean correlate with water temperature, and this is associated not only with a change in the taxonomic composition of phytoplankton but also with the difference in its enzymatic activity depending on temperature [44]. This dependence was determined for the temperature range from −2 to +33 °C, but at the same time, the authors showed that above 18 and up to 33 °C, the value of $\delta^{13}C$ practically does not change. During the period of our study, the water temperature was from 18 to 24 °C (Table 1). This apparently was the reason that we did not get a significant effect of temperature on the concentration of ^{13}C in suspended particulate organic matter (Table 3). Therefore, the dependence obtained for oceanic waters is also valid for the Neva Estuary.

$^{13}C/^{12}C$ fractionation varies depending on physiological characteristics and enzyme composition in phytoplankton [4,18,19,44]. This is well shown for marine phytoplankton consisting of dinoflagellates and diatoms [20,21]. In our study, the analysis of pairwise correlations showed that the $\delta^{13}C_{POM}$ values increased synchronously with the increase in the biomasses of green algae, diatoms, and cyanobacteria (Figure 3). Multi-regression analysis showed that the biomass of algae of only three groups (Euglenophyceae, Chlorophyceae, and Cryptophyceae) significantly influenced the $\delta^{13}C_{POM}$ values (Table 3). Moreover, the biomasses of algae of these groups acted as negative predictors of the model, i.e., an increase in their role in the phytoplankton community led to a decrease in $\delta^{13}C_{POM}$ values. Therefore, in our opinion, the ^{13}C concentration in the Neva Estuary is affected not only by the total amount of algae but also by the taxonomic composition of phytoplankton assemblages. However, based on the F-value, which varied from 2.4 to 4.2, one can conclude that the taxonomic composition of the phytoplankton community plays a smaller role in the change in $\delta^{13}C_{POM}$ values of suspended particulate organic matter compared to the concentration of chlorophyll *a* and water salinity (F-test was 57.4 for chlorophyll *a* and 46.0 for salinity, $p = 0.001$).

4.2. Nitrogen Isotope Ratio in Suspended Particulate Organic Matter

Among environmental factors, $\delta^{15}N_{POM}$ values in the Neva Estuary did not correlate with salinity, redox conditions, or temperature, but only with pH, chlorophyll *a* concentration, and the level of primary production (Figure 3). At higher values of these parameters, the concentration of ^{15}N in POM was higher. However, these significant correlations may be indirect, because an analysis of pairwise correlations showed that the $\delta^{15}N_{POM}$ values in the Neva Estuary changed synchronously with the $\delta^{13}C_{POM}$ values; with an increase in the proportion of one isotope in suspended particulate organic matter, the proportion of the second one also increased (Figure 4). The same synchronous change is shown with an increase in the trophic status of freshwater ecosystems, and it is associated with biochemical and biogeochemical processes caused by the intensive development of phytoplankton [45].

Multi-regression analysis showed that pH was the main predictor of the model; its increase most of all increased the $\delta^{15}N_{POM}$ values (according to the results of the F-test, $F = 11.76$, $p = 0.018$; Table 4). At the same time, the salinity of the water did not affect the pH value, as evidenced by the lack of correlation between these variables (Figure 3), apparently this was due to a slight change in this parameter in the current dataset (salinity median 0.14 PSU) (Table 1). It is important to take into account that the increase in pH in the Neva Estuary was apparently caused by the active photosynthesis of algae, since the concentration of Chl *a* and PP correlated significantly with the pH values. Moreover, it is known from the literature that the pH of water increases with active photosynthesis [46]. This result is interesting, since pH is not considered as the main factor influencing the $\delta^{15}N_{POM}$ values, but it does not contradict the previously obtained results. For example, in a recent review and model study on the effect of ocean acidification on the nitrogen cycle, Wannicke et al. [47] showed that a decrease in water pH leads to a decrease in the rate of nitrification, and in this connection, the authors predict an essential increase in the proportion of ammonia nitrogen in the upper water layers. In turn, an increase in the concentration of ammonium nitrogen and low pH values shift the isotopic signature of nitrogen in the negative direction [45]. For instance, $\delta^{15}N$ values of phytoplankton ranged from −7.8 to −3.4‰ in Japanese volcanic lake with the pH of about 2, and an increase in ammonia concentration statistically significantly negatively correlated with ^{15}N concentration [48].

The taxonomic composition of phytoplankton also had less effect on $\delta^{15}N_{POM}$ compared to $\delta^{13}C_{POM}$. Only the biomasses of Cryptophyceae and Euglenophyceae were significantly positively correlated with the nitrogen isotope ratio. According to multi-regression analysis, the second most important predictor was the biomass of cyanobacteria. An increase in the biomass of cyanobacteria led to a decrease in $\delta^{15}N_{POM}$ values (Tables 3 and 4). This is consistent with data on the isotopic signature of suspended particulate organic matter in lakes, for which it was shown that the isotope signature of nitrogen decreases to ≈0‰ when nitrogen-fixing bacteria dominate in the phytoplankton community [49]. The biomass of Chrysophyceae was also a significant negative predictor in our model. We could not find in the literature studies on the relationship between the isotopic composition of suspended particulate organic matter and Chrysophyceae, since this group is usually not considered as important or dangerous to humans. However, our research shows that these algae may play a role in the nitrogen cycle, and the study of this group would be useful for understanding the biogeochemical nitrogen cycle in aquatic ecosystems.

In our study, the primary production of plankton was a positive predictor of $\delta^{15}N_{POM}$ values (Table 4), and the same result was obtained when analyzing Pearson's pairwise correlations (Figure 3). Earlier, the analysis of data on 36 lakes with various morphometric and physicochemical characteristics, and trophic status from oligotrophic to hypereutrophic showed a similar trend [49]. It was found that the $\delta^{15}N$ values of suspended particulate organic matter increased from oligotrophic to eutrophic lakes, but their values decreased in hypereutrophic lakes. The author concluded that this was due to the cyanobacteria, which dominated in hypereutrophic lakes, fixed nitrogen from the air and, as a result, reduced the ^{15}N concentration in POM. According to our data, this is apparently also true for estuaries with a significant runoff of river waters, since the $\delta^{15}N$ values of POM increased with an increase in PP and chlorophyll *a* concentration, and an increase in the proportion of Cyanophyceae in the total phytoplankton biomass led to a decrease in the $\delta^{15}N_{POM}$ values.

An interesting result was obtained on the effect of Euglenophyceae on the isotopic ratios of nitrogen and carbon in POM in the Neva Estuary. Both the method of pairwise correlations and multi-regression analysis showed that with an increase in their biomass, the values of $\delta^{15}N_{POM}$ increased, while the $\delta^{13}C_{POM}$ values decreased. This may be due to the fact that these organisms are equally capable of both photosynthesis and phagotrophy [50]. As a source of nutrients, Euglenophyceae can consume bacteria and other protozoa, as well as POM entering the Neva Estuary with wastewater and thereby increase the ^{15}N pool and reduce the ^{13}C pool, since wastewater is characterized by high nitrogen and relatively low carbon isotope ratios [12,26,45,51]. In addition, some Euglenophyceae are able to feed on cyanobacteria [52], the increase in biomass of which led to a decrease in ^{15}N in POM of the Neva Estuary. However, a special study is required to clarify this issue.

Analysis of pairwise correlations also showed that the biomass of Cryptophyceae in the Neva Estuary was positively correlated with $\delta^{15}N_{POM}$. As with Euglenophyceae, these algae can switch to heterotrophic nutrition and consume suspended particulate organic matter [53,54]. Perhaps this is the reason why they can increase the ^{15}N pool in the Neva Estuary. However, this requires additional research.

5. Conclusions

The study showed that the concentrations of ^{13}C and ^{15}N change synchronously in suspended particulate organic matter of the Neva Estuary; with an increase in one, the other also increases. This suggests that the nature of their accumulation in the suspended particulate organic matter in the estuary is closely related to the processes of production of organic matter by phytoplankton. Pairwise correlations and multi-regression showed that for both isotopes, and especially for $\delta^{13}C_{POM}$ values, the photosynthetic activity of algae is important, the indicator of which is the concentration of chlorophyll *a* and the level of plankton primary production. However, statistical analysis showed that for $\delta^{13}C_{POM}$ values, the second most important factor is water salinity, which is apparently associated with the transition of algae during active photosynthesis from CO_2 consumption to HCO_3. For $\delta^{15}N_{POM}$ values, the most important abiotic factor was pH, the fluctuations of which were associated with the photosynthetic activity of plankton. The change in pH apparently affects the microbiological activity in the water column, which affects the nitrogen cycle in water and the predominance of certain mineral forms (NH_4, NO_3, etc.) that algae consume as a source of nitrogen. The analysis showed that the taxonomic composition of phytoplankton also influenced the concentrations of ^{13}C and ^{15}N isotopes in suspended particulate organic matter. The biomasses of certain taxonomic groups of phytoplankton acted as additional predictors of multiregression models for ^{13}C and ^{15}N changes in suspended particulate organic matter in the Neva Estuary. However, additional research is required, especially on the effect on the isotope signature of Euglenophyceae and Cryptophyceae, which are capable of heterotrophic feeding. In addition, it can be concluded that the dependences (the influence of trophic status, salinity, temperature, pH on isotopic ratios in POM) obtained for continental freshwaters and oceanic waters are also valid in transitional zones, such as the Neva Estuary.

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