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Declarations

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Abstract

Carbon and nitrogen stable isotope ratios are increasingly used to study long-term change in food web structure and nutrient cycling. We retrospectively analyse elemental composition (C, N and P) and stable isotopes ($\delta^{13}C$, $\delta^{15}N$) in archived monitoring samples of two important taxa from the bottom of the food web; the filamentous ephemeral macroalgae *Cladophora* spp. and the blue mussel *Mytilus edulis trossulus* from three contrasting regions in the Baltic Sea (coastal Bothnian Sea and Baltic Proper, open sea central Baltic). The aim is to statistically link the observed spatial and interannual (8-24 years' time-series) variability in elemental and isotope baselines with their biomass trends and to the oceanographic monitoring data reflecting the ongoing environmental changes (i.e., eutrophication and climate) in this system. We find clear differences in isotope baselines between the two major Baltic Sea basins. However, the

temporal variation in *Mytilus* $\delta^{13}C$ was similar among regions and, at the open sea station, mussels and algae $\delta^{13}C$ also correlated over time, likely reflecting a global (Suess) effect, whereas $\delta^{15}N$ of both taxa varied with local and regional dissolved organic nitrogen concentrations in water. $\delta^{15}N$ in source amino acids allowed detection of diazotrophic N in *Mytilus*, which was masked in bulk $\delta^{15}N$. Finally, *Cladophora* N:P reflected regional nutrient levels in the water while P %, which differed for both taxa, was linked to food quality for *Mytilus*. This study highlights the potential of a multi-taxa and multi-stable isotope approach to understand nutrient dynamics and monitor long-term environmental changes.

1. Introduction

Coastal seas are highly involved in fundamental biogeochemical processes controlling nutrient and organic matter cycling (Middelburg and Herman 2007; Bouwman et al. 2013; Carstensen et al. 2020). They receive nutrients and organic matter from both marine (e.g., primary production) and terrestrial (e.g., riverine inputs of terrestrial material, anthropogenic outfalls) origins and act as a filter between both realms (Asmala et al. 2017). In addition to climate change, human activities influence nutrient cycling through eutrophication, resulting in changes in both absolute values and ratios between nitrogen (N) and phosphorus (P). Major changes in nutrient supply are likely to affect requirements of primary producers with regard to elemental building blocks and have repercussions on processes regulating elemental homeostasis of consumers (ecological stoichiometry sensu Sterner and Elser 2002), with consequences for food web functioning and biogeochemical cycling.

Archived biological samples from environmental monitoring programs can be retrospectively analyzed for elemental composition (C, N, P) and stable isotope ratios of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) to study nutrient cycling and reconstruct food webs in relation to a changing environment. Carbon isotopes provide information about ultimate carbon sources for primary production (Fry and Sherr 1984). Nitrogen isotopes can be used to trace specific nitrogen sources, such as anthropogenic (e.g., Connolly et al. 2013) or diazotrophic ones (Rolff 2000; Karlson et al. 2015), and to quantify trophic position in consumers, if estimates of trophic discrimination factors are available (Vander Zanden and Rasmussen 1999, 2001; Post et al 2002). Organisms at the base of the food web such as filter-feeding bivalves or grazing snails, with low motility and long-life span, are commonly used as proxies for isotope baselines (i.e., the ultimate C and N sources) since they integrate intra-annual variability of nutrients (e.g., Vander Zanden and Rasmussen 1999; Post 2002) over longer time spans, several months or even year(s) (e.g., Gorman et al. 2017), compared to organisms with shorter life spans such as zooplankton or phytoplankton. Perennial macrophytes can be used as isotope baselines when no primary consumers are available (e.g., Haubrock et al. 2020). However, perennial macrophytes typically reflect nutrient sources in the water column during the growth period (e.g., Savage and Elmgren 2004), hence the relatively fast turnover rates of their tissue should reflect a seasonal or shorter baseline.

In the Baltic Sea, the suspension-feeding blue mussel, the *Mytilus edulis trossulus* species complex (Kijewski et al. 2006; Stuckas et al. 2009; hereafter referred to as *Mytilus* or blue

mussel) and ephemeral filamentous green algae from the genus *Cladophora*, are highly abundant. *Mytilus* occur in densities of up to ~100 000 individuals m^{-2} (Westerbom et al. 2008) and constitute, through their efficient suspension-feeding, an important link between the pelagic and benthic ecosystem, promoting nutrient cycling (Kautsky and Wallentinus 1980; Kautsky and Evans 1987; Attard et al. 2020). *Cladophora* is widely distributed in the Baltic Sea and mainly occurs from the surface down to 1 or 2 m depths, providing food and shelter for invertebrates (Snoeijs-Leijonmalm 2017). It is perennial, but overwinters as a small tuft attached to shallow rocky substrates. During summer it reaches its full growth and benefits from nutrient enrichment (Thybo-christesen et al. 1993). Both taxa are found throughout the Baltic Sea; *Cladophora* occurs from the Bothnian Bay down to the southern Baltic Proper while *Mytilus* distribution is restricted in the northernmost basin, the Bothnian bay, due to low salinity levels (Kautsky and Kautsky 2000). On a smaller scale, they co-occur on hard substrates at shallow depths and are often the dominant taxa based on biomass (Snoeijs-Leijonmalm 2017). Isotope composition in bivalves, including *Mytilus*, is commonly used to study pelagic organic matter origin (Magni et al. 2013) and its variability over time and space (Briant et al. 2018; Corman et al. 2018). Bivalves are considered suitable baselines for food web studies (e.g., Abrantes and Sheaves 2009; Willis et al. 2017) and in contaminant monitoring (Karlson and Faxneld 2021), while *Cladophora* is mainly used as a proxy of nutrient levels in coastal waters, in the Baltic and elsewhere (Mäkinen and Aulio 1986; Planas et al. 1996).

The pronounced latitudinal gradients of temperature, salinity and nutrients combined with eutrophication and ongoing climate change in the Baltic Sea provide an ideal study system to link environmental changes to resultant food web change and ecosystem functioning. In this system, retrospective chemical analyses (i.e., elemental and isotope composition) of archived samples help to quantify such potential changes in biota as a consequence of their changing environment. Riverine inputs of organic carbon and nutrients have increased in the recent time period, especially in the Bothnian Sea (Wikner and Andersson 2012; Asmala et al. 2019), and is expected to continue (Andersson et al. 2015a). In the Bothnian Sea, the N:P ratio of the dissolved inorganic pool is similar to the Redfield molar ratio of 16 (ca. 13, although the slight N limitation has increased in recent years; Rolff and Elfwing 2015), while it is considerably lower in the Baltic Proper (ca. 4), indicating strong N limitation (Savchuk 2018). Diazotrophic primary producers, such as some species of bloom forming cyanobacteria, can bypass this N-limitation by directly fixing N_2. Satellite images of surface accumulations indicate that these blooms have increased since the 1980s (Kahru and Elmgren 2014), and this internal N loading

now exceeds external loadings from rivers and atmospheric deposition in the Baltic Proper (Olofsson et al. 2020). Cyanobacterial blooms benefit from denitrification and phosphate release from hypoxic sediments, which exacerbate the N:P imbalance in a 'vicious cycle' (Vahtera et al. 2007). The most recent decade has also seen the regular occurrence of cyanobacterial blooms in the Bothnian Sea (Olofsson et al. 2020). Salinity and temperature are both lower in the Bothnian Sea compared to the Baltic Proper and are the primary factors affecting species distribution, including that of *Cladophora* and *Mytilus*. Predicted increase in temperature and decrease in surface salinity of the Baltic Sea (Räisänen 2017 and references therein) are hence expected to affect organisms, food webs and ecosystems (Andersson et al. 2015a; Vuorinen et al. 2015). Recent studies have shown a decrease in mussel populations over recent decades in the southern Baltic Proper, linked to increasing water temperature and changes in food quantity (Franz et al. 2019; Westerbom et al. 2019) and quality (Liénart et al. 2020). A shift in dominance from the canopy-forming perennial macrophyte *Fucus* towards opportunistic ephemeral *Cladophora* has been reported since the 1980s in different areas of the Baltic Sea (Kraufvelin and Salovius 2004), likely linked to eutrophication (Kautsky et al. 1986; Råberg 2004; Torn et al. 2006). However, *Fucus* recovery has been observed recently in some areas (Rinne and Salovius-Laurén 2020). Nonetheless, higher temperature and declining salinity promote filamentous green algae (Takolander et al. 2017) while also disadvantaging *Mytilus* (Westerbom et al. 2019), suggesting climate change will enforce the shift towards ephemeral macrophytes and fewer mussel beds in the Baltic.

Tracing C and N origin in the Baltic Sea is complex, due to multiple interacting sources, especially in coastal areas. In general, eutrophication is associated with elevated $\delta^{15}N$ values, for instance in Baltic Proper sediment (Voss et al. 2000). Also, sewage waters that are enriched in ^{15}N are traceable in macrophytes (Savage and Elmgren 2004). However, the depleted ^{15}N signal of synthetic N fertilizers used in agriculture (Bateman and Kelly 2007) can be confounded with the similar signal of diazotrophic cyanobacteria (Rolff 2000), the latter considered an indirect effect of eutrophication (Vahtera et al. 2007). Eutrophication can result in higher $\delta^{13}C$ values in plankton and mussels due to increasing plankton biomass (Oczkowski et al. 2018) with the exception of cyanobacteria (generally low $\delta^{13}C$; Rolff 2000). In the Bothnian Sea, the naturally low $\delta^{15}N\text{-}NO_3$ from pristine rivers (Voss et al. 2005) should equally be reflected in relatively depleted ^{15}N baselines. Furthermore, the typically low $\delta^{13}C$ both from particulate organic and dissolved inorganic terrestrial carbon (i.e., $\delta^{13}C_{DIC}$) from extensive riverine input in the north (Rolff and Elmgren 2000), is similarly expected to be reflected in a

low $\delta^{13}C$ baseline in the Bothnian Sea. However, low temperatures and low light availability (the latter from the dark, humus rich water in the north) can also result in lower $\delta^{13}C$ values for macrophytes (Wiencke and Fischer 1990; Hemminga and Mateo 1996). As an additional process, the global decrease in atmospheric $\delta^{13}C$ linked with fossil fuel burning over the 20th century also has an influence on carbon isotope composition in the Baltic Sea (Gustafsson et al. 2015). An organism's physiology (e.g., rapid growth, nutritional stress, reproductive stages) can also lead to substantial isotope variability in consumers (Doi et al. 2017; Gorokhova 2018). For instance, osmoregulation is an especially N-demanding process for *Mytilus* experiencing low-saline conditions in the Baltic Sea (Tedengren and Kautsky 1987) and likely influences ^{15}N fractionation in mussels' tissues, hence confounding the dietary origin of the $\delta^{15}N$ signal (Liénart et al. 2020). To better trace ultimate N sources, the end-member $\delta^{15}N$ signal can be measured in source amino acids (e.g., phenylalanine), which show negligible ^{15}N fractionation during assimilation, physiological processes or trophic transfer (McClelland and Montoya 2002) compared to the bulk $\delta^{15}N$ signal. Finally, regarding N and P elemental composition of algae and mussels, taxa-specific physiological requirements may override nutrient background in the water. Fast-growing algae such as *Cladophora* are under little homeostatic control compared to slow growing consumers like mussels (Smaal and Vonck 1997), and may therefore better reflect basin-specific nutrient conditions.

The general aim of this study is to test whether eutrophication and climate-related changes are mirrored in two widely distributed and abundant key taxa: the filamentous algae *Cladophora* and the blue mussel *Mytilus*. This main aim is divided into three objectives: to identify (I) potential region-specific differences and (II) potential consistency in temporal changes in elemental and isotope baselines of these two taxa and (III) link the observed year-to-year and long-term variability in the isotope and elemental data to environmental and oceanographic variables, and ultimately to population level data for each taxa.

We expect that (1) spatially, elemental and isotope composition of both taxa will reflect the latitudinal gradient in nutrients, with lower values of $\delta^{13}C$, $\delta^{15}N$, N% and P% in the more oligotrophic, Bothnian Sea compared to the Baltic Proper. However, lower $\delta^{13}C$, $\delta^{15}N$, N% and P% values in the Bothnian Sea of both taxa may reflect increased influence of riverine (terrestrial) input in this basin; (Table S1 and Liénart et al. 2020); (2) temporally, the decrease in eutrophication in recent years should be reflected in decreases in $\delta^{13}C$, $\delta^{15}N$, N% and P% in our longest time series, though potential increase in riverine influence in the system is also expected to lead to decreasing $\delta^{13}C$ but in increasing values of $\delta^{15}N$, N% and P% (Table S1

and Liénart et al. 2020); (3) N and P contents of *Cladophora* should reflect water nutrient concentrations in the water earlier in the year whereas *Mytilus* should have a greater homeostatic control over N and P content (and thus no link to water nutrient concentrations); (4) osmotic stress, especially in the low-saline Bothnian Sea, will influence blue mussel N requirements and hence confound $\delta^{15}N$ values (Lienart et al 2020). Table S1 describe the rationales for predictor selection that supports our hypotheses.

To explore these expectations, we take advantage of long-term oceanographic and land-based monitoring data and archived biological samples for the well-studied Baltic Sea. We retrospectively analyse elemental (C, N and P) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) composition of *Cladophora* and *Mytilus* from three contrasting regions (coastal Bothnian Sea, coastal Baltic Proper and open sea Baltic Proper), in time series spanning 8 to 24 years and link these biotic proxies to potential drivers. Finally, we correlate chemical, environmental and oceanographic data to population level biomass data in both taxa.

2. Material and methods

2.1. Study areas and sampling

This study used data from the following study regions: 1) the Bothnian Sea coastal region (Höga Kusten (HK)), 2) the Baltic Proper coastal archipelago area (Askö (A)), and 3) the Baltic Proper open sea (island of Gotland (G)) (Fig. 1, details Table S2). Within the Swedish marine monitoring program of the phytobenthic community, both the blue mussels *Mytilus* and filamentous green algae *Cladophora* have been sampled once a year at different stations and archived over the periods 1993-2016 for A, 2000-2016 for G, and 2008-2016 for HK. These taxa were sampled in three replicates (three quadrates of 20×20 cm) collected every year along the same land-to-sea transects (different depths, three quadrates per depth) in late August/early September by SCUBA divers. Samples were oven-dried at 60˚C and stored in the dark, dry and at room temperature. From each year and region, *Mytilus* individuals, n = 3 (G and HK) n = 15 (A), from similar size class (length: 10.0 ± 1.5 mm, mean ± sd; Table S2) from 5 m depth at the most exposed station in each region (two stations at HK to allow n = 3 per year; Fig. 1), were selected for biometric and chemical analyses (details below). *Cladophora* material (a few grams dry weight homogenised material, coming from many specimens) from 1 meter depth was selected for chemical analysis for three to six stations per region (Fig. 1). The rationale behind using several stations within each region for *Cladophora* was to avoid variability from

local nutrient conditions (such as bird guano on a particular rock), whereas one station was deemed sufficient for mussels, which actively filter-feeds large amounts of water.

Temperature (˚C), salinity, dissolved inorganic nitrogen and phosphorus (DIN, DIP, µmol L^{-1}), total nitrogen and phosphorus (total N, total P, µmol L^{-1}; i.e., includes dissolved inorganic, dissolved organic, and particulate fractions), and the phytoplankton community were measured by the Swedish national monitoring program for the nearby pelagic ecosystem: the station C3 close to Höga Kusten, station B1 close to Askö island in Stockholm's archipelago and the open sea station BY31 north of Gotland island (Fig. 1, Table S2). For more details about sampling and analytical methods, see supplemental material Table S2, Andersson et al. (2015b) and Liénart et al. (2020). All environmental and phytoplankton community data are available at http://www.smhi.se (Marine environmental monitoring data - SHARK database). Surface terrestrial total organic carbon transport (TOC, total loadings in tons) for each watershed in Sweden is measured monthly within the freshwater monitoring program "river mouths" (chemical analyses) and is available at http://webstar.vatten.slu.se/db.html (HAVET 2015/2016).

2.2.Elemental and isotope analysis

Dry ground material from both *Cladophora* and *Mytilus* soft tissue (individual whole mussel soft tissues, shells not included but weight of shells taken for later condition index calculations) were used for elemental carbon, nitrogen and phosphorus (% of C, N, P expressed per dry weight), bulk carbon and nitrogen isotope ($\delta^{13}C$, $\delta^{15}N$) and amino-acid-$\delta^{15}N$ measurements (AA-$\delta^{15}N$). Individual specimens of *Mytilus* soft tissue were used for C and N elemental and isotope measurements and P % was measured individually in different specimens, since the mussels were too small for all analyses in the same individual (soft tissue was on average 2.1±1.5 mg in mussels in this size range; Table S2). Phosphorus in *Cladophora* was only measured for 4 of the 6 stations at A (not measured from Lac and StA) and 3 of the 4 stations at HK (not measured for Dön). Amino-acid-$\delta^{15}N$ analysis was performed on pooled *Mytilus* material (ca. 5 individuals per region and year).

Phosphorus analyses of individual mussels were performed at the Department of Ecology, Environment and Plant Sciences (Stockholm, Sweden) using a Alpkem SFA system to analyse phosphate (SS-EN ISO 15681-2:2018), after combustion and persulfate digestion, following Larsson et al. (2001). C and N elemental and bulk stable isotopes were measured at the Center

for Physical Science and Technology (Vilnius, Lithuania) with a Flash EA 1112 Series Elemental Analyzer (Thermo Finnigan) connected to a DeltaV Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher).

In order to validate ultimate nitrogen sources independently of potential physiological effect, we further compared bulk $\delta^{15}N$ composition with a smaller data set of $\delta^{15}N$ in source amino acids for both taxa. Amino-acid-$\delta^{15}N$ analysis of *Mytilus* was conducted at the Center for Physical Science and Technology (Vilnius, Lithuania) and details are presented in supplemental material. In brief, the extraction and derivatization of amino acids were performed following the method described in Ledesma et al. (2020), then analysed by GC-C-IRMS with a Trace GC Ultra Gas Chromatograph (Thermo scientific) coupled to a Delta Advantage Isotope Ratio Mass Spectrometer via GCC III combustion interface (ThermoFinnigan). *Cladophora* amino-acid-$\delta^{15}N$, which had a lower N content, was analysed at the University of California Davis Stable Isotope Facility (UC Davis) following the method described on https://stableisotopefacility.ucdavis.edu/gcamino.html (details in suppl. material).

All isotope data are expressed using the conventional delta notation: $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ = $[(R_{sample}/R_{standard})-1]$, where $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$, with per mil deviation (‰) from international standards, Vienna Pee Dee belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. External and internal standards were analysed as references within each batch of samples (details in suppl. material). Analytical uncertainties were <0.15‰ and <0.20‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively and <0.30‰ for AA-$\delta^{15}N$. The overall analytical precision was 0.9% and 0.2% for elemental C and N respectively.

2.3. Data analyses

2.3.1. Condition index and population data

The Condition Index ($CI_{Mytilus}$ = dry weight soft tissue (g) / dry weight shell (g) × 100) was calculated as an indicator of mussels' health status (e.g., Filgueira et al. 2013; Irisarri et al. 2015) on the same individual *Mytilus* used for stable isotope analysis (n.b. each mussel's soft tissue and shell weight were taken separately). The N:P ratio of both taxa ($N:P_{Cladophora}$, $N:P_{Mytilus}$) was calculated to be compared to Redfield ratio in the water.

For each station of the three regions (Fig. 1), and for each year, i) *Cladophora* total biomass (g dry weight per m^2) from ca. 1 meter depth, ii) *Mytilus* total biomass (g dry weight including

shells per m^{-2}) and abundance (individual per m^2), as well the ratio between the two ($Bm:Ab_{Mytilus}$, mg dry weight per individual, a proxy for average mussel size in the population), were calculated for all size classes (from juveniles ≥1 mm to bigger mussels >10 mm, only a few individuals had a shell length exceeding 20 mm, Åkermark et al. in prep) at ca. 5 m depth. Calculations for each region (average of stations) were based on geometric mean for the total biomass of both taxa and the $Bm:Ab_{Mytilus}$ ratio, to avoid influence from extreme values, and on the median for *Mytilus* abundance.

2.3.2. Environmental and oceanographic data

For each station, environmental data were first integrated over 0-10 m depth (average) for each sampling date, then averaged per month (linear interpolation was performed for missing sampling dates or months). The average values per period were then calculated: the annual mean (January to August 31st, i.e., approximate date of biota sampling each year) for temperature, salinity, total N and P and the winter mean (January-February, i.e., reliable period to measure nutrient concentrations and reflect the general conditions of each region) for DIN and DIP concentrations (Table S2). The DIN:DIP ratio in the water (mol:mol) was calculated for each year based on winter concentrations. The maximum summer temperature (T_{max}) was considered over the period June-August for the Baltic Proper stations and July-August for the Bothnian Sea (Table S2). The total biovolume (mm^3 L^{-1}) of phytoplankton community (total Phyto.) integrated over 0-10 m depth (multiplied by 2 at stations B1 and BY31 to adjust for the tube sampling depth of 0-20m compared to the 0-10 at C3, Olofsson et al. 2020) was calculated over the productive period (March-August, see details Table S2). In addition, and over the same period, the biovolume of four groups representing most of the phytoplankton community biomass (diatoms, dinoflagellates, cyanobacteria and the ciliate *Mesodinium rubrum*) were recalculated as an integrated sum (area under the curve (AUC) linear interpolation), following Liénart et al. (2020) (see details Table S2). A lag in the productive period was considered for the northern station (April-August). Summer AUC values, for only cyanobacteria, were also calculated to be later compared with *Cladophora* variables. Yearly averages of total organic carbon loadings (TOC_{terr}, tons) were calculated from the different watersheds of each region (Table S2). The average day of the year the shift in water temperature from 8 to 10-12˚C occurred (i.e., temperature rise triggering spawning, Kautsky pers. com.) was calculated for each region (T_{shift}, in Julian days) and used as a proxy of climate change (with this shift

supposedly occurring earlier in recent years). Finally, to capture the variations in the Ocean-Atmosphere Regime of the Baltic Sea (i.e., large-scale climatic index related to the North Atlantic Oscillation), the Baltic Sea Index (BSI) was calculated according to Lehmann et al. (2002).

2.4. Statistical analysis

Data treatment and statistical analysis were performed using R free software (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/, version 4.0.3 (2020-10-10)), except for the DistLM models, which were performed in PERMANOVA+ of PRIMER version 6 (Anderson et al. 2008). For all statistical univariate tests, critical *p*-value was set to 0.05.

2.4.1. Pre-analysis of *Cladophora* data

Since *Cladophora* were sampled at 3 to 6 stations within each region, local variability in elemental and isotope composition among these stations within each region was investigated prior to subsequent analyses. Unidirectional trends in time series using Mann-Kendall tests (details below) were explored for the individual stations within a region. Potential station effect over the entire time period was tested with a Kruskal-Wallis test followed by Bonferroni corrected Mann-Whitney pairwise comparison tests. Since little difference among stations was found (see results 3.1.1.), the annual mean of the 3 to 6 stations was computed for each variable and region for use in further analyses.

2.4.2. Region-specific differences and temporal trends in all data sets

To test for region-specific differences in elemental and isotope baselines (objective I) the last 9 years of each time period (Höga Kusten is only 9 years long) was compared using a Kruskal-Wallis test followed by Bonferroni corrected Mann-Whitney pairwise comparison tests.

To test for unidirectional trends in elemental and isotope baselines (objective II) as well as in the other biological and environmental variables for each region, Mann-Kendall tests were used. Autocorrelation was checked (acf(), {stats}), and if required, a modified version of the test for autocorrelated time series was applied (mmkh3lag(), {modifiedmk} package, version

1.5.0, allowing autocorrelations). To further test similarities in temporal development among regions (objective II), correlations were calculated for each unique variable of the same taxa over the same time series length. Correlations were evaluated, for % C, N, P, N:$P_{Cladophora}$ with Spearman's rank correlation while Pearson's correlation were used for stable isotope and biometric data on *Mytilus* ($\delta^{13}C$, $\delta^{15}N$, $CI_{Mytilus}$). To test similarities between the two taxa within each region, correlations (as described above) were calculated for each unique variable between *Mytilus* and *Cladophora*.

Finally, in order to summarize the major patterns of variation among the different regions, principal component analyses (PCA, scaled data) were performed for i) elemental and isotope composition of *Cladophora* and *Mytilus* (separate analyses) and ii) environmental data. The results of these analyses are presented in Supplemental Material.

2.4.3. Linking biological and environmental and oceanographic data

Several statistical approaches were used in order to explore linkages between the observed year-to-year and long-term variability from chemical biomarkers to population level data with environmental and oceanographic variables (objective III).

First, to identify which of the environmental variables (11 for *Cladophora*, 15 for *Mytilus*, Table S1) are correlated to the combined dataset of elemental and isotope composition of *Cladophora* or *Mytilus*, distance-based linear models were used (DistLM, resemblance matrix created using Euclidean distances, evaluated using both forward and stepwise selection of explanatory variables based on Akaike information criterion). The final DistLM model was plotted as a redundancy analysis.

Second, to model the relationship between a single response variable in each taxon ($\delta^{15}N$, $\delta^{13}C$, N%, P%) or the population biomass (and for *Mytilus*, the abundance and Bm:$Ab_{Mytilus}$) with the environmental and phytoplankton explanatory variables (11 to 17 predictors, the rationales for including predictors in the different models are presented Table S1, see also our general expectations in the end of introduction), partial least squares regression (PLSR) were used. PLSR analyses were performed on mean-centred and variance-standardized data, with models optimized to two components (as detailed in Lienart et al. 2020). Selection and removal of predictors were performed stepwise i) according to the variable of importance for projection (VIP) scores values (with a cut-off of 0.8 Wold et al. (2001) extended to 0.9 when needed for

more parsimonious models i.e., ≤ 7 predictors) and ii) trying to maximize both R^2Q (indicates model predictive capacity) and R^2Y (explanatory capacity, analogous to coefficient of determination in regression analysis, R^2X is the explained variance). Quality of the model was evaluated based on both explanatory $R^2Y > 0.6$ and predictive $R^2Q > 0.4$ capacities. Standardized regression coefficients were calculated for each predictor from the two-component model to ascertain the significance of environmental factors explaining and predicting the variance in response variables. PLSR analyses were performed using a modified version of the {pls} R package (v. 2.7-0, August 2018) as detailed in Liénart et al. (2020).

Finally, in order to test the direct relationship between the N:P ratio of the biota and the DIN:DIP in the water of each region over time (expectation 3), linear models were applied for each taxon (lm(), allowing the interaction region x N:P water). Variability in $N:P_{Cladophora}$ was also investigated locally (4 stations, Fur, Str, Jus, Iss) for our longest time series only (A), using distance to the shore (in km from an arbitrary runoff point selected with regard to the agricultural landscape occupying the bay and its position relative to the stations), and compared with the local DIN:DIP in the water (inner station, sö28 and outer station sö25; 2004-2016 August only; Fig. 1).

3. Results

3.1. Region specific differences in elemental and isotope baselines

3.1.1. Comparison of *Cladophora* within regions

There were no significant differences in *Cladophora* $\delta^{13}C$ and $\delta^{15}N$ between stations in any of the regions with the exception of $\delta^{13}C$ in the coastal Bothnian Sea ($p = 0.05$, between Bön and Sjä at Höga Kusten; Table S3, Fig. S1). There were no significant trends in any of *Cladophora* elemental and isotope variables over time (Table S4). Elemental composition differed only between some stations of the coastal archipelago (Askö) and the open Baltic Proper (Gotland, Table S3). Thus, mean values for *Cladophora* $\delta^{13}C$ and $\delta^{15}N$ and C, N and P % were calculated within regions, and used in subsequent analyses.

3.1.2. Comparisons among regions (expectation 1)

Both taxa had significantly higher $\delta^{15}N$ values from the Baltic Proper coastal archipelago station (Askö) compared to the other regions (Fig. 2, Fig. S2, Table S5). *Cladophora* $\delta^{13}C$ was

lower in the coastal Bothnian Sea station (Höga Kusten) and *Mytilus* $\delta^{13}C$ was higher in the open Baltic Proper station (Gotland) compared to the other regions (Fig. 2, Fig. S2, Table S5). For both taxa, N % and N:P ratio did not differ between the regions and P % was generally lower at Gotland compared to Höga Kusten (Fig. 2, Fig. S2, Table S5). The condition index of *Mytilus* ($CI_{Mytilus}$) was significantly higher for the mussels of Höga Kusten than for the Baltic Proper, and was significantly lower for Askö archipelago than for Gotland (Fig. 2, Fig. S2, Table S5). Within each region, $\delta^{13}C$ and $\delta^{15}N$ were never significantly correlated for the mussels nor algae (Table S6).

3.1.3. Ultimate nitrogen sources: $\delta^{15}N$ in amino acids for both taxa (expectation 1)

There was a significant correlation between bulk $\delta^{15}N$ and $\delta^{15}N$-Phe for both taxa (*Mytilus*: $p = 0.028$, $rho = 0.61$; Cladophora: $p = 0.008$, $rho = 0.83$, Fig. 3). Between regions, *Mytilus* $\delta^{15}N$-Phe was lowest for Gotland in the open Baltic Proper (-2.9±1.6 ‰) and for Höga Kusten in the coastal Bothnian Sea (-1.9±0.4 ‰) and higher for the coastal archipelago of Askö (-0.7±2.0 ‰). *Cladophora* $\delta^{15}N$-Phe was lowest at Höga Kusten (-0.7±0.03 ‰), intermediate in Gotland (1.1±3.5 ‰), and highest for Askö (4.6±3.4 ‰, Fig. S3). Regardless of region, *Mytilus* had generally negative $\delta^{15}N$ values of the source amino acid Phenylalanine ($\delta^{15}N$-Phe) compared to more positive values for *Cladophora* (Fig. 3).

3.2. Comparison between the two taxa within regions (expectation 1)

Regardless of region, the % P and N were higher in the mussels (N: 9.5±1.0 %, P: 0.8±0.1 %) than in the green algae (N: 1.9±0.4 %, P: 0.1±0.04 %), and $\delta^{13}C$ was higher for *Cladophora* (-15.9±1.8 ‰) than *Mytilus* (-22.3±0.8 ‰). $\delta^{15}N$ was generally higher for *Mytilus* (5.1±1.3 ‰), except for *Cladophora* from Askö, which showed high values comparable to the mussels at this site (5.8±1.2 ‰), but was lower in the other regions (ca. 2.0±1.5 ‰) (Fig. 2). *Cladophora* and *Mytilus* $\delta^{13}C$ were positively correlated at Gotland ($r = 0.65$, $p = 0.01$), and for their $\delta^{15}N$ values at Askö ($r = 0.41$, $p = 0.04$), while no significant correlation was observed at Höga Kusten (please note n = 9; Table S7).

3.3. Temporal changes in elemental and isotope baselines (expectation 2)

Over time, *Mytilus* $\delta^{13}C$ and $\delta^{15}N$ at Askö ($tau = -0.37$, $p = 0.01$, $tau = -0.41$, $p = 0.005$ respectively) and N % at Gotland ($tau = -0.50$, $p = 0.01$) in the Baltic Proper were significantly decreasing, while no patterns in isotopes could be identified for the shorter time series of the

Bothnian Sea station Höga Kusten (Table S8, Fig. 2). There was no significant trend for any *Cladophora* variables at the regional level (Table S8). *Mytilus* $\delta^{13}C$ was positively correlated between the two coastal stations from different basins: Askö and Höga Kusten (r = 0.82, p = 0.01), and also between the two stations of the Baltic Proper (Gotland and Askö, r = 0.59, p = 0.02; Table S9). *Mytilus* N % was positively correlated between Gotland and Höga Kusten (*rho* = 0.78, p = 0.02; Table S9, no correlations between regions for *Cladophora* variables).

3.5. Environmental and oceanographic and population data

The biomass of *Cladophora* was on average similar at all regions (A: 7.7±7.6 g m^{-2}, G: 6.6±5.4 g m^{-2}, HK: 5.6±4.7 g m^{-2}; Fig. 4, Table S8), and there was no temporal trend over time nor correlation between regions (Table S8 and S9). *Mytilus* biomass was high at Askö in the coastal Baltic Proper archipelago (453±163 g m^{-2}), about four times lower at Gotland in the open Baltic Proper (129±43 g m^{-2}), and 100 times lower at Höga Kusten in the coastal Bothnian Sea (4±3 g m^{-2}, Fig. 4, Table S8). Further details are presented in supplemental material (Fig. S4, Table S5 and S8).

The station C3 in the Bothnian Sea was characterized by high total organic carbon loadings (TOC_{terr}), low salinity and water temperature, low total nitrogen (N) and phosphorus (P), and low dissolved inorganic phosphorus (DIP) concentrations (Fig. 5, Fig. S5). Generally, the stations B1 and BY31, in the coastal and open Baltic Proper respectively, had higher salinity, total and dissolved N and P concentrations and total phytoplankton biovolumes (total Phyto.) compared to C3 (Fig. 5, Fig. S5). The station B1 was characterized by higher concentrations of dissolved inorganic N (DIN), while BY31 had more dinoflagellates, N_2-fixing cyanobacteria and larger phytoplankton biovolumes (Fig. 5, Fig. S5). Over time (Fig. S5, Table S10), C3 showed a significant decrease in DIN:DIP. Both B1 and BY31 showed a significant increase in total P and significant decrease in temperature shift (T_{shift}), indicating that the rapid temperature rise from 8 to 10-12 ˚C is now occurring earlier in the year in the Baltic Proper. Total N and phytoplankton biomass, especially dinoflagellates, significantly increased at BY31. DIN, DIN:DIP and the ciliate *Mesodinium rubrum* significantly decreased and TOC_{terr} significantly increased at B1. Environmental variables were rarely correlated (Table S11).

3.7. Links between elemental, isotope, population, and environmental data

Generally, elemental and isotope composition of *Cladophora* ($R^2_{adj.}$ = 0.35) and *Mytilus* ($R^2_{adj.}$ = 0.31) were associated in the DistLM analyses with 5 environmental variables, of which three were the same (DIN, salinity, T_{max}, Fig. 6, Table S12). Higher $\delta^{13}C$ values in *Cladophora* were linked to high salinities and maximum water temperatures in summer (T_{max}). For *Mytilus*, high $\delta^{13}C$ was linked to high dinoflagellate biovolumes. Higher $\delta^{15}N$ values were linked to high dissolved inorganic nitrogen (DIN) in both *Cladophora* and *Mytilus*, driven by the high values in samples from the coastal station of Askö archipelago.

When isotope and elemental response variables were tested separately using PLSR across regions (Table 1), the $\delta^{15}N$ models of both taxa met the Lundstedt criteria (predictive capacity $R^2Q > 0.4$; Lundstedt et al. 1998). In line with DistLM models, high values of $\delta^{15}N$ for both taxa were explained by high DIN. High $\delta^{15}N$ in *Cladophora* was generally linked to high nutrient levels (DIP, total N) and to an earlier warming of waters (T_{shift}). Large blooms of N_2-fixing cyanobacteria were linked to low $\delta^{15}N$ of *Mytilus*. The condition index ($CI_{Mytilus}$) was also included as a predictor in the mussel's $\delta^{15}N$ model, with better condition (high $CI_{Mytilus}$) linked to lower $\delta^{15}N$. In turn, *Mytilus* condition was best explained by $\delta^{15}N$ (negative relationship). It was furthermore linked to the Baltic Sea Index, a proxy for global climate change, and to earlier warming of water (T_{shift}), which negatively affected the mussels' condition. Higher TOC_{terr} was associated with higher $CI_{Mytilus}$. High $\delta^{13}C$ for *Cladophora* was linked to high water temperature (annual and summer maximum T_{max}), high phosphorus concentrations (dissolved inorganic), and high total organic carbon (TOC_{terr}), the latter a proxy for low light penetration (Table S2). Low $\delta^{13}C$ in *Mytilus* was linked to high DIN, high TOC_{terr}, a proxy for food quality (i.e., degraded food; Attermeyer et al. 2018), bearing a low $\delta^{13}C$ signal, Table S2), and to higher salinities as well as greater total N and P. The models for elemental content for both taxa generally had low predictive and explanatory performance. High N % in *Cladophora* was linked to high nutrients (DIN, DIP), and to low phytoplankton bloom (more light availability, a proxy for lower competition for nutrients, Table S2). In the mussels, high N % was linked to higher $CI_{Mytilus}$ and lower water temperatures, as well as earlier warming of water. High P % in *Cladophora* was linked to high summer water maximum temperature, low total phytoplankton bloom and low TOC_{terr} loadings (higher light availability). *Mytilus* P % was positively linked to DIN and to proxies for diet quality (diatoms and dinoflagellates).

The simpler linear models testing for stoichiometric relationship between the elemental ratio N:P of the biota and water between different regions did not show any effect for *Mytilus*. However, *Cladophora* N:P was explained by region-specific differences in water nutrient

DIN:DIP ratio (Fig. S6). These results are supported by a local dataset from Askö archipelago, where $N:P_{Cladophora}$ is low in the inner station (Fur) compared to high values for the outer archipelago (Iss, significantly higher than the inland stations), with this pattern also observed for the DIN:DIP in the water between these two locations (Fig. S7). Regarding population models; the *Mytilus* biomass model met the Lundstedt criteria (predictive capacity $R^2Q > 0.4$) and had high explanatory power ($R^2Y \geq 50$ %; Table 1). High *Mytilus* biomass was linked to high $\delta^{15}N$, high nutrient concentrations (DIN, DIP, total N) and to low TOC_{terr}.

4. Discussion

4.1. Spatial $\delta^{13}C$ pattern differs among taxa

The $\delta^{13}C$ values for *Cladophora* followed a latitudinal pattern, with most depleted values in the north, in support of expectation 1. This pattern was mainly explained by physical variables: the low $\delta^{13}C$ in the algae from Höga Kusten, compared with algae from the Baltic Proper, was linked to low water temperature and terrestrial loading, which was our proxy for light penetration but might also indicate dissolved inorganic carbon with a depleted endmember ^{13}C value. Light and temperature are known to drive growth and C uptake in macrophytes, thereby influencing their $\delta^{13}C$ values. Enriched ^{13}C (less negative $\delta^{13}C$) is associated with enhanced photosynthetic activity in warm areas like the tropics, experiencing high irradiance, compared to temperate and polar regions (Wiencke and Fischer 1990; Hemminga and Mateo 1996; Stepien 2015). *Cladophora* $\delta^{13}C$ was further linked to phosphorus levels in the water (DIP), which likely influence its growth condition and hence $\delta^{13}C$. In limnic systems, P is the limiting factor for *Cladophora* growth (Howell 2018 and references therein) but, to our knowledge, no studies have tested whether N or P limit *Cladophora* growth in the Baltic Sea.

The $\delta^{13}C$ of *Mytilus* showed no latitudinal pattern, but instead differed between coastal areas and open sea, in contrast to expectation 1. Similarly depleted ^{13}C values were observed for the two coastal stations (Askö archipelago and Höga Kusten), despite them being situated in different basins. In the statistical models, low $\delta^{13}C$ was linked to higher eutrophication (dissolved inorganic nitrogen: DIN) and total organic carbon loadings from land (TOC_{terr}), and to lower salinity. Terrestrial or organic particles from resuspended old sediment, both bearing a low $\delta^{13}C$ signal (Rolff and Elmgren 2000; Voss et al. 2000), have a higher contribution to the pool of suspended particulate organic material in shallow areas (i.e., close to the coast) or at the vicinity of river mouths (Liénart et al. 2017). This signal can be mirrored in the tissues of

552 filter-feeders (Lefebvre et al. 2009; Pernet et al. 2012; Briant et al. 2018). In a recent study
553 comparing the isotope signal of benthic and pelagic food web components in different sub-
554 basins of the Baltic Sea, Kiljunen et al. (2020) found a progressive north to south ^{13}C
555 enrichment pattern in both benthic and pelagic baselines, supposedly influenced by the amount
556 of allochthonous organic material from freshwater inflows. However, it is less likely that the
557 low $\delta^{13}C$ signal of *Mytilus* from the coastal station of Askö archipelago, nearly identically to
558 Höga Kusten, can be explained by temporally similar TOC_{terr} inputs, since total organic carbon
559 loadings are 10-fold lower in the archipelago area compared to Höga Kusten. Other processes
560 are likely involved, and the significant correlation over time between $\delta^{13}C$ in mussels from
561 Askö archipelago and Gotland suggests a global driver influencing the pelagic ecosystem, such
562 as the Suess effect (Gruber et al. 1999; Quay et al 2007; Gustafsson et al. 2015, see below).
563 Despite correlation over time, higher $\delta^{13}C$ for *Mytilus* from the open sea region Gotland reflects
564 a more marine signal, less influenced by terrestrial runoff (Rolff and Elmgren 2000). An
565 explanation for the higher $\delta^{13}C$ in *Mytilus* from Gotland could be greater eutrophication of the
566 open Baltic Proper, since ^{13}C enriched values in mussels may be the result of high plankton
567 biomass (Oczkowski et al. 2018). However, since phytoplankton biomass was never selected
568 as an important predictor in our models, this explanation is less likely. In addition, P content
569 was also lowest in Gotland mussels (and algae from this region)).

570

571 **4.2. Common $\delta^{13}C$ temporal trends may reflect global environmental changes**

572 Over time, our longest dataset (1993-2016) from Askö archipelago in the coastal Baltic Proper
573 revealed a significant decrease in *Mytilus* $\delta^{13}C$, as reported in Lienart et al. (2020). A similar
574 decrease in *Mytilus* $\delta^{13}C$ was also demonstrated from the same time period in Kvädöfjärden, a
575 coastal station 100 km south of Askö archipelago (Ek et al. 2021). However, the Kvädöfjärden
576 time series was longer (starting in 1981), and there was no evident decrease when considering
577 the entire series of nearly 4 decades. Still, the nearly identical temporal variation (and absolute
578 values) in $\delta^{13}C$ for mussels from Askö archipelago and Höga Kusten, and the positive
579 correlation over time in $\delta^{13}C$ for *Mytilus* from Kvädöfjärden, Askö archipelago and Gotland,
580 suggest a large-scale effect on $\delta^{13}C$ values in recent decades (in contrast to our more local
581 expectation 2). Furthermore, even though not significant ($p < 0.08$ in both cases), the results
582 suggest a positive correlation trend between mussels and algae $\delta^{13}C$ at both Gotland and Askö
583 archipelago. This large-scale similarity in $\delta^{13}C$ over time may hence reflect common global or

584 at least northern hemisphere changes not included in our statistical approach. The worldwide
585 decrease in $\delta^{13}C$ of atmospheric CO_2 as a result of global anthropogenic activities (e.g., fossil
586 fuels use and deforestation; referred to as the Suess effect; Gruber et al. 1999; Quay et al. 2007
587 and references therein), has significantly affected marine biota over recent decades (Schloesser
588 et al. 2009). This global process is reflected in ultimate source of carbon in the Baltic Sea
589 (Gustafsson et al. 2015) and could underlie the more recent (since 1993 and onwards) decrease
590 in the $\delta^{13}C$ of phytoplankton mirrored in our mussels. We found a significant 1‰ depletion in
591 $\delta^{13}C$ values of the mussels over the 24 year time period (see calculations in suppl. material),
592 which is consistent with the decreases reported for other marine organisms (Druffel and
593 Benavides 1986; Bauch et al. 2000; Schloesser et al. 2009). Similar multi-decadal decreasing
594 trends for $\delta^{13}C$ have been observed in bivalves (including *Mytilus*) from the English Channel
595 and the Mediterranean Sea (Briant et al. 2018). A general decrease since the early 90s has also
596 been demonstrated from various organisms and for different trophic levels in coastal and
597 pelagic food webs of the in the North Sea and Baltic Sea, including Herring gull (Corman et
598 al. 2018) and Atlantic salmon (Torniainen et al. 2014).

599

600 **4.3. $\delta^{15}N$ pattern is region-specific for both taxa**

601 The high and similar $\delta^{15}N$ values of *Cladophora* and *Mytilus* from the coastal Baltic Proper
602 (Askö archipelago) were both linked to the high DIN concentration in this region. Savage and
603 Elmgren (2004) described high $\delta^{15}N$ values in *Fucus vesiculosus* close to a sewage outfall north
604 of Askö archipelago but reported that values were back to background level ($\delta^{15}N$ of 4‰)
605 towards the Askö island, 24 km away from the sewage outlet. The $\delta^{15}N$ of *Cladophora* in our
606 study, however, remained high around Askö island (ca. 6.5‰). This could be explained by the
607 higher nutrient uptake and growth rate of the seasonal filamentous algae, with a high
608 surface:volume ratio (Snoeijs-Leijonmalm 2017) compared to the slow growing perennial
609 *Fucus* spp. (Wallentinus 1984), which makes *Cladophora* a more effective N-sink. The
610 significant decrease in $\delta^{15}N$ of both taxa in the Askö archipelago over time is statistically linked
611 to the significant decrease in DIN concentration in this area, probably reflecting the general
612 decrease in land-based nutrient loadings over recent decades as a result of eutrophication
613 mitigation (Savage et al. 2010; Elmgren et al. 2015). An alternative explanation is that it mirrors
614 changes in the internal denitrification potential from increased hypoxic bottom waters since the
615 90s resulting in less DIN (Carstensen et al. 2014). Natural features such as coastal upwellings

of bottom water, which are frequent on the Swedish east coast of the Baltic Proper (Lehmann et al. 2012), bear an enriched ^{15}N signal that can contribute to high $\delta^{15}N$ values in macrophytes (Marconi et al. 2011; Viana and Bode 2013). Bivalves including *Mytilus* can also capture the high $\delta^{15}N$ signal from eutrophication (Fukumori et al. 2008; Carmichael et al. 2012; Thibault et al. 2020; Liénart et al. 2020) or from upwellings indirectly via plankton ingestion, as observed for invertebrates in other systems (e.g., Hill and McQuaid 2008).

In the Askö archipelago, both bulk $\delta^{15}N$ and $\delta^{15}N$ of phenylalanine ($\delta^{15}N$-Phe) for *Cladophora* where generally high and correlated, supporting the assimilation of ^{15}N enriched nutrients in this area. For *Mytilus*, there was also a positive correlation between $\delta^{15}N$-bulk and $\delta^{15}N$-Phe, but $\delta^{15}N$-Phe values were much lower than for *Cladophora*, demonstrating differences in utilization of ultimate N sources, with negative values indicating diazotrophic N (Rolff 2000; Eglite et al. 2018). Alternatively, this may indicate different turnover time of N sources between these taxa. Indeed, low $\delta^{15}N$ in *Mytilus* was explained by high amounts of N_2-fixing cyanobacteria and also linked to the mussel's condition index, indicating the role of physiology on bulk $\delta^{15}N$ variability in the mussels, with lower $\delta^{15}N$ indicative of better condition (Liénart et al. 2020). For *Mytilus*, slow N turnover (Smaal and Vonck 1997), and hence likely large ^{15}N fractionation, may confound this signal in bulk $\delta^{15}N$ values. In addition, the low salinity (expectation 4) of the Baltic Sea reduces the scope for growth and affects respiration rates of the mussels (Tedengren and Kautsly 1986), thus influencing N requirements and possibly confounding $\delta^{15}N$ signal.

Cyanobacterial N supports zooplankton and deposit-feeders during summer in the Baltic Proper (Karlson et al. 2015), but there is currently no published study reporting active feeding of *Mytilus* on cyanobacteria. However, a recent experimental study suggests it is a relevant food source for the mussels during summer (Liénart et al. in prep). At the open sea station of the Baltic Proper, cyanobacterial N fixation is higher than in the coast (Olofsson et al. 2020). *Mytilus* bulk $\delta^{15}N$ in this region was accordingly lower than in the Askö archipelago, better resembling the low $\delta^{15}N$-Phe in the mussels, and also lower in algae, possibly indirectly utilizing leaked N of cyanobacterial origin. Negative $\delta^{15}N$-Phe for particulate organic matter in surface water of Gotland has been confirmed previously (-1.7 to -6‰, Eglite et al. 2018). The large year-to-year variability in *Cladophora* bulk $\delta^{15}N$, and possibly in $\delta^{15}N$-Phe, could also reflect the occasional influence of local upwellings at the southern tip of Gotland (Lehmann et al. 2012) bringing up more enriched ^{15}N waters.

Finally, in the Bothnian Sea Höga Kusten, the low $\delta^{15}N$ of *Cladophora* is likely related to the low $\delta^{15}N$ signal of NO_3 from pristine Nordic rivers ($\delta^{15}N$-NO_3 of 0.6±1.1‰, $\delta^{15}N$-PON of 2.9±2.1‰; Voss et al. 2005). Similarly, Kiljunen et al. (2020) show increasing $\delta^{15}N$ values from north to south of both pelagic and benthic baselines in the Baltic Sea, likely related to eutrophication patterns. However, increased cyanobacteria blooms in the coastal zone of the Bothnian Sea over the past decade (Andersson et al. 2015b; Olofsson et al. 2020) could additionally contribute to the low bulk $\delta^{15}N$ and negative $\delta^{15}N$-Phe observed in both taxa here, especially in *Mytilus*.

4.4. Different nutrient turnover rates in taxa reflect system nutrient dynamics

There were no significant differences in *Cladophora* and *Mytilus* N % or N:P across regions, with only P % significantly lower for both taxa in Gotland compared to the other regions (Table S5). This is surprising since the overall Baltic Sea is classified as a 'problem area', with eutrophication status rated as mostly poor for the Central Baltic/Baltic Proper compared with moderate to poor for the Bothnian sea according to the HELCOM Eutrophication Assessment Tool. However, the N:P ratio of *Cladophora* was statistically linked to the water N:P ratio at a regional scale (simple linear model, Fig. S6), and this was supported at a local scale within the Askö archipelago (inner versus outer stations, Fig. S7), in accordance with expectation 3. In our PLSR models, elemental composition for *Cladophora* was mainly linked to nutrient levels and, for P %, to summer maximum water temperature and phytoplankton bloom intensity, with a larger bloom driving higher competition for nutrients and resulting in less light available. Nutrient concentrations in the water were generally higher for the Baltic Proper stations, and the DIN:DIP ratio was close to Redfield values in the Bothnian Sea (ca. 13), but N limited in the Baltic Proper (ca. 6-7, Fig. S5). The high nutrient absorption rates of *Cladophora* to sustain rapid growth during summer (Wallentinus 1984) can thus explain the correlation between the algal N:P ratio and nutrient levels in the water.

N:P ratio in *Mytilus* did not differ among regions, despite differences in nutrients and salinity (expectation 3 and 4), reflecting the relatively higher degree of homeostatic control consumers have over their elemental ratios. *Mytilus* has a low N turnover (Smaal and Vonck 1997), which likely explains the general absence of significant temporal trends and spatial pattern in elemental content of the mussels. A high N % in the mussels, used here as a proxy for protein content (Sterner and Elser 2002), was linked to higher condition index and lower temperature,

with negative effects of higher temperatures on protein content, as expected (see Liénart et al. 2020 and references therein). Resembling the $\delta^{15}N$ model, this highlights the importance of physiological processes for interpreting consumers' N elemental and isotope values. Nonetheless, *Mytilus* P %, used here as a proxy for growth (Elser et al. 2003), was significantly lower at the open sea station (Gotland), despite the potential for highest P exposure here. Still, P content was best explained by eutrophication and diet related predictors (dinoflagellates, diatoms). This emphasizes the importance of food quality for the growth of the mussels (e.g., Bracken 2017), with implications for their role as an ecosystem P sink.

4.5. From nutrients to populations: understanding population level effects

Our data showed a rather similar biomass per m^2 of *Cladophora* across the Baltic Sea, even though a strong year to year variability was observed within regions. There are no recent studies reporting *Cladophora* distribution, biomass or chemical composition in the Baltic Sea. Although *Cladophora* has been reported as increasing in the Baltic since the 1980s (Kraufvelin and Salovius 2004), our data did not show any significant temporal trends in *Cladophora* over the past two decades. That *Cladophora* thrive in shallow areas has been mainly discussed in relation to the eutrophication-induced decline of perennial brown macroalgae *Fucus* (Kautsky et al. 1986; Torn et al. 2006). The two macrophytes compete for space, light and nutrients (Kautsky et al. 1986), which could explain the rather high biomass of *Cladophora* in the Bothnian Sea where *Fucus* is absent due to low salinity. In our analyses, the biomass of *Cladophora* was linked to large-scale environmental conditions (i.e., Baltic Sea Index), and stable isotopes were also included as main predictors, with high $\delta^{15}N$ reflecting eutrophication (positive correlation between $\delta^{15}N$ and DIN in the model, Table 1). However, the biomass model had low explanatory and predictive capacities, and should be interpreted with caution. Adding predictors related to top-down control, such as grazing, and biomass of competitors for nutrients, like *Fucus*, and ice cover and duration would likely improve the model.

The biomass and abundance of *Mytilus* populations were low in the Bothnian Sea, as expected due to the low salinity of this area (i.e., affect growth, Kautsky et al. 1990; Westerbom et al. 2002). High mussel biomass was positively linked to higher nutrient concentration (DIN, DIP), with both decreasing over time as a result of eutrophication mitigation, although no corresponding decrease in total phytoplankton biovolume has been shown for this time period (opposite trend apparent at all regions, see Fig. S5 and Ek et al. 2021). However, compositional

changes of phytoplankton have occurred (Fig. S5; Hjerne et al. 2019), which influence diet quality and hence the growth or survival of mussels as discussed by Liénart et al. (2020). For instance, the decrease in average mussel size (Bm:$Ab_{Mytilus}$) in the Baltic Proper was linked to decrease in DIN but also to increase in N_2-fixing cyanobacteria blooms, a food source for *Mytilus* whose quality is still debated. Finally, high biomass and large average mussel size were also linked to high $\delta^{15}N$, although $\delta^{15}N$ was simultaneously linked to a lower condition index in individual mussels, which is contradictory. In general, the condition index was highest for the mussels at Höga Kusten. This is surprising considering the low salinity of the Baltic Proper, which is already at the *Mytilus* distribution area salinity limit of 4 (Snoeijs-Leijonmalm et al. 2017). One ecological explanation could be related to spawning, which is characterised by dramatic weight loss during early summer (up to 50%, Kautsky 1982). It is possible that spawning may not occur for mussels living at the salinity margin at Höga Kusten, hence resulting in a high condition index contrary to Askö archipelago mussels, which are likely now undergoing two spawning events per year (Westerbom pers. com.). Experimental studies testing the effects of the predictors discussed here on *Mytilus* individual growth and condition are hence needed to provide a mechanistic understanding for observed population biomass declines.

5. Conclusion

Our study documents large-scale and long-term patterns in elemental and isotope composition in the ephemeral filamentous macroalgae *Cladophora* spp. and the suspension-feeding blue mussel *Mytilus* sp., two key-taxa from the Baltic Sea. We statistically link the observed patterns to environmental, oceanographical and terrestrial monitoring data. The $\delta^{15}N$ of both taxa responded to regional and local drivers (mainly water nutrient concentrations), yet $\delta^{15}N$ in the source amino acid Phenylalanine ($\delta^{15}N$-Phe) revealed clear differences between algae and mussels regarding ultimate N source. Diazotrophic N signal was detected in *Mytilus*, which was masked in bulk $\delta^{15}N$. Clear differences in carbon isotope baselines between the two major Baltic Sea basins were found: the $\delta^{13}C$ followed a latitudinal gradient for the algae, while it instead differed between the coastal and open sea environment for mussels. Nonetheless, mussels from the different regions had similar temporal development in $\delta^{13}C$, suggesting a global driver influencing $\delta^{13}C$ dynamics in the pelagic ecosystem. Elemental composition only differed among regions for P %, which was lower at the open sea station for both taxa. *Cladophora* N:P reflected regional nutrient levels in the water, while *Mytilus* P % was linked to food quality. This study highlights the use of a multi-taxa and combined elemental and

isotope approach to quantify the effects of eutrophication and climate-related environmental changes in food webs and ecosystem functioning.

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Figures and table

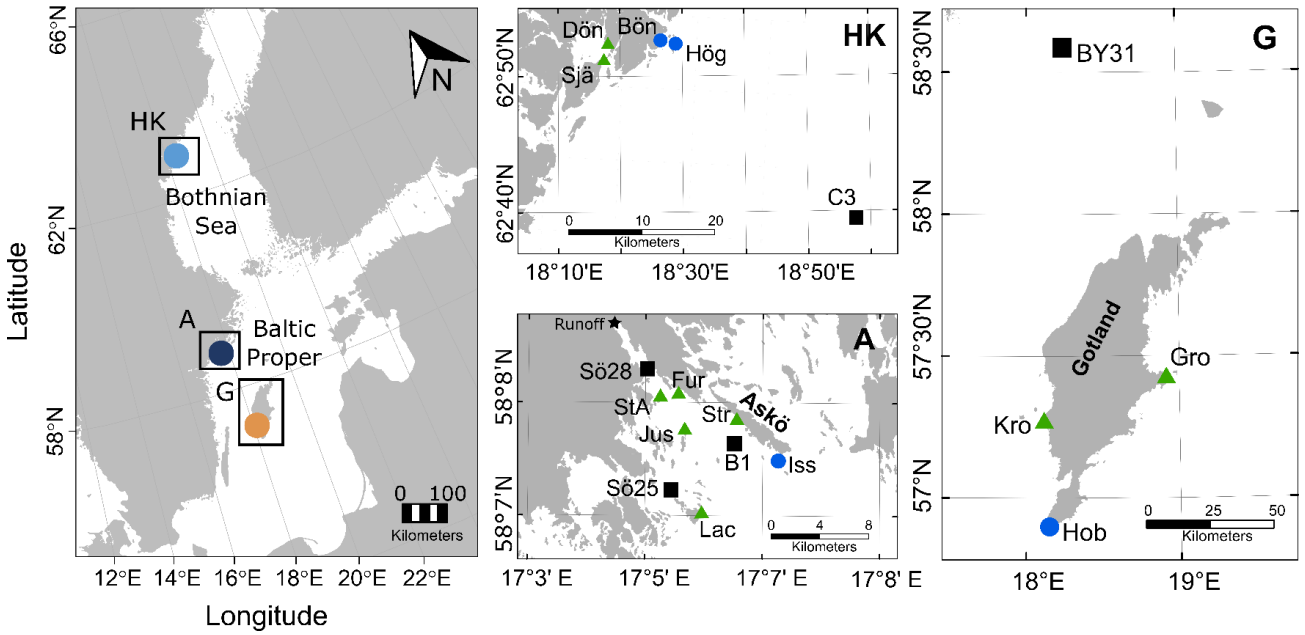

Figure 1: Study regions of the Bothnian Sea (HK: Höga Kusten) and the Baltic Proper (A: Archipelago close to Askö field station, and G: Gotland island). Stations within each region are shown in the three right panels: green triangles are *Cladophora* sampling stations only, blue circles are *Mytilus* and *Cladophora* sampling stations, black squares are pelagic environmental monitoring stations.

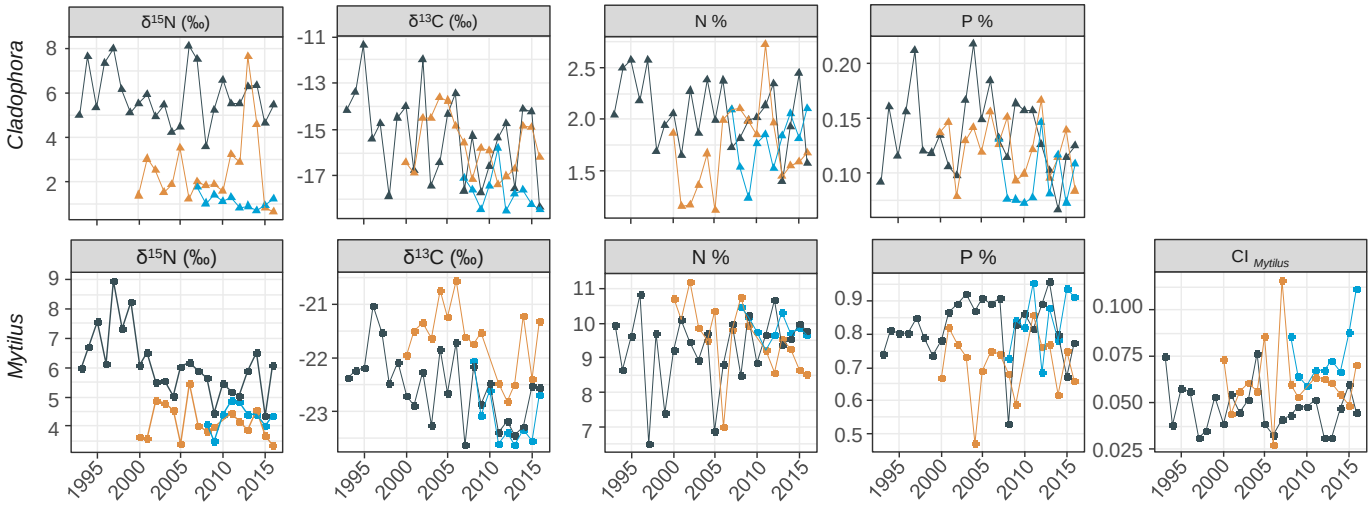

Figure 2: Temporal trends in elemental and isotope composition of *Cladophora* (upper panels, triangles) and *Mytilus* (lower panels, circles) from the 3 regions (Archipelago: black, Gotland: orange, Höga Kusten: blue). For a multivariate summary of region-specific pattern see Fig S2.

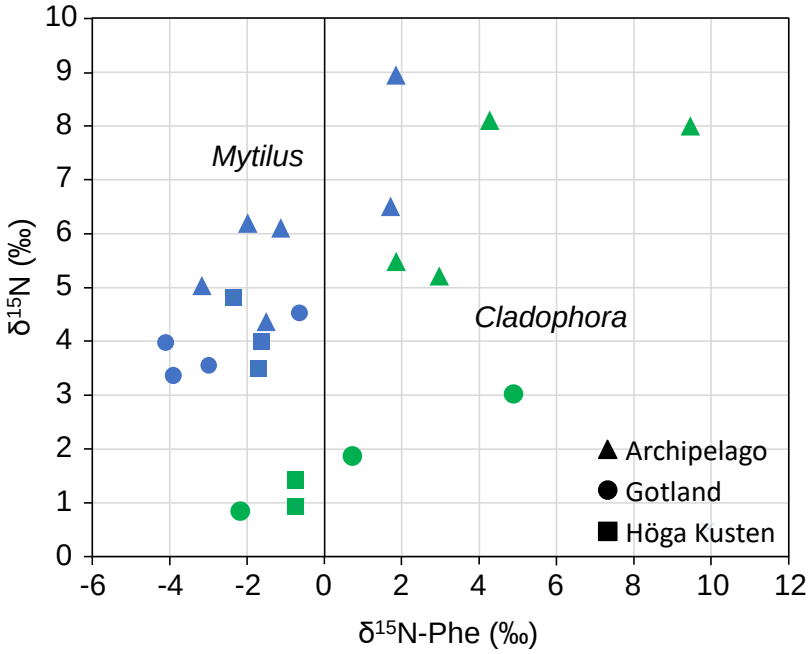

Figure 3: Nitrogen isotope ratio of the source amino-acid phenylalanine (δ^{15}N-Phe) for *Mytilus* (blue) and *Cladophora* (green) of the 3 regions.

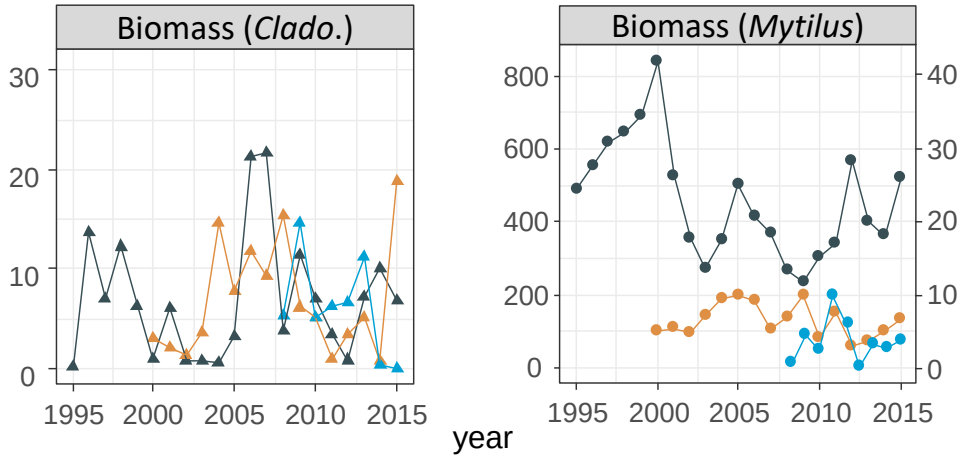

Figure 4: Temporal trends in *Cladophora* (triangles) and *Mytilus* (circles) population biomass (g dry weight per m^2) from the 3 regions (Archipelago: black, Gotland: orange, Höga Kusten:

blue). For *Mytilus* biomass, the left axis indicates Askö archipelago and Gotland and the axis on the right indicates Höga Kusten.

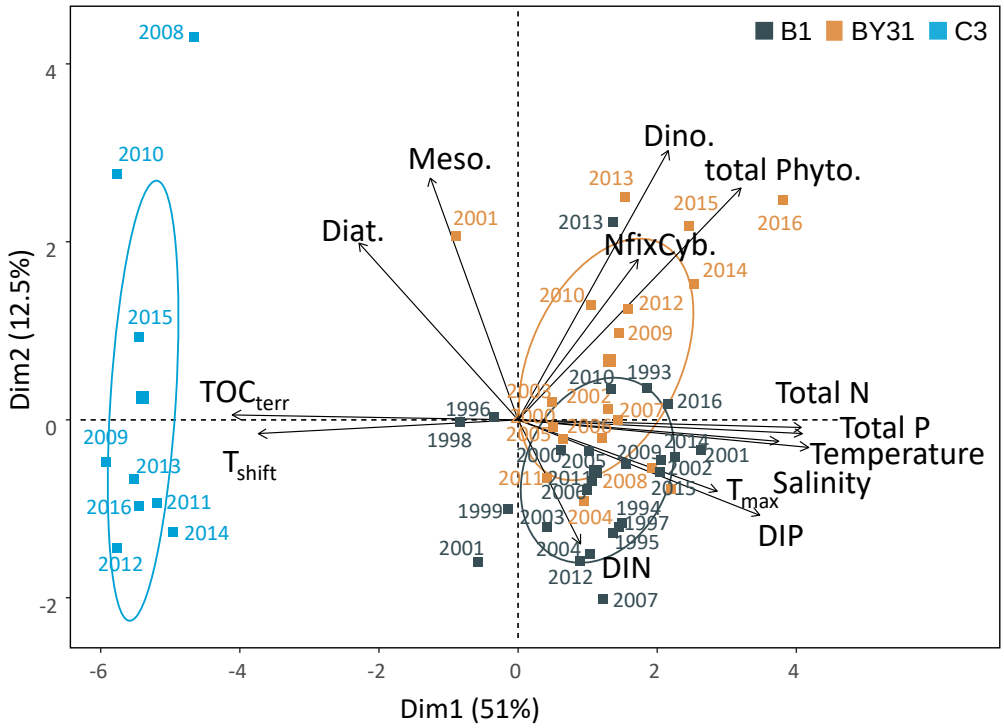

Figure 5: Principal component analysis (PCA) of environmental variables for B1 (black), BY31 (orange) and C3 (blue). Scaled data. Ellipses represent 50% of the data. See Fig. S5 for the raw dataset over time. T_{max}: water maximum temperature in summer, DIN or DIP: Dissolved Inorganic Nitrogen / Phosphorus, TOC_{terr}: total organic carbon from land, T_{shift}: water temperature shift, Diat.: diatom, Dino.: dinoflagellates, Meso.: *Mesodinium rubrum*, NfixCyb.: N_2-fixing cyanobacteria.

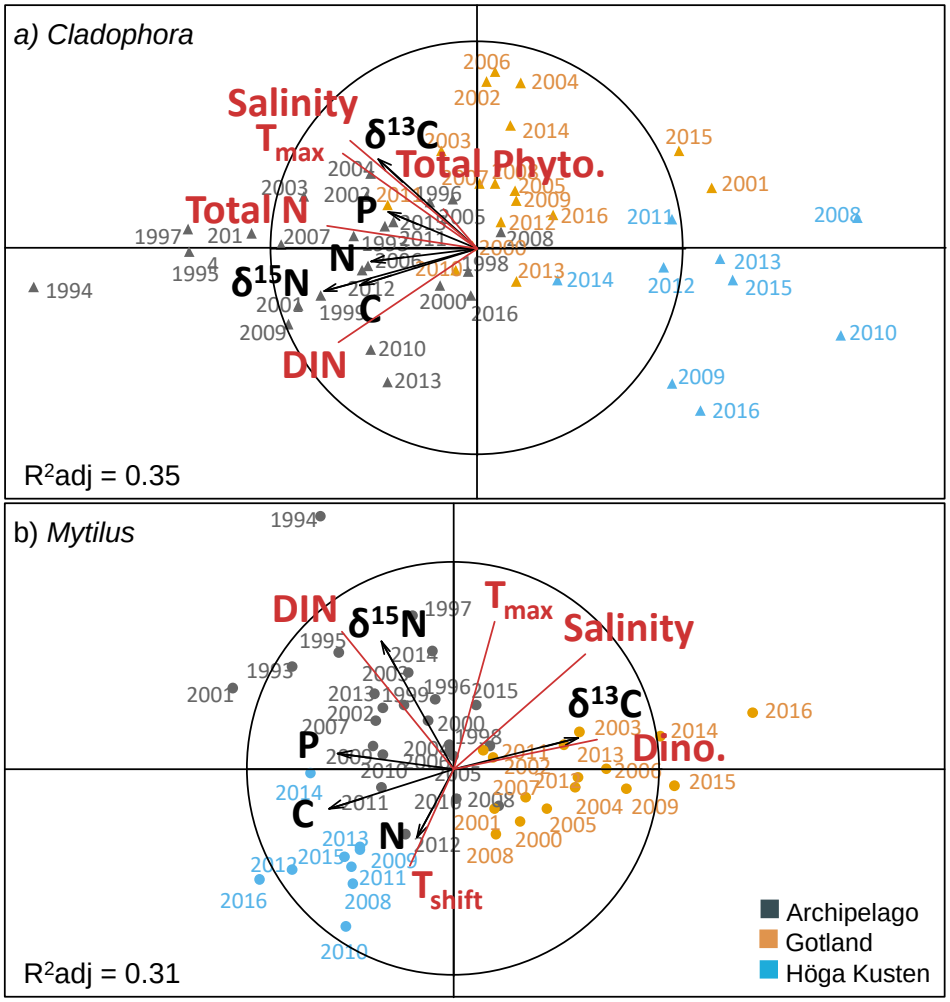

Figure 6: Redundancy analysis of a) *Cladophora* and b) *Mytilus* elemental and isotope data (black arrows) for the 3 studied regions (Archipelago: black, Gotland: orange, Höga Kusten: blue) constrained by environmental variables (red vectors) selected from the DistLM models based on Akaike information criterion. DIN: dissolved inorganic nitrogen, total Phyto.: total phytoplankton bloom, T_{max}: water maximum temperature in summer, Dino.: dinoflagellates, T_{shift}: water temperature shift. See Table S12 for model selection.

Table 1: Results of the PLSR models for the 6 *Cladophora* and 8 *Mytilus* response variables tested (in white: individual level, in dark grey: population level). R^2Y is the model explanatory capacity, R^2Q is the model predictive capacity, R^2X is the explained variance. Models with high prediction capacity according to Lundstedt evaluation criteria (Lundstedt et al. 1998; $R^2Y > 0.6$ and $R^2Q > 0.4$) are in bold. Shaded grey cells are predictors with negative influence. The '-' sign means no additional predictors. Predictors are ranked by importance based on absolute value of regression coefficient (in italics). $CI_{Mytilus}$: condition index, Abund.: abundance, Temp.: water temperature, T_{max}: maximum summer water temperature, T_{shift}: water temperature shift, DIP and DIN: dissolved inorganic nitrogen or phosphorus, TOC_{terr}: total organic carbon loadings, Tot. Phyto.: total phytoplankton biomass, NfixCyb.: N_2-fixing cyanobacteria, Diat.: diatoms, Dino.: dinoflagellates, BSI: Baltic Sea Index.

Response variable		Model evaluation parameters			Predictors (reg. coefficients)						
		R^2Y	R^2Q	R^2X	1	2	3	4	5	6	7
$\delta^{13}C$	*Cladophora*	0.4	0.3	0.8	T_{max} *0.92*	Temp. *0.32*	DIP *0.14*	Total P *-0.09*	Salinity *-0.08*	TOC_{terr} *0.06*	Total N *-0.05*
$\delta^{15}N$	*Cladophora*	**0.6**	**0.5**	**0.9**	**DIN** ***0.92***	**DIP** ***0.29***	**T_{shift}** ***-0.27***	**Total N** ***0.24***	**Total P** ***-0.07***	**Salinity** ***0.04***	-
N %	*Cladophora*	0.3	0.2	0.8	DIN *0.12*	DIP *0.08*	Tot. Phyto *-0.05*	Total N *0.02*	-	-	-
P %	*Cladophora*	0.3	0.2	0.8	Tot. Phyto *-0.01*	T_{max} *0.007*	Salinity *0.004*	TOC_{terr} *-0.004*	-	-	-
Biomass	*Cladophora*	0.2	0.0	0.7	$\delta^{15}N$ *1.26*	BSI *-1.25*	$\delta^{13}C$ *-0.93*	Total N *0.77*	DIP *0.61*	-	-
$\delta^{13}C$	*Mytilus*	0.4	0.3	0.9	DIN *-0.31*	Total P *0.11*	Salinity *0.10*	TOC_{terr} *-0.08*	Total N *0.02*	-	-
$\delta^{15}N$	*Mytilus*	**0.6**	**0.5**	**0.8**	**DIN** ***0.59***	**$CI_{Mytilus}$** ***-0.49***	**NfixCyb.** ***-0.28***	-	-	-	-
N %	*Mytilus*	0.2	0.1	0.8	$CI_{Mytilus}$ *0.35*	T_{shift} *-0.18*	T_{max} *-0.08*	Total N *0.01*	-	-	-
P %	*Mytilus*	0.3	0.2	0.7	DIN *0.025*	Dino. *-0.017*	Total P *-0.016*	Diat. *0.014*	Salinity *-0.013*	-	-
$CI_{Mytilus}$	*Mytilus*	0.4	0.3	0.7	$\delta^{15}N$ *-0.005*	BSI *0.004*	Total N *-0.003*	T_{shift} *0.002*	TOC_{terr} *0.002*	Salinity *-0.001*	-
Biomass	*Mytilus*	**0.7**	**0.7**	**0.8**	**$\delta^{15}N$** ***75***	**DIN** ***68***	**DIP** ***41***	**Total N** ***29***	**TOC_{terr}** ***-24***	-	-
Abund.	*Mytilus*	**0.6**	**0.5**	**0.8**	**DIN** ***-2638***	**$\delta^{13}C$** ***2635***	**Total P** ***2320***	**Salinity** ***2238***	**TOC_{terr}** ***-1827***	-	-
Bm:Ab	*Mytilus*	**0.6**	**0.5**	**0.8**	**$\delta^{15}N$** ***0.007***	**NfixCyb.** ***-0.007***	**DIN** ***0.005***	-	-	-	-