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Spatiotemporal carbon, nitrogen and phosphorus stoichiometry in planktonic food web in a northern coastal area

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ABSTRACT

The concentrations of ambient nutrients and dissolved organic carbon (DOC) in northern coastal ecosystems often show large variations, due to the spatiotemporal differences in terrestial inputs. How these variations affect the stoichiometry of coastal planktonic organisms is, however, poorly known. Here we assessed the spatiotemporal variability of C, nitrogen (N), and phosphorous (P) concentrations of the seawater on the elemental stoichiometry of seston and dominant mesozooplankton taxa in a coastal area of the northern Baltic Sea. The freshwater inflow peaked in spring following the snowmelt and brought a significant amount of DOC, but not N and P to the coastal system. DOC was the main environmental descriptor for seston C:N stoichiometry. The C:N ratio of seston from 0.7 to 50 μm and mesozooplankton followed the temporal pattern of water C:N ratio, while the temporal trend of bacteria C:N showed an opposite pattern. Our results also indicated that the C:N ratio of seawater controlled both seston and mesozooplankton C:N ratios. Our findings imply that inflows of terrestrial DOC alter the stoichiometry and reduce the nutritional quality of planktonic food webs in northern coastal ecosystems.

1. Introduction

Coastal ecosystems connect land with the open ocean, and provide important habitats for diverse organisms. In northern Europe, the coastal ecosystems are increasingly threatened by climate change which is expected to result in more precipitation in the region within the next hundred years, followed by concomitant increases in freshwater input to the estuaries (Kjellström and Ruosteenoja, 2007; Andersson et al. 2015; Reusch et al. 2018). Consequently, larger amounts of colored terrestrial organic matter and nutrients (such as inorganic and organic nitrogen (N) and phosphorus (P)) could be transported from land to coastal waters, which will alter the physicochemical environment and potentially affect the ecological interactions, basal production and therefore ecosystem functions (Walve and Larsson, 2010; Stenzel et al., 2017; Moreno and Martiny, 2018).

The carbon (C), N and P concentrations are generally more variable in coastal waters than in the open ocean (Klausmeler et al., 2004; Sardans et al. 2012), due to the dual influence from land and offshore water. In northern Europe, the largest freshwater inflow usually occurs in spring when the snowmelt creates large floods to the coastal areas. In comparison to the offshore water, the freshwater inflow is usually browner and richer in terrestrial dissolved organic carbon (DOC) (Bauer et al., 2013; Reader et al. 2014; Harvey et al. 2015) instead of N and/or P (Stepanauskas et al. 2002; Fleming-Lehtinen et al. 2015), thus it can potentially change the water C:N:P ratios in the estuaries.

Changes in elemental stoichiometry of the water can affect the structure at the base of the coastal pelagic food web, i.e. seston (Walve and Larsson, 2010; Stenzel et al. 2017; Moreno and Martiny, 2018). Heterotrophic bacteria and phytoplankton are major seston components that differ in size, C sources, and metabolic and nutrient demands, thus they potentially respond to the stoichiometric changes in water differently (Elser et al. 2002; Kendrick and Benstead, 2013; Yvon-Durocher et al. 2017). Bacteria are generally considered as N-rich and have C:N ratios between 5:1 and 7:1 (Cotner et al. 2010), while phytoplankton stoichiometry is thought to be more variable (Klausmeier et al. 2008). Also, with increases in inputs of terrestrial DOC, the production of

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heterotrophic bacteria may be favored since they can utilize DOC for maintenance, energy and growth (Cotner et al. 2010; Wikner and Andersson, 2012). However, the stoichiometric changes of bacteria and phytoplankton in response to changes in DOC and ambient nutrient concentrations in these ecosystems have not been explored. This knowledge gap hinders our understanding of the climate-change consequences for dietary stoichiometric constraints of zooplankton, which are the key prey of fish.

Alterations in the elemental composition of bacteria and phytoplankton caused by increased DOC inputs can potentially influence the stoichiometry of zooplankton. Consumers (e.g. zooplankton) can generally maintain relatively stable elemental stoichiometry, i.e. homeostasis (Sterner and Elser, 2002; Persson et al. 2010). This means that the variability of C:N:P ratios in the diet has little effect on the stoichiometry of consumers within species (Andersen and Hessen, 1991; McManamay et al. 2011). However, the stoichiometry of different consumers can differ, for example, cladocerans have generally lower N:P and C:P ratios than copepods (Sterner and Elser, 2002). Therefore, alterations in the seston stoichiometry could influence the abundance of different zooplankton species (Van De Waal et al. 2010; Meunier et al. 2016). In northern aquatic ecosystems, copepods and cladocerans are common mesozooplankton taxa (Johansson et al. 2004). Copepods are N-dependent and able to selectively feed on large phytoplankton (<80 μm), while cladocerans are relatively P-rich and opportunistic filter feeders on the smaller seston size fractions (<30 μm) that contain mainly small phytoplankton (Sommer and Sommer, 2006). Cladocerans also feed more on flocculated DOC than do copepods when DOC is increasingly available (Tanentzap et al. 2017). It is likely that the elemental composition of cladocerans and copepods is affected differently by the stoichiometry of their prey and the DOC availability. Increases in DOC inputs will potentially increase the C:nutrient ratios of mesozooplankton, i.e. lower their food quality for fish, and the effects on the stoichiometry of cladocerans are expected to be stronger than those of copepods in northern coastal ecosystems. However, these effects are yet to be verified.

In this study, we used coastal sites of the northern Baltic Sea as the study systems. The Baltic Sea is one of the largest brackish water systems in the world. The northernmost Baltic Sea is generally nutrient poor (Andersson et al., 1996), and hence, altered nutrient inputs from adjacent landscapes resulting from anthropogenic activities and climate-driven processes are likely to affect the structure and function of the coastal ecosystems there. Our knowledge of how fluctuations in coastal physicochemical characteristics will affect organism stoichiometry is necessary before we can quantify the impacts of environmental pressures. Thus, we assessed the effects of spatial and temporal changes in the physicochemical environment, especially in the concentrations of DOC and ambient nutrients, on plankton stoichiometry in four bays of the northern Baltic Sea, which receive varying amounts of freshwater from relatively undisturbed catchments. We aimed to answer how fluctuations in water physicochemical characteristics, especially its elemental stoichiometry, will affect the seston and zooplankton stoichiometry in these coastal ecosystems.

2. Methods

2.1. Study bays and approach

Four bays in the northern Baltic Sea were sampled monthly from May to September 2018; Ängerån (63°34.400N, 19°50.666E), Kalvarsskatan (63°36.072N, 19°53.140E), Stadsviken (63°33.026N, 19°47.647E), and Valviken (63°32.468N, 19°46.725E) (Fig. S1). These bays received variable amounts of freshwater inflow particularly in spring (Fig. S2). Samples for all variables were collected at a station with a water depth of 3 m. Stadsviken and Valviken were not sampled in May due to logistic reasons.

2.2. Physiochemical characteristics

Salinity and water temperature were measured at 0.5 m below surface with a WTW ProfiLine Cond 3110 conductivity-meter. For chemical measurements, water samples were collected at 0.5 m depth and filtered through Supor membrane syringe filters (0.2 μm pore size, non-pyrogenic, Acrodisc®, Pall). Concentrations of dissolved inorganic phosphorus (DIP, i.e. phosphate PO_4) and dissolved inorganic nitrogen (DIN, i.e. nitrate, nitrite and ammonium) of the samples were analyzed using the Seal QuAAtro39 auto-analyzer (Grasshoff et al. 1999). Samples for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were analyzed in the same way as the DIN after an oxidation step using peroxodisulphate. The dissolved organic nitrogen (DON) or phosphorus (DOP) concentrations were determined by subtracting DIN or DIP from TDN or TDP. Water samples for DOC concentration measurements were acidified (18 mM HCl, final concentration) and analyzed using a Shimadzu TOC-5000.

2.3. Primary production

Primary production rates were measured using the ^{14}C uptake method (Gargas, 1975) at 0.5, 1.5 and 2.5 m depth. For each depth, 20 ml of seawater were placed in four glass vials (three light and one dark) and incubated *in situ* at their sampling depth with $NaH^{14}CO_3$ (activity 100 μCi ml^{-1}, DHI lab products, Denmark) at a final concentration of 0.1 μCi ml^{-1} for a minimum of 4 h. After incubation, 5 ml of each sample were transferred to a scintillation vial and gently bubbled with 300 μl of 6 M HCl for 30 mins. Subsequently, 15 ml of Optiphase HiSafe 3 scintillation fluid were added, and samples were measured in a PerkinElmer TriCarb 2910 TR scintillation counter. Dissolved inorganic carbon was calculated based on temperature and salinity according to Gargas (1975). Daily net primary production was calculated using the "light factor method" as described in Gargas (1975) and Andersson et al. (1996).

2.4. Seston and mesozooplankton stoichiometry

At each bay, approximately 1 l of water from 0.5 m depth was filtered onto glass-fiber filters of different pore sizes to collect four size fractions of seston: 0.3–0.7 μm, 0.7–10 μm, 10–50 μm and >50 μm (hereafter Size 1, Size 2, Size 3 and Size 4, respectively) (Table S1). Size 1 mainly consisted of heterotrophic bacterioplankton, Size 2 comprised pico- and nano-phytoplankton and heterotrophic nanoflagellates, and Size 3 contained large nano-phytoplankton, microphytoplankton, ciliates and rotifers. Larger microphytoplankton dominated in Size 4, where larger grazers (e.g. copepods) could also occasionally be present (Tables S1 and S3). Flocculated terrestrial DOC might be present in all seston classes (Bauer and Bianchi, 2012). In previous studies, most of the filtration methods for natural bacterial assemblages used a size range from 0.7 to 1.0 μm (Makino et al. 2003; Cotner et al. 2010; Hall et al. 2011; Scott et al. 2012; Stenzel et al. 2017). However, since the 0.7–1.0 μm size fraction contained not only bacteria but also small picophytoplankton (Table S1), we used the seston size fraction 0.3–0.7 μm to represent mainly bacteria. To our knowledge, this smaller seston size class has not been assessed in marine ecosystems, and thus its importance for the stoichiometry of overall seston and mesozooplankton remains unknown.

Mesozooplankton samples were collected using a plankton net of 90 (May–June) or 200 μm mesh size (July–September). These mesh sizes were judged most suitable for the sampling occasions, i.e. smaller mesozooplankton were dominant in the spring while larger mesozooplankton became abundant in summer. The collected mesozooplankton samples were first starved at 4 °C in 0.2 μm filtered seawater overnight to empty their guts before sorting individual taxa. The abundant mesozooplankton taxa, each constituting >10% of total mesozooplankton abundance in each bay, were sorted under a stereomicroscope. Individuals of the same taxon were pooled to ensure sufficient materials for the stoichiometric analyses. Overall, the analyzed mesozooplankton genera included two copepods (*Acartia* sp. (Acartiidae) and *Eurytemora* sp. (Temoridae)) and three cladocerans (*Bosmina* sp. (Bosminidae), *Podon* sp. (Podonidae) and *Evadne* sp. (Podonidae)) (Table S3). All mesozooplankton samples were then freeze-dried.

The mesozooplankton samples of approximately 1.0 mg dry mass and the filter samples of different seston size fractions were packed into tin capsules (Säntis Analytical, Teufen Switzerland). They were then analyzed for their C and N content using an elemental analyzer (PDZ Europa ANCA-GSL; Sercon Limited, Cheshire, UK) at the University of California Davis Stable Isotope Facility (U.S.A.). The P content of mesozooplankton and seston was quantified using the ash-hydrolysis method (Solórzano and Sharp, 1980).

2.5. Statistical analyses

All stoichiometric ratios (C:N, C:P and N:P) of the water, seston fractions and mesozooplankton are molar ratios. The water C:N:P ratios were calculated based on the concentrations of DOC, TDN, and TDP in the water samples. Principal component analysis (PCA) was used to analyze the spatial and temporal patterns of the physicochemical characteristics and primary production of the bays.

We calculated the relative C, N, and P mass content of different size fractions to evaluate the seston size composition. Two-way ANOVA was used to analyze the spatial and temporal differences in C:N:P ratios of seston and mesozooplankton, using bay and month, from June to September, as fixed factors. The May samples were excluded because of the unbalanced number of observations for each bay. Due to the lack of sample replicates, we did not include the interaction between bay and month in the ANOVA. To compare the differences of C:N:P ratios between two major mesozooplankton groups, copepods and cladocerans, a paired *t*-test from May to September was performed by using the stoichiometric ratios of copepods and cladocerans.

We identified the environmental descriptors for the stoichiometry of individual seston size fractions using redundancy analyses (RDA). Separate RDA were conducted for seston C:N, C:P, and N:P. Prior to the RDA, we examined the correlations between the environmental data (i.e. physicochemical characteristics and primary production of the bays), as RDA is sensitive to multicollinearity of explanatory variables (Dormann et al. 2013). Some highly correlated environmental variables (when the Pearson's $r \geq 0.7$) were removed to avoid overfitting the RDA models. For example, primary production was strongly correlated with DOC ($r = 0.7$) and therefore not included in the RDA. The RDA model-building procedure started from the unconstrained model, i.e. without any environmental variable, and used stepwise forward selection based on the Akaike's information criterion to select the best model. Permutation tests were used to assess whether the explanatory environmental variables, i.e. constraints, were significant ($p < 0.05$) at each step when a constraint was added. The RDA with forward selection showed that no environment variables could significantly explain the seston C:P and N:P ratios. Therefore, the RDA results of only seston C:N were presented.

To explore the relationship between the C:N ratios of 1) water and seston, and 2) seston and individual mesozooplankton groups, i.e. copepods and cladocerans, we used a combination of Size 2 and Size 3 to represent seston, as these size fractions were most dominant in seston. Four regression models (up to order 4 polynomial regression) were evaluated, and the linear models were selected as the best models in all regression analyses based on the *F*-test (analysis of variance). Tthe nonlinear, polynomial models were not significantly different from the linear model $p > 0.05$, therefore, the simpliest model was selected. The significant relationships between seston C:N and zooplankton C:N (the whole mesozooplankton group as one) was also evaluated using partial correlation to assess whether the relationships was controlled by the water C:N.

Data were ln-transformed to approximate normal distribution for all regressions, PCA, ANOVA, and RDA. All variables used in PCA and RDA were also *Z*-score standardized. The autocorrelation was not analyzed for individual sites before the PCA because of the limited number of time points, i.e. five months. All statistical analyses were conducted using R version 3.5.2 (R Core Team, 2019). We used the “vegan” package (Oksanen et al. 2020) for PCA and RDA. The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Temporal and spatial variation of physicochemical variables

The physicochemical variables showed larger temporal than spatial variation among the study bays. In May, the water temperature was 10–12 °C (Fig. 1a), while maximum values of 20–23 °C were observed in July. The temperature then dropped to below 16 °C in September. The salinity of Ängerån and Kalvarsskatan was ca. 2.5 in May and increased to ca. 4 in later months (Fig. 1b). Primary production was highest in May, decreased during the summer and was generally lowest in August, and then increased slightly again in September (Fig. 1c). Contrary to the temporal trend of salinity, DOC concentrations were substantially higher in May than in the subsequent sampling months (Fig. 1d). This was likely a consequence of the spring flood after the snowmelt, which had transported substantial amounts of terrestrial DOC into the bays (Fig. S2). During the spring flood, the DOC concentrations of the bays reached ca. 6 mg C l^{-1}, while during summer they dropped to ca. 4 mg C l^{-1}. The dissolved inorganic nitrogen (DIN) concentration was the lowest in August (Fig. 1e). The dissolved organic nitrogen (DON) concentration reduced from May to June but then increased to higher levels again in July–September (Fig. 1f). Phosphate concentrations remained low (<1 μg P l^{-1}) throughout the whole study period (Fig. 1g). The dissolved organic phorsphrus (DOP) concentration was the lowest in May and increased afterward, which may suggest that the high freshwater inflow in spring did not contribute to DOP loads in the coastal area (Fig. 1h).

Principal component analysis (PCA) showed a clear spatiotemporal pattern of the environmental conditions in the study bays, where the first two axes together explained 58.1% of the total variance (Fig. 2). The first axis was negatively correlated to DOC and primary production, and positively correlated to DOP and salinity. Along this axis, the May samples were separated from the August–September samples, and the June–July samples appeared as an intermediate group. The second axis was positively correlated with DIN and PO_4, and negatively correlated with temperature. The June and July samples were mainly separated on this axis. Overall, the PCA results confirmed that the temporal variation was larger than the spatial variation of environmental variables in the bays, i.e. the samples from different bays were not clearly separated in the PCA.

3.2. Stoichiometry of water, seston and mesozooplankton

The C:N:P ratios of water, all individual seston size fractions (Size 1: 0.3–0.7 μm, bacteria dominated; Size 2: 0.7–10 μm, dominated by pico- and nanoplankton; Size 3: 10–50 μm, dominated by nano-microplankton; and Size 4: >50 μm, dominated by larger microplankton; see details in Table S1 (Olenina et al. 2006; Telesh et al. 2009), and the mesozooplankton groups generally did not differ between bays, except that the C:P ratio of Size 4 and cladocerans differed between bays (Table 1). However, there were temporal differences in C:N ratios of water, Size 2 seston and copepods, and in the C:P ratio of cladocerans among sampling months from June to September (Table 1). The C:N ratios of water and copepods decreased over the study period (Fig. 3). A similar but statistically non-significant decreasing trend in C:N ratios over time was observed for cladocerans (Table 1; Fig. 3). Similarly, the C:N ratios of Size 2 seston was the highest in spring (May–June), and

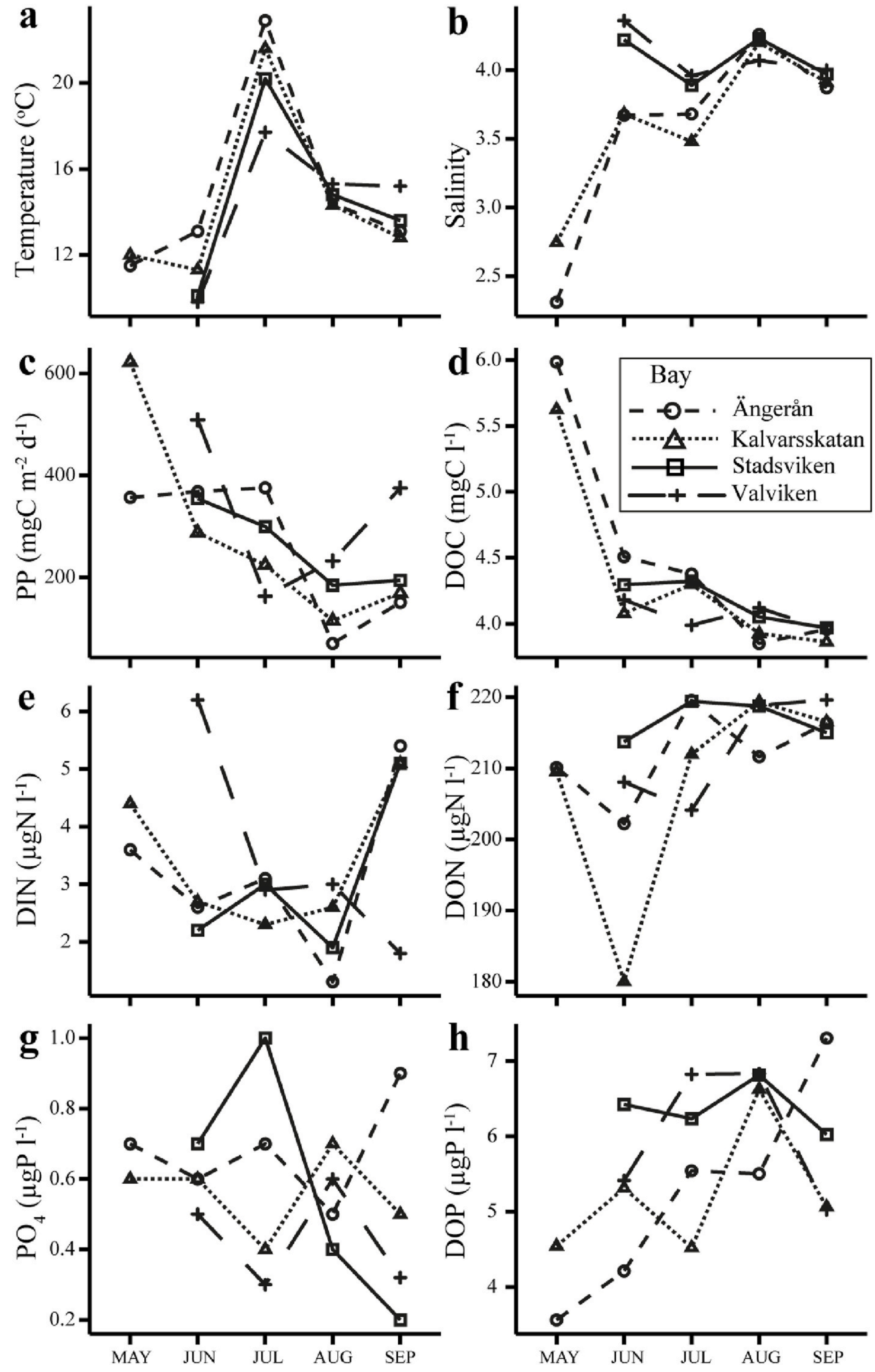

Fig. 1. Temporal variation of potentially explanatory variables in the study bays. a) temperature, b) salinity, c) primary production (PP), d) dissolved organic carbon (DOC), e) dissolved inorganic nitrogen (DIN), f) dissolved organic nitrogen (DON), g) dissolved inorganic phosphorus, i.e. PO_4, and h) dissolved organic phosphorus (DOP).

then decreased in summer (July–September). A similar temporal pattern was observed for Size 3 and Size 4 seston (Table S2; Fig. 3), but it was not statistically significant (Table 1). In contrast to the C:N patterns found in other seston fractions, the C:N ratio of Size 1 seston, i.e. bacteria, showed a trend to increase from May to September, although the temporal differences in C:N of Size 1 seston were not significant (Table 1). The C:N ratios were generally higher in water than in seston and mesozooplankton (Fig. 3), and those of seston tended to decrease from the smallest to the largest size fraction, with Size 2 and Size 3 seston having similar values. The C:N ratio of Size 4 seston was relatively similar to that of mesozooplankton (Fig. 3). The C:N ratios of water and Size 1–3 seston were higher than the Redfield ratio (7), but those of Size 4 seston and mesozooplankton were similar to or below the Redfield ratio.

The C:P ratios were generally much higher in the water than in seston and mesozooplankton (Fig. 3). Significant temporal differences in C:P were observed for cladocerans, but not for water, seston, and copepods (Table 1). The C:P ratios of all seston fractions and mesozooplankton groups were similar to the Redfield ratio (106; Fig. 3). The C:P ratio of

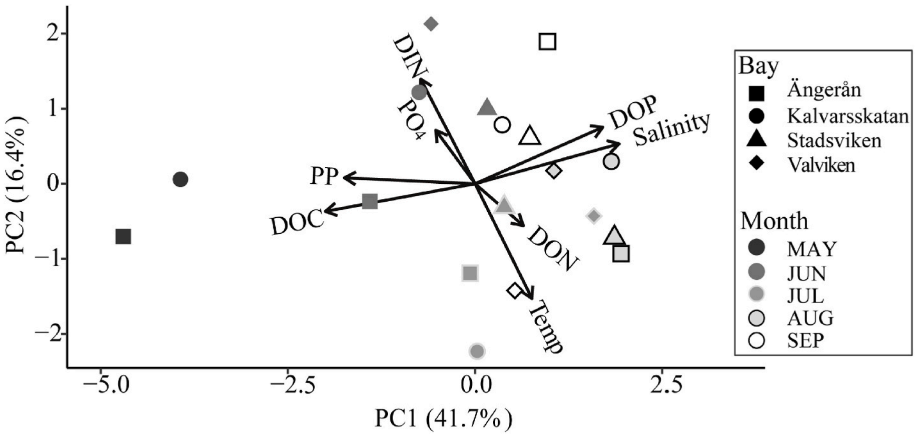

Fig. 2. Principal component analysis (PCA) of the relation between potentially explanatory variables in the study bays, from May to September 2018. Abbreviations of variables are explained in Fig. 2. The percentage of the total variation explained by the PC axes is indicated in parentheses. Different shapes represent data from different bays and different color represent data from different months.

Table 1

Summary of two-way ANOVAs of temporal (between month) and spatial (between bays) effects on water, seston and mesozooplankton stoichiometry at the study bays in June–September 2018. Signifcant diferences at *p* value * ≤ 0.05, ** ≤ 0.01 and *** ≤ 0.001.

		Between Month		Between Bay	
		F	p	F	p
Seawater	C:N	13.280	0.001**	0.477	0.706
	C:P	1.206	0.362	0.454	0.721
	N:P	0.358	0.785	0.344	0.794
Size 1	C:N	0.129	0.941	0.700	0.575
	C:P	3.377	0.068	1.591	0.259
	N:P	3.049	0.085	1.352	0.318
Size 2	C:N	33.887	<0.001***	1.421	0.299
	C:P	1.215	0.359	0.910	0.474
	N:P	0.592	0.636	0.811	0.519
Size 3	C:N	0.555	0.659	0.443	0.729
	C:P	0.405	0.754	0.305	0.822
	N:P	0.329	0.805	0.050	0.984
Size 4	C:N	2.044	0.209	0.459	0.721
	C:P	8.707	0.105	42.859	0.023*
	N:P	0.112	0.949	2.006	0.255
Copepods	C:N	8.292	0.015*	1.319	0.352
	C:P	2.190	0.190	1.042	0.439
	N:P	2.514	0.155	1.002	0.454
Cladocerans	C:N	1.607	0.406	1.382	0.420
	C:P	30.950	0.032*	49.38	0.020*
	N:P	8.948	0.102	17.645	0.054

Size 1 seston increased over time and was higher in September than in earlier months, but this was not statistically significant (Table 1; Fig. 3). The differences in C:P ratios between Size 1 and Size 2 or Size 3 seston were generally larger in spring than in summer (Table S2).

No significant temporal difference in N:P ratio was detected in water, seston and mesozooplankton (Table 1; Fig. 3), and there was no consistent temporal trend in N:P among seston size fractions (Fig. 3). The N:P ratios were higher in water than in seston and mesozooplankton (Fig. 3). Size 3 and Size 4 seston had larger variability in N:P than did Size 1 and Size 2 seston. The N:P of Size 1 seston remained much lower than the Redfield ratio (16) from May to September (Fig. 3), possibly because the bacteria fraction was deficient in N and/or rich in P (Fig. S3).

The relative C, N and P content of individual seston size fractions varied over time (Fig. S3). Size 2 seston fraction was the most dominant in all months. It had higher C, N and P relative content than did any of the other seston size fractions, reflecting that its biomass was also higher (Fig. S3). Its dominance in seston C and N gradually increased from May to September. Its contribution to overall seston P was also high, but more variable in the study period (Fig. S3). These results suggest that small phytoplankton (<10 μm) were the most abundant basal resource in the pelagic food web. The contribution of Size 3 to overall seston C and N was high in May but decreased in the later months. In contrast, the contribution of Size 1 to overall seston C, N and P increased from May to September.

Overall, cladocerans generally had higher C:N ratios than did copepods (Table 2 & S4; Fig. 3). The C:N, C:P and N:P ratios of all mesozooplankton taxa were close to or below the Redfield ratios (Fig. S4). The variability of C:P and N:P ratios of individual mesozooplankton group were larger than that of the C:N ratio.

3.3. *Environmental descriptors for seston and mesozooplankton stoichiometry*

Results of the redundancy analysis (RDA) showed that 79.7% of the total variance in seston C:N ratios was explained by the first two axes of the constrained model, in which DOC, water temperature and phosphate were identified as significant environmental descriptors (Fig. 4). The C:N ratios of Size 2, Size 3, and Size 4 seston increased with increasing DOC concentration and decreasing water temperature in the bays. The C:N ratio of Size 1 seston increased with decreasing phosphate concentration, however, this relationship was mainly driven by an extreme value of phosphate concentration in July. Overall, the spatial variation (among-bays ordination distance) was smaller in June than in other months (Fig. 4). No significant RDA models were found for seston C:P and N:P.

The seston C:N (Size 2 and 3) showed a strong positive linear relationship with the water C:N (Fig. 5a). The C:N ratios of both copepods and cladocerans increased with increasing seston C:N (Fig. 5b). However, the partial correlation analysis indicated that the mesozooplankton C:N was not correlated with the seston C:N ($r = 0.533$, $p = 0.061$) after controlling the effect of water C:N. This result indicated that the changes in both seston and zooplankton C:N were mainly caused by changes in the water C:N.

4. Discussion

Our results showed that the temporal variation in elemental stoichiometry of coastal water, seston and zooplankton was more pronounced than the spatial variation, with the largest temporal changes generally occurring during the transition from spring to summer (i.e. from May to July). The stoichiometry of coastal water was highly influenced by freshwater and terrestrial organic matter inputs in spring,

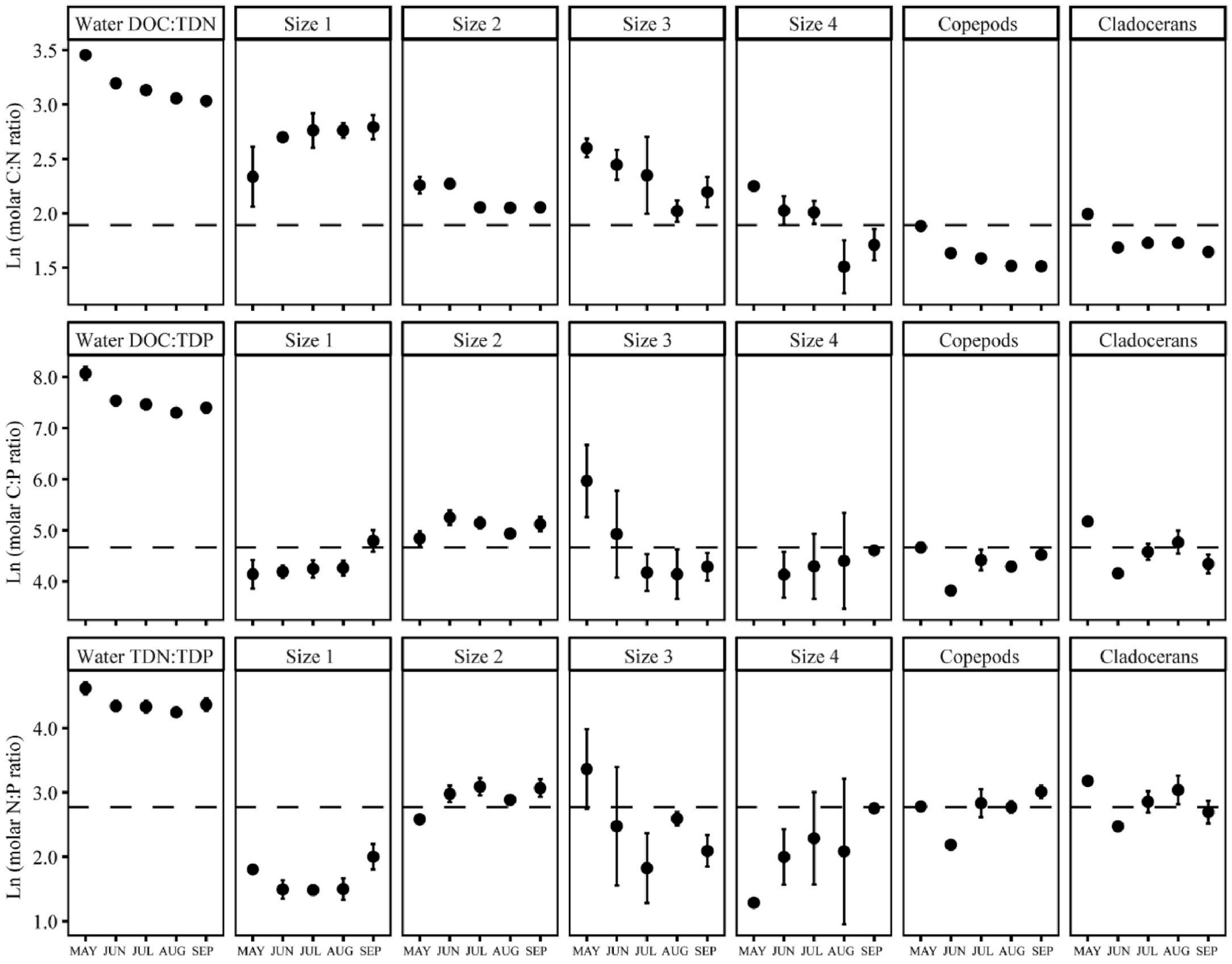

Fig. 3. Temporal variation of C:N, C:P and N:P ratios in water, seston size fractions 1–4 and mesozooplankton (copepods and cladocerans). All the data are ln-transformed and the average values for the 4 bays are presented. Error bars represent standard error. Dashed horizontal lines show the Redfield ratios.

Table 2

Paired *t*-test comparing copepods and cladocerans stoichiometry at the study bays in May–September 2018. Signifcant diferences at *p* value * ≤ 0.05, ** ≤ 0.01 and *** ≤ 0.001.

Ratio	Between Group	
	t	p
C:N	−6.701	<0.001***
C:P	−1.620	0.144
N:P	−0.488	0.638

during which the DOC concentrations in the bays also increased. Corresponding to the larger DOC inputs in May–June, the C:N and C:P ratios of seston (particularly Size 3 and Size 4) and zooplankton were also higher, suggesting that the water stoichiometry influenced the seston and zooplankton stoichiometry. DOC was the main descriptor of changes in seston C:N stoichiometry. Zooplankton stoichiometry also changed most drastically in the spring-summer transition, although the C:N:P ratios of zooplankton were less variable than those of seston. The C:N stoichiometry of both seston and zooplankton was determined by that of water, as the relationships between seston C:N and mesozooplankton C:N were not significant after controlling the effect of water C:N. Our findings imply that increases in inputs of DOC will likely result in lower nutritional quality (i.e. higher C:N) of crustacean zooplankton, which may have repercussions for the pelagic food web and fish production.

In the coastal areas of the northern Baltic Sea, the DOC concentration in the seawater is strongly affected by the increased terrestrial inputs from nearby landscapes during the snowmelt in spring (i.e. May) (Reader et al. 2014). Our results showed that the increased inputs of terrestrial DOC to the bays in spring had strong effects on water and seston C:nutrient ratios. As the supply of terrestrial DOC is expected to increase in the future due to increased precipitation and higher temperature (Larsen et al., 2011; Andersson et al., 2015), and as the catchments inputs to the northern Baltic Sea coast are already P-poor (Andersson et al. 1996; Stepanauskas et al. 2002, 2008; Tamminen and Andersen, 2007; Liu et al. 2017; Voss et al. 2021), our results imply that climatic changes will lead to increased C:nutrient ratios in the coastal planktonic food webs, and the coastal ecosystems in the northern Baltic Sea may become more P-limited in the future.

The stoichiometric responses of seston to temporal changes were size specific. The temporal variability in C:N:P ratios of the bacterial fraction (Size 1) were constrained within a relatively small range, while the other size fractions of seston were more variable, especially in their C:P and N:P ratios. The larger seston fractions (Size 2–4) were predominant and had higher C:N ratios than the Redfield ratio in May, and their C:N ratios became close to the Redfield ratio in autumn. These results could have

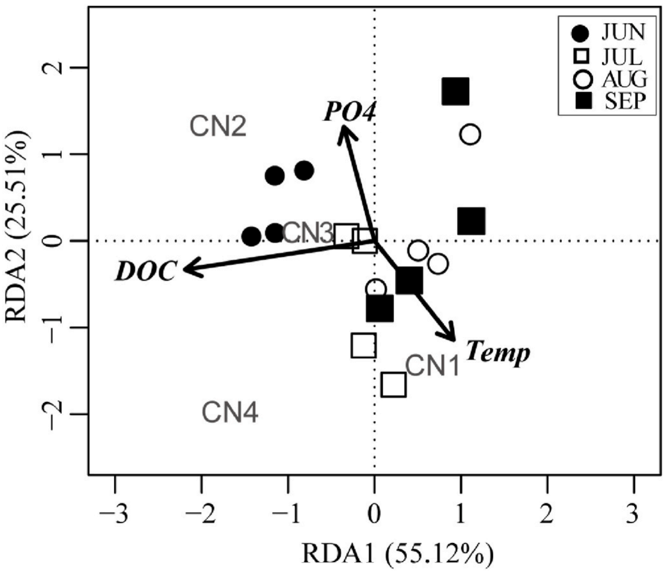

Fig. 4. Redundancy analysis (RDA) with forwards selection of the relationships between seston molar C:N ratio of different size fractions and significant explanatory variables from June to September. Adjusted *R*-square after forward selection was 0.52. CN1 to CN4 are the C:N ratios of specific seston size fractions (e.g., CN1 is the C:N ratio for Size 1 seston).

been due to the temporal differences in the taxonomic composition of seston, as not only autotrophs but also heterotrophs and mixotrophs composed Size 2–4 seston (Table S1), and the largest change of seston size classes occurred from May to June. In May, Size 2 and Size 3 seston together represented more than 90% of the overall seston C biomass (Fig. S1), but in later months the Size 2 seston became increasingly dominant. Our data showed that primary production decreased from spring to autumn. Autotrophic organisms have been shown to dominate the coastal seston biomass in the Baltic Sea in spring, while autotrophic and heterotrophic organisms have similar biomass during autumn (Andersson and Rudehall, 1993). In comparison to the autotrophs, the heterotrophic organisms have generally lower C:N ratios (Sterner and Elser, 2002). When the proportion of heterotrophic organisms increased in Size 2–4 seston, the C:N ratios of these seston fractions were likely reduced concomitantly, indicating the importance of community composition to the stoichiometry of different seston size fractions.

In this study, the C:N ratios of Size 1 seston (bacterial fraction) were ca. 15:1 on average, much higher than the commonly reported ratio for natural bacterial assemblages (ca. 5:1 to 7:1) (Elser et al. 1995; Cotner et al. 2010; Zimmerman et al. 2014; Stenzel et al. 2017). Such high C:N ratio of bacteria may indicate that the bacterial community was relatively deficient in N, although bacteria in the study area have been shown to be either C- or P-limited based on nutrient addition experiments (Zweifel et al. 1993). Another possible explanation is that we measured bacterial stoichiometry using a size fraction different from that in previous studies where bacterial stoichiometry was usually analyzed using the seston size 0.7–1 μm. However, in natural seawater, the size range 0.7–1 μm commonly contains mostly picophytoplankton (generally 0.5–2 μm) and possibly makes such stoichiometric measurements not specific for bacteria. In this study, we analyzed the elemental composition of bacteria by using the seston size range 0.3–0.7 μm (i.e. Size 1), which had excluded most picophytoplankton and likely better represented the heterotrophic bacterial community. In contrast to seston Size 2–4, the C:N ratio of Size 1 seston, tended to increase from spring to autumn and did not directly reflect the changes in the water C:N. During the spring flood, the lower bacterial C:N ratio may be mainly due to 1) the competitive advantage of bacteria in taking up N over phytoplankton and 2) bacteria taking advantage of the exudates produced by the phytoplankton spring bloom. Nevertheless, our temporal C:N data of Size 1 seston suggest that bacteria are possibly not N-rich as previously proposed (Cotner et al. 2010).

Our data did not support that mesozooplankton stoichiometry is strictly homeostatic. The C:N ratios of copepods and cladocerans, at both group and genus levels, followed the seston C:N pattern caused by the temporal changes in water C:N ratio. The relationships between C:N ratios of Size 2–3 seston (i.e. the most dominant seston size classes) and mesozooplankton were especially strong. Also, the C:N ratio of cladocerans was generally higher than that of copepods. This could be due to the higher N demand of copepods (Sommer and Sommer, 2006). Our results are in line with the findings of Walve and Larsson (1999) which showed that cladocerans have a higher C:N ratio than copepods in the Baltic Sea, and that copepods have a more stable C and N content. The stoichiometric differences between copepods and cladocerans might result from their different feeding modes: copepods are selective feeders of relatively large nano-and microplankton, while cladocerans are opportunistic filter feeders that prefer small pico- and nanoplankton below 20 μm (Sommer and Sommer, 2006; Herstoff et al. 2019). Also, the less selective feeding of cladocerans could have led to their greater use of flocculated DOC than did the copepods (Tanentzap et al. 2017), leading to the high C:N of cladocerans. Even though there is a prey-size overlap among copepods and cladocerans, the Size 2 seston was potentially a more important food source for cladocerans, while Size 3 seston was more favorable for copepods. The C:N ratios of both copepods and cladocerans were elevated in spring, likely caused by the concurrent increases in C:N ratios of their major diet (seston Size 2 and Size 3). These results imply that Size 2–3 seston had relatively lower food quality for zooplankton in spring than in summer. Therefore,

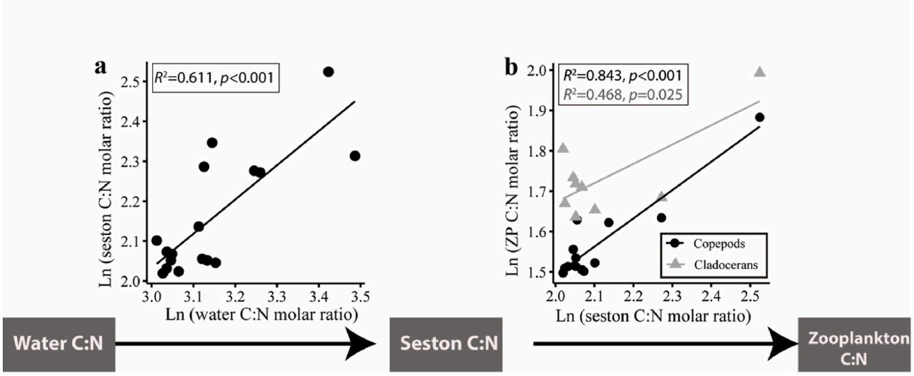

Fig. 5. A hypothesized flow chart of elemental stoichiometry from water to mesozooplankton, with the linear relationships between a) water and seston C:N ratios, and b) seston and mesozooplankton (copepods and cladocerans) C:N ratios from the study bays in May–September 2018. Seston C:N ratios used for this flow chart were from the main mesozooplankton prey fractions: Size 2 and Size 3 seston. All data were ln-transformed.

increasing inputs of DOC to the coastal waters might consequently reduce the overall food quality of mesozooplankton for their predators.

The environmental conditions of the study bays were unexpectedly homogeneous, possibly because precipitation was unusually low compared to the same period in previous years. Yet, after excluding the May samples in the RDA, DOC still was the main descriptor for C:N ratios of larger seston fractions (Size 2–4). The C:N ratio of Size 1 seston (bacterial fraction) was not correlated with DOC but instead weakly correlated with water temperature in the study area. Earlier study (Cotner et al. 2006) showed that increasing temperature could led to increased cellular C and N content of bacteria resulting in minor variation of their C:N ratio.The low temporal variability of the C:N ratio of Size 1 seston indicated a certain degree of homeostasis in bacteria compared to the larger seston size groups, and that terrestrial DOC inflows to the study bays had weaker effects on C:N ratios of bacteria than those of the larger seston fractions. Overall, the seston and mesozooplankton C:N ratios were not affected by variations of N but rather of DOC, whose concentrations in the bays were dependent on the temporal changes in terrestrial inflow. Altogether, our results suggest that DOC is important in affecting the seston and mesozooplankton C:N stoichiometry regardless of the magnitude of freshwater inflow and terrestrial organic matter inputs in the nutrient-deficient coastal areas of the northern Baltic Sea. Increasing DOC concentration in the coastal area would impose stronger effects on C:N ratios of larger seston fractions than the bacteria, and will likely result in higher C:N ratios of larger seston.

5. Conclusion

The high DOC concentration after the spring flood was found to alter the C:N ratio in the coastal water, which in turn affected the stoichiometry of seston and mesozooplankton. DOC was the main descriptor for the stoichiometry of seston especially the larger size fractions, i.e. > 0.7 μm. Copepods generally had a lower C:N ratio than cladocerans, but both copepods and cladocerans showed similar temporal changes in C:N as the water and the >0.7 μm seston. Knowledge on stoichiometric changes of individual seston size fractions is important for understanding the impacts of environmental stressors on the zooplankton stoichiometry. Climate change-induced increases in precipitation and inflow of freshwater and DOC would possibly increase the C:N ratios of the planktonic food chains in northern coastal ecosystems, which in turn might affect the quality and production of higher trophic levels.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Junwen Guo: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sonia Brugel:** Writing – review & editing, Formal analysis, Conceptualization. **Agneta Andersson:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Danny Chun Pong Lau:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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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Appendix A. Supplementary data

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References

Andersen, T., Hessen, D.O., 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. 36, 807–814.

Andersson, A., Hajdu, S., Haecky, P., Kuparinen, J., Wikner, J., 1996. Succession and growth limitation of phytoplankton in the Gulf of Bothnia (Baltic Sea). Mar. Biol. 126, 791–801.

Andersson, A., Meier, H.E.M., Ripszam, M., Rowe, O., Wikner, J., Haglund, P., et al., 2015. Projected future climate change and Baltic Sea ecosystem management. Ambio 44, 345–356.

Andersson, A., Rudehall, A., 1993. Proportion of plankton biomass in particulate organic carbon in the northern Baltic Sea. Mar. Ecol. Prog. Ser. 95, 133–139.

Bauer, J.E., Bianchi, T.S., 2012. Dissolved organic carbon cycling and transformation. In: Treatise on Estuarine and Coastal Science. Elsevier Inc., pp. 7–67

Bauer, J.E., Cai, W.J., Raymond, P.A., Bianchi, T.S., Hopkinson, C.S., Regnier, P.A.G., 2013. The changing carbon cycle of the coastal ocean. Nature.

Cotner, J.B., Hall, E.K., Scott, J.T., Heldal, M., 2010. Freshwater bacteria are stoichiometrically flexible with a nutrient composition similar to seston. Front. Microbiol. 1, 132.

Cotner, J.B., Makino, W., Biddanda, B.A., 2006. Temperature affects stoichiometry and biochemical composition of Escherichia coli. Microb. Ecol. 52, 26–33.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., et al., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop.). 36, 27–46.

Elser, J.J., Chrzanowski, T.H., Sterner, R.W., Schampel, J.H., Foster, D.K., 1995. Elemental ratios and the uptake and release of nutrients by phytoplankton and bacteria in three lakes of the Canadian shield. Microb. Ecol. 29, 145–162.

Elser, J.J., Frost, P., Kyle, M., Urabe, J., Andersen, T., 2002. Effects of light and nutrients on plankton stoichiometry and biomass in a P-limited lake. Hydrobiologia 481, 101–112.

Fleming-Lehtinen, V., Räike, A., Kortelainen, P., Kauppila, P., Thomas, D.N., 2015. Organic carbon concentration in the northern coastal Baltic Sea between 1975 and 2011. Estuar. Coast 38, 466–481.

Gargas, E., 1975. A manual for phytoplankton primary production studies in the Baltic. Publs. Balt. mar. Biol.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1999. Methods of Seawater Analysis, third ed. Wiley-VCH.

Hall, E.K., Maixner, F., Franklin, O., Daims, H., Richter, A., Battin, T., 2011. Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. Ecosystems 14, 261–273.

Harvey, E.T., Kratzer, S., Andersson, A., 2015. Relationships between colored dissolved organic matter and dissolved organic carbon in different coastal gradients of the Baltic Sea. Ambio 44, 392–401.

Herstoff, E.M., Baines, S.B., Boersma, M., Meunier, C.L., 2019. Does prey elemental stoichiometry influence copepod movement over ontogeny? Limnol. Oceanogr. 64, 2467–2477.

Johansson, M., Gorokhova, E., Larsson, U., 2004. Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. J. Plankton Res. 26, 67–80.

Kendrick, M.R., Benstead, J.P., 2013. Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. Freshw. Biol. 58, 1820–1830.

Kjellström, E., Ruosteenoja, K., 2007. Present-day and future precipitation in the Baltic Sea region as simulated in a suite of regional climate models. Clim. Change 81, 281–291.

Klausmeier, C.A., Litchman, E., Daufresne, T., Levin, S.A., 2008. Phytoplankton stoichiometry. Ecol. Res.

Klausmeler, C.A., Litchman, E., Daufresne, T., Levin, S.A., 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429, 171–174.

Larsen, S., Andersen, T., Hessen, D.O., 2011. Climate change predicted to cause severe increase of organic carbon in lakes. Global Change Biol. 17, 1186–1192.

Liu, Y., Markus Meier, H.E., Eilola, K., 2017. Nutrient transports in the Baltic Sea - results from a 30-year physical-biogeochemical reanalysis. Biogeosciences 14, 2113–2131.

Makino, W., Cotner, J.B., Sterner, R.W., Elser, J.J., 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Funct. Ecol. 17, 121–130.

McManamay, R.A., Webster, J.R., Valett, H.M., Dolloff, C.A., 2011. Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams. J. North Am. Benthol. Soc. 30, 84–102.

Meunier, C.L., Boersma, M., Wiltshire, K.H., Malzahn, A.M., 2016. Zooplankton eat what they need: copepod selective feeding and potential consequences for marine systems. Oikos 125, 50–58.

Moreno, A.R., Martiny, A.C., 2018. Ecological stoichiometry of ocean plankton. Ann. Rev. Mar. Sci 10, 43–69.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al., 2020. Package "Vegan" Title Community Ecology Package. Version 2.5-7. *cran.ism.ac.jp*.

Olenina, I., Agency, E.P., Hajdu, S., Andersson, A., Wasmund, N., 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea helsinki commission. Balt. Sea Environ. Proc. 106, 144.

Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J., Kato, S., 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119, 741–751.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Reader, H.E., Stedmon, C.A., Kritzberg, E.S., 2014. Seasonal contribution of terrestrial organic matter and biological oxygen demand to the Baltic Sea from three contrasting river catchments. Biogeosciences 11, 3409–3419.

Reusch, T.B.H., Dierking, J., Andersson, H.C., Bonsdorff, E., Carstensen, J., Casini, M., et al., 2018. The Baltic Sea as a time machine for the future coastal ocean. Sci. Adv. 4, eaar8195.

Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. Biogeochemistry 111, 1–39.

Scott, J.T., Cotner, J.B., LaPara, T.M., 2012. Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. Front. Microbiol. 3, 42.

Solórzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25, 754–758.

Sommer, U., Sommer, F., 2006. Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia 147, 183–194.

Stenzel, B., Rofner, C., Pérez, M.T., Sommaruga, R., 2017. Stoichiometry of natural bacterial assemblages from lakes located across an elevational gradient. Sci. Rep. 7, 5875.

Stepanauskas, R., Jorgensen, N.O.G., Eigaard, O.R., Žvikas, A., Tranvik, L.J., Leonardson, L., 2008. Summer inputs of riverine nutrients to the Baltic Sea : bioavailability and eutrophication. America (NY) 72, 579–597.

Stepanauskas, R., Jørgensen, N.O.G., Eigaard, O.R., Žvikas, A., Tranvik, L.J., Leonardson, L., 2002. Summer inputs of riverine nutrients to the Baltic Sea: bioavailability and eutrophication relevance. Ecol. Monogr. 72, 579–597.

Sterner, R., Elser, J., 2002. Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere.

Tamminen, T., Andersen, T., 2007. Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. Mar. Ecol. Prog. Ser. 340, 121–138.

Tanentzap, A.J., Kielstra, B.W., Wilkinson, G.M., Berggren, M., Craig, N., Del Giorgio, P. A., et al., 2017. Terrestrial support of lake food webs: synthesis reveals controls over cross-ecosystem resource use. Sci. Adv. 3, e1601765.

Telesh, I., Postel, L., Heerkloss, R., Mironova, E., Skarlato, S., 2009. Zooplankton of the Open Baltic Sea: Extended Atlas. BMB Publ, p. 21.

Voss, M., Asmala, E., Bartl, I., Carstensen, J., Conley, D.J., Dippner, J.W., et al., 2021. Origin and fate of dissolved organic matter in four shallow Baltic Sea estuaries. Biogeochemistry 154, 385–403.

Van De Waal, D.B., Verschoor, A.M., Verspagen, J.M.H., Van Donk, E., Huisman, J., 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Front. Ecol. Environ. 8, 145–152.

Walve, J., Larsson, U., 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. J. Plankton Res. 21, 2309–2321.

Walve, J., Larsson, U., 2010. Seasonal changes in Baltic Sea seston stoichiometry: the influence of diazotrophic cyanobacteria. Mar. Ecol. Prog. Ser. 407, 13–25.

Wikner, J., Andersson, A., 2012. Increased freshwater discharge shifts the trophic balance in the coastal zone of the northern Baltic Sea. Global Change Biol. 18, 2509–2519.

Yvon-Durocher, G., Schaum, C.E., Trimmer, M., 2017. The temperature dependence of phytoplankton stoichiometry: investigating the roles of species sorting and local adaptation. Front. Microbiol. 8, 2003.

Zimmerman, A.E., Allison, S.D., Martiny, A.C., 2014. Phylogenetic constraints on elemental stoichiometry and resource allocation in heterotrophic marine bacteria. Environ. Microbiol. 16, 1398–1410.

Zweifel, U.L., Norrman, B., Hagstrom, A., 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar. Ecol. Prog. Ser. 101, 23–32.