

Long-term changes in trophic ecology of blue mussels in a rapidly changing ecosystem

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Abstract

Ocean climate change strongly affects organisms and ecosystems, and the causes, consequences, and underlying mechanisms need to be documented. In the Baltic Sea, a marginal sea under severe eutrophication stress, a longer productive season, and changes in the phytoplankton community over the last few decades have likely impacted diet and condition of keystone species, from individual to population level. This study uses stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and derived isotope niche metrics) to trace energy and nutrient flows in archived samples of blue mussel (*Mytilus edulis trossulus*) spanning 24 yr (1993–2016). We test if long-term changes in isotope and elemental composition in mussels, as well as population abundance and biomass, can be explained by changes in abiotic and biotic variables, using partial least square regressions and structural equation modeling. We found decreasing trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as well as in mean size and total biomass of mussels, but no unidirectional changes in their stoichiometry or condition index. Changes in isotope composition were best explained by nitrogen-fixing cyanobacteria, by increased terrestrial organic carbon from land runoff (reflecting precipitation) and by decreases in dissolved inorganic nitrogen (indicative of successful eutrophication mitigation) and in biomass of a mixotrophic ciliate species. The trophic niche (assessed from isotope niche) was included as the best predictor for both mussel body condition and the observed decline in their total biomass. This study reveals that altered trophic relationships from climate-induced changes in the productivity base may strongly impact keystone species, with potential knock-on effects on ecosystem functions.

Coastal oceans experience effects of climate change and intensified anthropogenic activities, but consequences for the ecosystems and the underlying mechanisms are largely unknown (Cloern et al. 2016). The Baltic Sea, the world's largest body of brackish water, has for decades been under multiple interacting stressors (e.g., hazardous substances, eutrophication, overfishing leading to trophic cascades, climate change; Elmgren et al. 2015), many of which are expected to increase. Eutrophication has already caused an increase in primary production while the general warming of surface waters during the last decades (Belkin 2009) has resulted in a longer productive season (Kahru et al. 2016). In particular, there has been a shift in maximum

production from spring to the summer cyanobacterial blooms (Kahru et al. 2016). Also, climate models predict increased precipitation in the north, which would lower surface salinity and increase organic matter inputs of terrestrial origin (Asmala et al. 2019).

The Baltic Sea region is one of the best studied coastal areas with long-term data sets, making it an ideal case study when linking environmental variables to ecological effects (Reusch et al. 2018). Measuring carbon (C) and nitrogen (N) stable isotope composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) in archived biological samples from monitoring programs enables the tracing of nutrient flows and the quantification of temporal changes in food web structure (Glibert et al. 2018 and references therein). These tools are commonly used to reconstruct organisms' diet: $\delta^{13}\text{C}$ indicating ultimate carbon source and $\delta^{15}\text{N}$ trophic position (Fry and Sherr 1984). Stable isotopes can also be used to quantify subtle changes in population-level trophic structure (Layman et al. 2007; Jackson et al. 2011), where the “ δ -space” area, or isotopic niche (i.e., distance between individuals in a $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplot), provides insight on the resource type and species habitat (sensu Hutchinson niche concept,

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e.g., Newsome et al. 2007). Elemental composition of the main building blocks C, N and phosphorus (P) can be used as indicators of condition and growth status of organisms, as it reflects the balance between the organisms requirements and elements availability (i.e., ecological stoichiometry, Elser et al. 2003). Although consumers have a high degree of homeostatic control of N and P, stoichiometric relationships have been linked to diet quality (e.g., Bracken 2017). Dry samples without fixatives are optimal for stable isotope and elemental measurements (Correa 2012), making specimens from archived collections suitable for analysis (Sarakinis et al. 2002). However, organism isotope and elemental composition depend on physiological status, and isotopes must therefore be interpreted with caution as simple diet tracers (Wolf et al. 2009 and references therein). Starvation, for instance, generally increases $\delta^{15}\text{N}$ values, but can either increase or decrease $\delta^{13}\text{C}$ (Doi et al. 2017).

Sessile long-lived bivalves provide a time-integrated measure of the highly dynamic pelagic environment and are routinely used as an isotopic baseline for aquatic food web studies (e.g., Fukumori et al. 2008). Blue mussels (*Mytilus* spp.) are common in temperate marine coastal ecosystems, making them suitable for comparative studies and as a bioindicator of environmental status (e.g., Kanduč et al. 2018). In the Baltic Sea, the ubiquitous species complex *Mytilus edulis trossulus* (one single population with mixed gene pool from both *M. edulis* and *M. trossulus*; Snoeijs-Leijonmalm et al. 2017 and references therein, hereafter referred to as “*Mytilus*” or “blue mussel”) is a keystone species that structures benthic communities (Koivisto and Westerborn 2010) and drives ecosystem functioning, including nutrient cycling and benthic-pelagic coupling (Kautsky and Wallentinus 1980; Kautsky and Evans 1987). Changes in quantity or quality of the pelagic organic matter pool will influence the growth, body condition and tissue chemistry of these suspension feeders (Bracken 2017), and are expected to modify *Mytilus* tissue's isotope composition. Among potential food sources, N_2 -fixing cyanobacteria exhibit a characteristic depleted ^{15}N signature of ca. -2‰ (Rolff 2000), which is mirrored in consumers throughout the food web (Karlson et al. 2015, although blue mussels were not measured). Some other plankton groups have distinct isotope signatures (e.g., ciliates with enriched ^{15}N and depleted ^{13}C values, Brutemark and Granéli 2011) that could also be reflected in consumers. Particulate organic matter of terrestrial origin has a lower $\delta^{13}\text{C}$ signal (ca. -27‰) and can contribute to a large part of the suspended particle pool in the Baltic Sea (Rolff and Elmgren 2000). Also, N from sewage or manure leads to higher $\delta^{15}\text{N}$ signal in primary producers (e.g., Savage and Elmgren 2004), which could indirectly influence consumer $\delta^{15}\text{N}$.

Baltic Sea *Mytilus* live at the margin of their distribution range in terms of salinity (Westerborn et al. 2002). Moreover, climate change may affect their physiology; increasing water temperature during both winter and summer (Hiebenthal

et al. 2013; Waldeck and Larsson 2013) and decreasing salinity (Tedengren and Kautsky 1986) are likely to reduce growth rates and, ultimately, population size. Increasing temperature and chlorophyll *a* concentrations (a proxy for food quantity) and decreasing salinity have been linked to reductions in *Mytilus* populations in the south-west Baltic (Franz et al. 2019) and in the Gulf of Finland (Westerborn et al. 2019). Also, a general multidecadal decrease in *Mytilus* spp. elemental and/or isotope composition from the southern North Sea, Baltic Sea (Corman et al. 2018), and other European seas (Briant et al. 2018) has been linked to climate change. However, the mechanisms behind such decreases remain hypothetical and no study statistically link decadal environmental change(s) with isotope composition, including population data on trophic niche, or stoichiometric relationships in *Mytilus*, or any other species.

Based on the possible effects of environmental changes on *Mytilus*, we evaluate the following four hypotheses (see Table S1 for details) using time-series data. First, that an increase in N_2 -fixing cyanobacterial blooms should reduce mussel $\delta^{15}\text{N}$ values. Second, that an increase in organic material from land runoff should reduce mussel $\delta^{13}\text{C}$. Third, that increases in terrestrial organic material and cyanobacteria—two supposedly low-quality food sources (e.g., Müller-Navarra et al. 2000, but see Karlson et al. 2015)—should widen mussel trophic (isotope) niche and deteriorate mussel body condition. Fourth, that higher temperatures and lower salinity should be negatively linked to mussel abundance and population biomass, by increasing respiration and osmotic stress, respectively (see e.g., Tedengren and Kautsky 1986).

To test these hypotheses, we correlate *Mytilus* soft tissue stable isotope and elemental chemistry, as well as population biomass and abundance, to environmental changes over the last two decades, using a uniquely detailed and high-frequency monitoring data set of abiotic and biotic variables. To identify the environmental variables that contributed to the observed variability in *Mytilus* response variables, ranging from subcellular to population level, we applied partial least squares regressions (PLSR) to a 24-yr time series. Finally, all variables were classified into different categories and linked in a structural equation modeling (SEM) approach in order to study their direct and indirect links.

Material and methods

Study site and sampling

The study was conducted in the Northern Baltic Proper, close to Askö field station on the Swedish East coast. Since 1993, the Swedish Marine Monitoring program of the phytobenthic community (that includes *Mytilus*) has archived samples from the station Isskären (58.79245; 17.68692, Fig. S1); a small island in the outer archipelago southeast of Askö Island. *Mytilus* were annually sampled at different depths in late August/early September from 1993 to 2016 by SCUBA

divers. The mussels were oven-dried at 60°C and stored at room temperature (ca. 20°C) in plastic bags within cardboard boxes in dark and dry conditions. From each yearly archive, 15–20 mussels (mean length \pm SD: 10 \pm 1 mm) from 5 m depth were selected for chemical and biometric analyses.

Abiotic variables and phytoplankton community were measured by the monitoring program of the nearby pelagic ecosystem at station B1 (58.803; 17.62533, ca. 40 m depth, Fig. S1). Sampling occurred once a month during winter (November–February) and twice a month during the rest of the year, with higher frequency during the spring bloom (once a week from mid-March to end of April). Temperature (°C) and salinity (psu) profiles were measured with a CTD probe in the water column. Water samples for estimation of dissolved inorganic nitrogen (DIN, $\mu\text{mol L}^{-1}$), total nitrogen, and total phosphorus ($\mu\text{mol L}^{-1}$; includes dissolved inorganic, dissolved organic and particulate fractions) were collected every 5 m from the surface to the bottom and the ratio of the two latest (N:P_{pelag}) was calculated. Phytoplankton samples were collected using a plastic hose integrating the water column over 0–20 m depth, preserved with acidic Lugol's solution, and analyzed (cells $> 2 \mu\text{m}$) in an inverted microscope using the Utermöhl technique. For more details about sampling and analytical methods, see Andersson et al. (2015). All data for station B1 are available at <http://www.smhi.se> (marine environmental monitoring data—SHARK database).

Total organic carbon (TOC) for each watershed in Sweden is measured within the freshwater monitoring program “river mouths” (monthly, chemical analyses) and river flow is monitored by the Swedish Meteorological and Hydrological Institute. The yearly averages of flow-weighted TOC concentrations (TOC_{terr}, mg L^{-1}) from the counties of Södermanland and Stockholm, the closest to Askö field station, were used as a proxy for terrestrial organic carbon loading (data available at <http://webstar.vatten.slu.se/db.html>; HAVET 2015/2016).

Mussel elemental and stable isotope analyses

Dried soft tissues of each individual were removed from the shell and ground for chemical analysis. Individual *Mytilus* were analyzed for carbon and nitrogen elemental composition (C% and N% per shell-free dry weight) and bulk stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Phosphorus (P% per shell-free dry weight) was measured on different specimens than C and N elemental and isotope composition since mussels were too small to perform all analyses on the same individual (soft tissue was on average 1.6 \pm 0.9 mg in 10 \pm 1-mm mussels).

Analyses for C% and N% and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were performed at the Center for Physical Science and Technology (Vilnius, Lithuania), using an Elemental Analyzer (Flash EA 1112 Series, Thermo Finnigan) connected to an Isotope Ratio Mass Spectrometer (DeltaV Advantage, Thermo Finnigan). Isotope data are expressed using the conventional delta notation: $\delta^{13}\text{C}_{\text{sample}}$ or $\delta^{15}\text{N}_{\text{sample}} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R

= $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, in per mille deviation (‰) from international standards, Vienna Pee Dee belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$. External and internal standards were analyzed as references within each batch of samples. Analytical uncertainties were $< 0.15\text{‰}$ and $< 0.20\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, and the overall analytical precision 0.9% and 0.2% for C and N, respectively. Analysis of P% were performed at the Department of Ecology, Environment and Plant Sciences (Stockholm, Sweden) using a O.I. Analytical Flow Solution IV, SFA, Alpkem. In total, for elemental and/or isotope ratio, 432 *Mytilus* samples were analyzed for carbon and nitrogen (15–20 individuals yr^{-1}) and 72 for phosphorus (3 individuals yr^{-1}).

Data pretreatment

Mytilus individual and population descriptors

Detailed rationale about *Mytilus* descriptors are provided Table 1. Elemental composition (C%, N%, P%) and the stoichiometric relationship (C : N : P) as well as condition index (CI) were used as proxies for body condition and growth status. We calculated the Layman metrics (dN, dC; Layman et al. 2007) and Bayesian standard ellipse area (SEA_B, Jackson et al. 2011) using the SIBER package (version 2.1.3) based on isotope individual data for each year as a proxy of the resource type and species habitat (Table 1). Total biomass (in grams, Biomass_{tot5m}), abundance (Abundance_{tot5m}) and the biomass : abundance ratio (Bm : Ab_{tot5m}) of *Mytilus* per area (20 \times 20 cm quadrats) at 5 m depth at Isskären (all size classes, total dry weight including shells) was computed for each year (nontransformed data were used in models).

To generalize our results at population level, *Mytilus* population biomass and abundance as well as the Bm : Ab ratio were also computed over the same time period (standardized z-scored transformed data) from four other stations in the Askö region (see map Fig. S1; Furholmarna, Lacka, St. Arnholmen, N. Jutskär [biomass only] or Strömmingshallen [abundance only]) and for other depths when available (5 and 9–10 m, and 14 m at Isskären only).

Environmental variables

Since the mussels were sampled late August/early September, all environmental data were only considered until 31st August. For most environmental variables, yearly averages were calculated for spring (spr: March–May), summer (sum: June–August), and the total productive season (tot: March–August). This was motivated by the changing *Mytilus* physiology over time: rapid gametogenesis occurs in spring and spawning in May/June, and is followed by rapid growth to recover lost biomass (Pierścieniak et al. 2010). Similarly, phytoplankton community composition changes over the productive period. Seasonal averages were used as predictors for *Mytilus* condition, growth and population models while the total productive season was expected to better reflect the isotope composition, since mussels feed during the entire

Table 1. *Mytilus* descriptors used as response variables in PLSR and PLS-SEM models.

<i>Mytilus</i> descriptor	Proxy for	References
Condition index (CI)	<i>Mytilus</i> health status (CI = dry weight soft tissue (g)/dry weight shell (g) × 100)	Irisarri et al. (2015)
C%	Represent lipids (i.e., main components of cell membrane, provide high metabolic energy) or carbohydrate content (i.e., energy storage)	Sturner and Elser (2002)
N%	Reflects organism protein content (i.e., N-rich macromolecules)	Sturner and Elser (2002)
P%	The largest pool of P in the cell is related to RNA, which is correlated with growth rate	Elser et al. (2003)
C : N	Low C : N ratio in <i>Mytilus</i> was expected to reflect high protein content, as long as N% is high (blue mussels are generally N limited)	Smaal and Vonck (1997)
N : P, C : P	Low N : P or C : P ratios are indicators of good growth status, but in cases of N limitation (e.g., starvation) the relationship is more complicated	Sturner and Elser (2002) and Elser et al. (2003)
$\delta^{13}\text{C}$	Diet tracer	Fry and Sherr (1984)
$\delta^{15}\text{N}$	Diet tracer, also indicate food limitation or starvation (i.e., results in elevated values)	Wolf et al. (2009)
dC	Diet breadth	Layman et al. (2007)
dN	Trophic diversity and potential stress	Layman et al. (2007)
Bayesian standard ellipse area (SEA _B)	Overall size niche, total extent of trophic diversity of <i>Mytilus</i> population (based on Layman's total area but is a more robust measure of niche size than total area)	Jackson et al. (2011)
Biomass, abundance	Potential pseudofeces/feces reingestion by <i>Mytilus</i> (i.e. the more and the bigger the mussels the larger amount of pseudo-feces/feces produced and resuspended, the lastest having a higher $\delta^{15}\text{N}$) and food competition	Kautsky and Evans (1987)
Biomass : Abundance (Bm : Ab _{tot5m})	Average mussel size in the population	—

C, carbon; N, nitrogen; P, phosphorus and their related ratios; dC and dN, C and N range; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, stable isotope ratio of C and N.

productive season (Table S1). Water temperature was also calculated for winter (win: January–February) and for summer maximum (sum_max: June–August); high winter water temperature may negatively impact *Mytilus* growth and condition (Waldeck and Larsson 2013), and prolonged exposure to high summer temperatures generates physiological stress and mortality (Seuront et al. 2019). DIN concentration, a core indicator for eutrophication status (HELCOM 2018), was only averaged for winter (i.e., maximum of the pool), since it affects

phytoplankton isotope values (Rolff 2000; Oczkowski et al. 2018). Abiotic variables were calculated as integrated average over 0–10 m.

Phytoplankton biovolume ($\text{mm}^3 \text{L}^{-1}$) was calculated for each period (spr, sum, tot) as integrated sum by using the area under the curve (AUC, linear interpolation) for the total community (Bloom) and for the following groups separately: (1) diatoms, (2) dinoflagellates, (3) N_2 -fixing cyanobacteria (i.e., hereafter referred as “cyanobacteria”; the three bloom-

forming species *Aphanizomenon* sp., *Nodularia spumigena*, and *Dolichospermum* spp., Fig. S2), and (4) the mixotrophic ciliate *Mesodinium rubrum* with unusual photosynthetic properties (Johnson et al. 2006). The four groups represented most of the phytoplankton community biomass and were defined considering abundance/dominance, size, trophic type, and possible specific isotope signature (Table S1). The N₂-fixing cyanobacteria were also calculated as % of summer bloom total biovolume. Finally, a principal coordinate analysis was performed for each period including the entire phytoplankton community for each year (on AUC values, Bray–Curtis distance, capscale(), “vegan” package version 2.5-2). Annual values for the first two components (PC1 and PC2) explained together 34% to 42% of variability depending on the season considered and were used as proxies for interannual variability in phytoplankton community (Table S1).

Statistical analysis

Temporal trends and variables correlation

For all response and predictor variables, their respective time series were first checked for autocorrelation (acf(), for up to 3 yr [longer autocorrelations were considered of limited biological meaning]). Thereafter, unidirectional trends were tested using Mann–Kendall test or a modified version of the Mann–Kendall test when auto-correlation was detected (mmkh3lag(), “modifiedmk” package version 1.5.0). Mann–Kendall test was applied to evaluate paired-correlations in *Mytilus* descriptors and between each pair of environmental variables.

Influence of environmental variables

PLSR was used to model the relationship between each of the selected response variables describing *Mytilus* diet ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, SEA_B), condition and growth (CI, C : N, N%, Bm : Ab_{tot5m}, N : P, C : P, P%), and population (Biomass_{tot5m}, Abundance_{tot5m}) independently and a set of 18–33 predictors. *Mytilus* response variables are described in Table 1, rationale for including predictors in the different models are presented in details in Table S1. Abiotic conditions (i.e., temperature, salinity, nutrients) and possible food sources (i.e., the pre-determined phytoplankton groups, terrestrial inputs) were included as predictors in all models, whereas physiological proxies (CI, Bm : Ab_{tot5m}, elemental composition and ratio) were only included in the diet and population models. The rationale for including some of the elemental and/or stoichiometric variables as predictors in the isotope models was that physiological process strongly influences isotope composition of organisms. Time-lag effects on isotopic signature (sensu isotopic memory: the effect of the previous year isotopic value of *Mytilus* tissues on the observed isotopic signature for a given year) and condition (CI, Bm : Ab_{tot5m}; the effect of previous year condition on individual mussels and of previous year biomass and abundance on *Mytilus* population) were included as

an additional predictor in models, and kept if the model fit improved.

To conceptualize the link between the various response variables and predictors, structural equation modeling using PLSR (PLS-SEM) were performed based on the same hypotheses as in Table S1. The explanatory variables were classified into different groups of latent variables—abiotic and food/resources as environmental proxies and physiology and isotope composition for *Mytilus* proxies—and were connected in two different models, one with isotope composition as the dependent latent variable, the other with physiology as the dependent latent variable.

The choice of the methods, the settings used in PLSR and SEM models, as well as selection criteria, are detailed in the Methods section support of the Supporting Information.

Results

Mytilus: Changes on all levels of biological organization

There was considerable year-to-year variability in CI, carbon, nitrogen, and phosphorus content (C%, N%, P%) and stoichiometric ratios (C : N, N : P, C : P), but only stable isotopes ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) and biomass : abundance ratio (Bm : Ab_{tot5m}) significantly decreased (Fig. 1a,b,j; Table S2). *Mytilus* total biomass per area at 5 m depth significantly decreased over time at Iskären (Fig. 2n, Table S2) and at another station (St. Arnholmen, Table S3) and when all sites are combined (averaged biomass; Fig. 3a, Table S3). Averaged mussel abundance increased significantly over time at both 5 and 10 m depth (but not at Iskären, Fig. 3b, Table S3). Significant correlations were found between CI and C : N as well as between C : N, N%, $\delta^{15}\text{N}$ and the SEA_B and the Layman metrics dN and dC (Table 2). According to our condition index data, “good” condition for the mussels (i.e., high CI, high tissue biomass) is reflected by high N content (sensu protein rich, Sterner and Elser 2002), low C : N ratio (relatively larger amount of N, sensu proteins), narrow isotopic niche (SEA_B) and lower $\delta^{15}\text{N}$ (Table 2). Also, a high P content (sensu high RNA content, Elser et al. 2003) and, accordingly, low C : P and N : P ratios (relatively larger amount of RNA) reflected a higher growth rate and were related to low N content, high C : N ratio and low CI (Table 2). The strong correlation between SEA_B and dN and dC resulted partly from autocorrelation.

Environmental variables

A significant decrease was observed for DIN (Fig. 2h), while terrestrial TOC loading (TOC_{terr}), total phosphorus (P) over the total productive period (i.e., March–August), N : P ratio of pelagic particles over the total productive period and the total N in summer increased (Fig. 2 g,e,f,d; Table S2). The other variables had no significant long-term trends, even though changes in *M. rubrum* biomass (ciliate) and cyanobacteria (i.e., N₂-fixing) over the total productive period and total

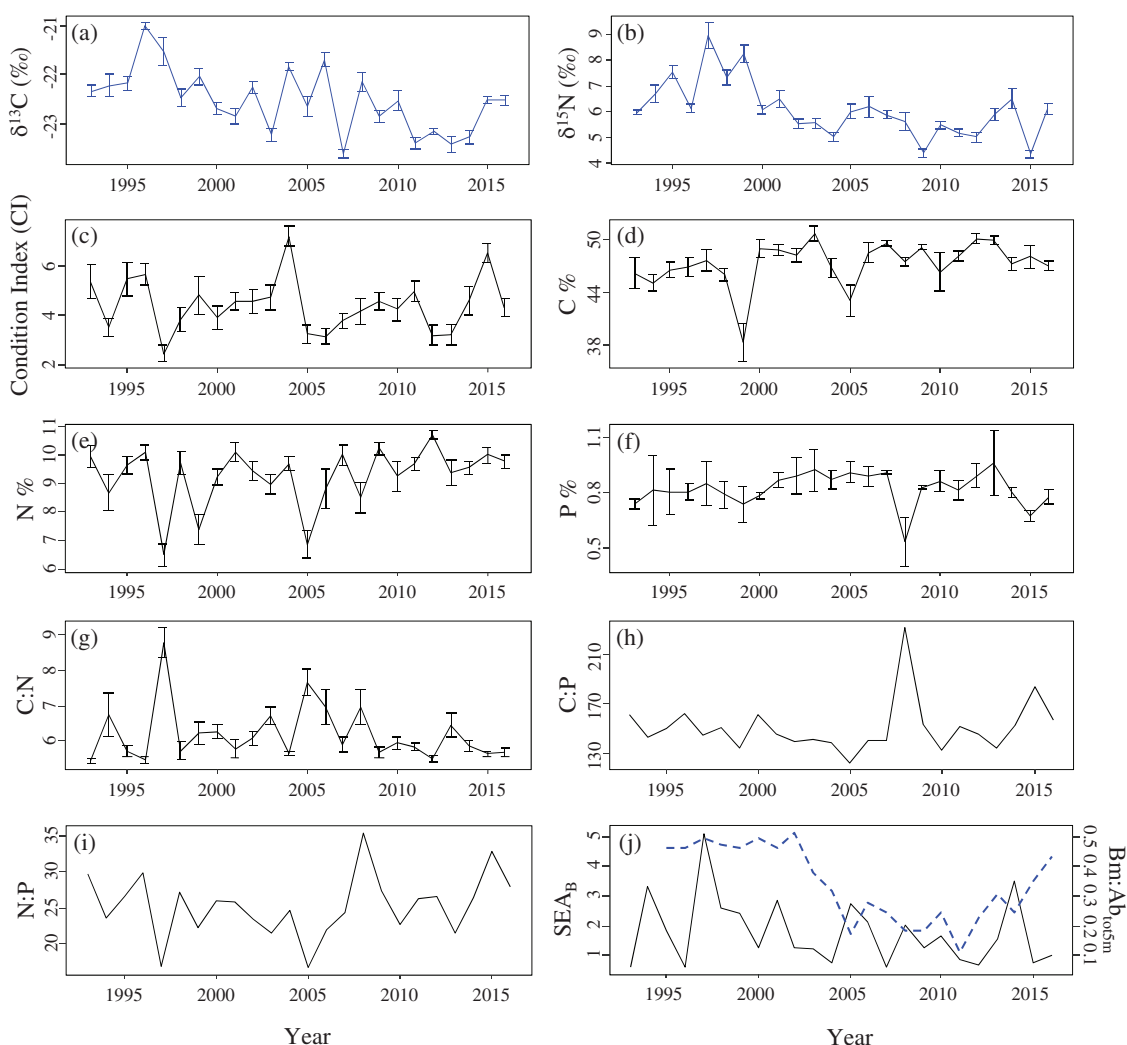


Fig 1. Time series for *Mytilus* proxies (annual mean values and standard error). $n = 15$ to 20 individuals per year for (a) $\delta^{13}\text{C}$, (b) $\delta^{15}\text{N}$, (c) condition index (CI), (d) C%, and (e) N% and related (g) C : N ratios and (j) Bayesian standard ellipse area (i.e., trophic niche of population, SEA_B , plain line). $n = 3$ for (f) P% data and related ratios (h) C : P and (i) N : P. (j) Biomass : abundance ratio ($\text{Bm} : \text{Ab}_{\text{tot5m}}$, dash line) calculated on *Mytilus* population at 5 m at Isskären. Black, non-significant; blue, significant decrease (Mann–Kendall or modified Mann–Kendall test for autocorrelated data, p -values and Kendall-tau given in Table S2).

phytoplankton community (Bloom) in summer were nearly significant ($p < 0.07$; Table S2). Among environmental variables, significant correlations were found (208 over 780, $p < 0.05$, Table S4) and the most relevant relationships ($\tau > 0.30$) are described hereafter. High water temperatures in winter and/or spring were positively correlated with high total P in spring and/or in summer, and high temperatures in summer with high total N in summer. High salinity in summer was correlated with low cyanobacteria in summer and with higher total P over the total productive period. High DIN was correlated to higher *M. rubrum* in spring and lower cyanobacteria in summer. Higher TOC_{terr} was correlated to higher total N over the total productive period and in summer. High cyanobacterial biomass over the total productive period and in summer was correlated with higher summer temperatures

and total N. Finally, over the total productive period high *M. rubrum* biomass was correlated with high dinoflagellate, while high cyanobacteria in summer was correlated to both lower *M. rubrum* in summer and dinoflagellate in spring.

Variables influencing *Mytilus*: From subcellular to population level

The Lundstedt evaluation criteria, a measure that indicates model predictive capacity ($R^2Q > 0.4$; Lundstedt et al. 1998, see Supporting Information) was met for 5 of the 12 isotopes, physiological and population response variables tested: $\delta^{15}\text{N}$, SEA_B , C : N, N% and biomass : abundance ratio ($\text{Bm} : \text{Ab}_{\text{tot5m}}$), and close to meeting the criteria for the $\delta^{13}\text{C}$ and N : P models (Table 3). Explanatory power (R^2Y , i.e., analogous to coefficient of determination in regression analysis) and explained

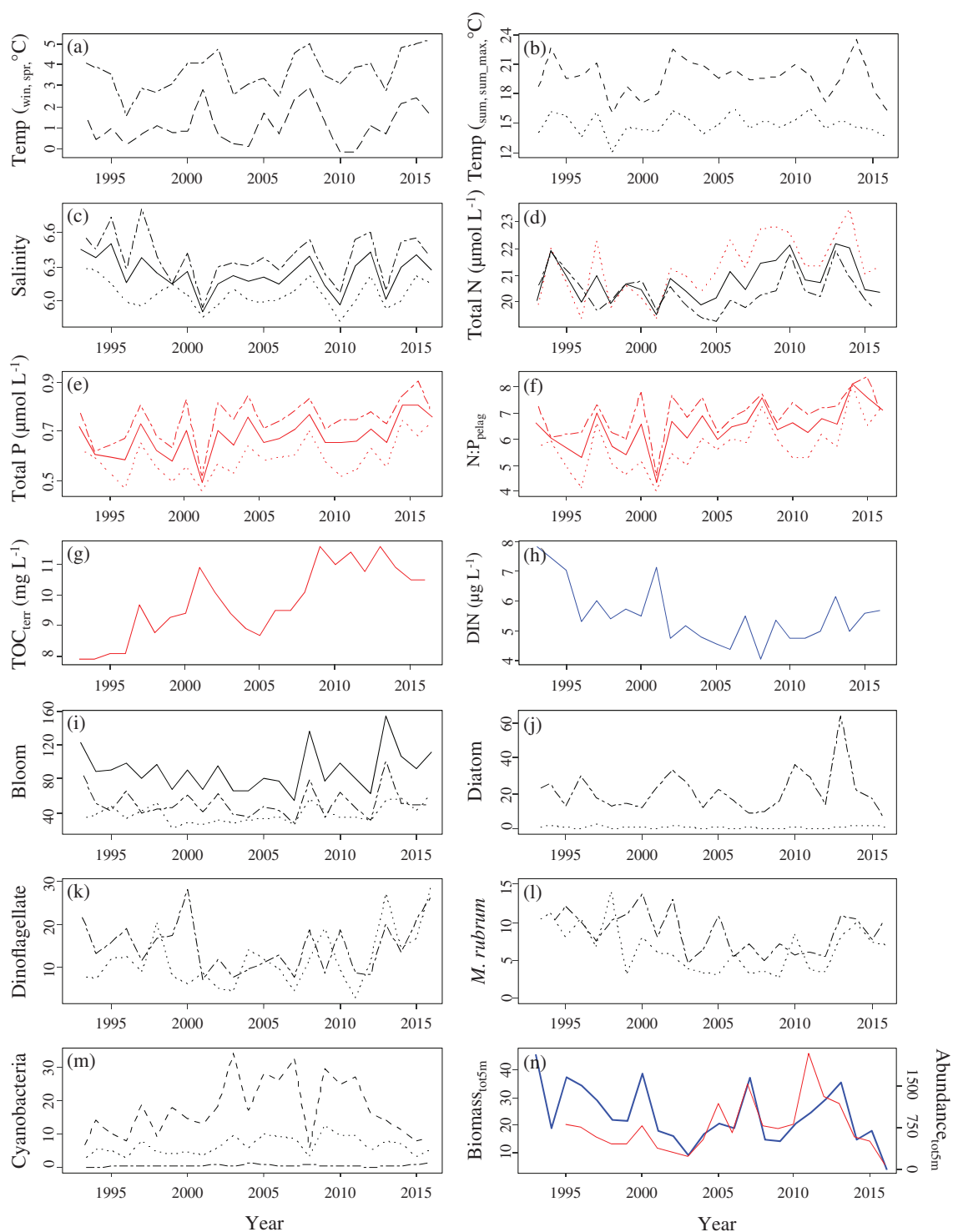


Fig 2. Time-series data for environmental parameters for phytoplankton groups over spring (spr ----), summer (sum) or total productive period (tot —), except for (g) terrestrial total organic carbon loading (TOC_{terr}, all year average) and (h) dissolved inorganic nitrogen (DIN; winter only). Temperature (temp) was split in two panels: (a) winter (win ----) and spring, (b) summer and summer maximum (sum_max ----). (c) is salinity, (d) and (e) are total nitrogen and total phosphorus and (f) their ratio. Units for all phytoplankton groups are given in terms of AUC calculated from biovolume: (i) Bloom is total phytoplankton biomass, (j) diatoms, (k) dinoflagellates, (l) *Mesodinium rubrum*, (m) N₂-fixing cyanobacteria (including % of summer bloom — —). Total values for phytoplankton proxies are not shown as it correspond to sum of spring and summer values. (n) Biomass (in bold) and abundance of *Mytilus* population at 5 m at Isskären. Blue, significant decrease; red, significant increase; black, non-significant (Mann–Kendall or modified Mann–Kendall test for autocorrelated data, *p*-values and Kendall-tau given in Table S2).

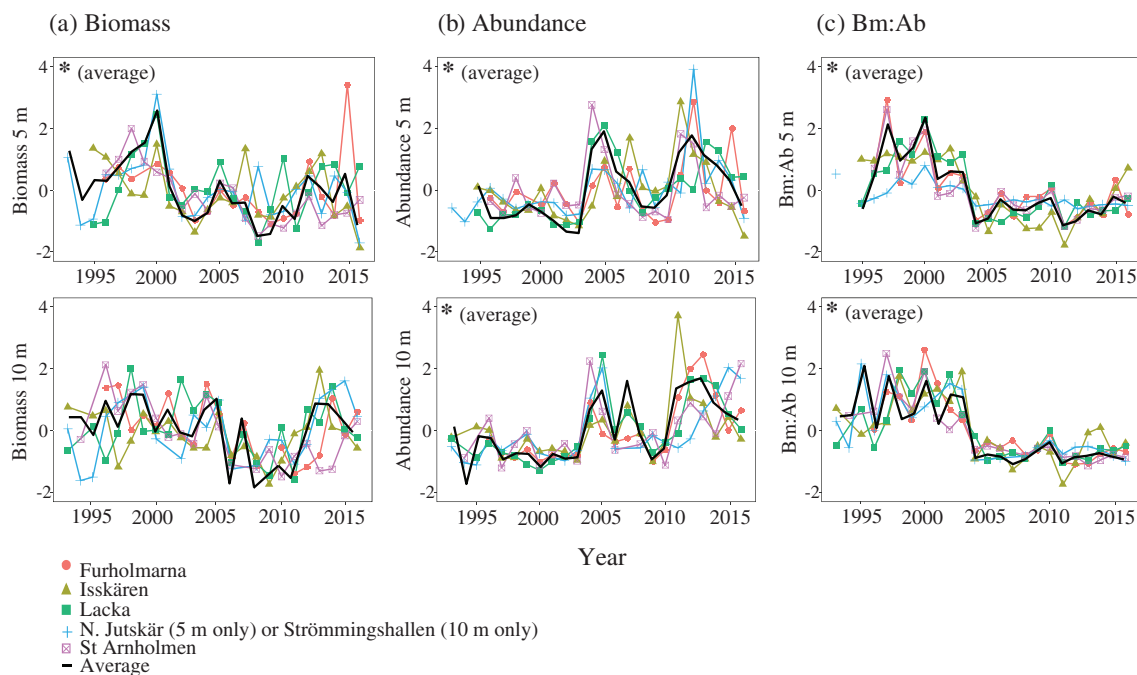


Fig 3. Total (a) biomass, (b) abundance, (c) biomass : abundance ratio (Bm : Ab) of *Mytilus* at 5 and 10 m depth for five different stations in the Askö area and their average (black line). Standardized z-score transformed data. Mann–Kendall or modified Mann–Kendall test for autocorrelated data; * p -values < 0.05 (only for averages), p -values for each stations are given in Table S4.

variance (R^2X) were always > 50% for all models, even though some had R^2Q around 0. Only the $\delta^{15}\text{N}$, Bm : Ab_{tot5m} and Abundance_{tot5m} models were improved by including lagged values (n.b. the previous year values as an additional predictor). Models results are described below, presented in detail in Table 3 and illustrated in Figs. 4 and 5.

Role of physiological processes

From the physiological predictors initially included in the isotope models (i.e., $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, SEA_B, diet models; see Table S1), condition proxies (N%, C : N, condition index (CI), Bm : Ab_{tot5m}) were the most important predictors in the final models (Table 3, Fig. 4a,b,c). A high N% in blue mussels tissue was linked to low $\delta^{15}\text{N}$ and high Bm : Ab_{tot5m} (i.e., large mean size) and isotope lag to high $\delta^{15}\text{N}$. High Bm : Ab_{tot5m} had a negative influence on $\delta^{13}\text{C}$. High C : N had a positive influence whereas low CI and N : P a negative influence on SEA_B (i.e., trophic niche). Even though initially included, no physiological predictors were selected as important in the abundance or biomass models; however, for the SEA_B model (also on population level) the first two predictors were physiological proxies (C : N ratio and CI).

Role of food availability and quality

Food availability was generally not an important predictor, apart from a positive influence on *Mytilus* C : P (total phytoplankton community: Bloom_{sum}, Table 3). From a food quality aspect (Table 3), N₂-fixing cyanobacteria were an important predictor in most models: high cyanobacteria

biomass had a positive influence in P% model and a negative influence in N : P, C : P, $\delta^{13}\text{C}$, SEA_B, CI and Bm : Ab_{tot5m} models (Figs 4b,c,d and S4g,h,i,j), and was also included in total biomass and abundance models (Fig. S4k,l). The biomass of the ciliate *M. rubrum* was an important predictor in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as well as in Bm : Ab_{tot5m} and total biomass models (positive influence for all) but had a negative influence on the abundance one. Variability in phytoplankton communities (PC1 and PC2) in spring or summer came out as predictors especially in Bm : Ab_{tot5m}, P%, C : P, N : P, N% and total biomass and abundance models. As another potential food source, terrestrial organic carbon (TOC_{terr}) was the first predictor in $\delta^{13}\text{C}$ model, the higher the TOC_{terr}, the lower the $\delta^{13}\text{C}$ of *Mytilus* and was important in condition models N% and Bm : Ab_{tot5m}, the higher the TOC_{terr} the higher the N% and average size. TOC_{terr} also had a negative influence on total biomass. Total N in spring or summer was an important predictor in condition models: summer values had a positive influence but spring values had a negative influence on C : N, also summer values had a negative influence on N%, CI and Bm : Ab_{tot5m} as well as in total biomass model. Finally, isotopic niche (i.e., trophic diversity at population level, SEA_B) was the most important predictor in all condition models (positive influence on C : N, negative on N% and CI) and in the N : P model (negative influence), the smaller the niche, the better the condition. The nitrogen isotopic range (dN; i.e., Layman metrics) was the most important predictor in total biomass and

Table 2. Correlation matrix for *Mytilus* proxies. Kendall-rank correlation test: Kendall-tau values and *p*-values significance in different blue/gray shades.

	CI	C%	N%	P%	C : N	N : P	C : P	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	SEA _B	dC	dN	Bm : Ab _{tot5m}	Biom _{tot5m}	Ab _{tot5m}
CI															
C%	-0.13														
N%	0.26	0.32													
P%	-0.23	0.28	-0.05												
C : N	-0.45	-0.07	-0.74*	0.21											
N : P	0.27	0.05	0.51	-0.54	-0.62*										
C : P	0.16	0.15	0.31	-0.57*	-0.32	0.70*									
$\delta^{13}\text{C}$	0.09	-0.24	-0.24	-0.27	0.04	0.09	0.09								
$\delta^{15}\text{N}$	-0.19	-0.23	-0.28	-0.11	0.23	-0.17	-0.05	0.26							
SEA _B	-0.27	-0.30	-0.52	-0.01	0.50	-0.29	-0.19	0.15	0.46						
dC	-0.13	-0.32	-0.50	-0.01	0.44	-0.27	-0.17	0.02	0.25	0.66*					
dN	-0.25	-0.26	-0.29	-0.05	0.36	-0.17	-0.05	0.20	0.39	0.67*	0.38				
Bm : Ab _{tot5m}	0.03	-0.01	-0.01	-0.10	-0.01	-0.07	0.05	0.28	0.37	0.12	0.06	0.15			
Biom _{tot5m}	-0.10	0.01	0.03	-0.01	-0.08	-0.03	-0.03	0.03	0.15	-0.15	-0.12	-0.28	0.11		
Ab _{tot5m}	-0.15	0.08	-0.01	0.13	0.04	-0.03	-0.07	-0.20	-0.19	-0.17	-0.04	-0.29	-0.47*	0.43	

Ab_{tot5m}: abundance; Biom_{tot5m}: biomass; Bm : Ab_{tot5m}: ratio biomass to abundance for total *Mytilus* population at 5 m at Iskkären.

*Autocorrelated variables.

abundance models, both with negative influence (Fig. S4k,l; Table 3).

Role of the abiotic environment

Temperature was an important predictor for most of the models (Table 3): higher winter and spring temperatures had a negative influence on $\delta^{13}\text{C}$ and P% and positive influence on N : P and C : P. High summer and summer maximum temperatures had a positive influence on C : N, CI, and N : P and negative influence on N%. Both summer and maximum temperatures also affected total abundance in opposite directions. High salinity in summer had a positive influence on N : P, C : P, and negative on P%; spring salinity had a positive influence on total biomass model. Higher DIN had a positive influence on both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

Linking *Mytilus* isotope composition, physiology, diet, and environmental factors

None of the PLS-SEM models had significant paths; however, the strength of the relation between the latent variables and their impact on each other (path coefficients, Fig. 5, Table S5) agreed with the PLSR results. *Mytilus* isotope composition was equally influenced by both physiology ($\beta = -0.53$) and food ($\beta = -0.54$), while abiotic factors had a strong positive influence on food resources ($\beta = 0.67$) and a negative influence on the *Mytilus* physiology ($\beta = -0.38$) (Fig. 5a). *Mytilus* condition and growth were strongly and positively influenced by resource quality and availability ($\beta = 0.58$) with abiotic conditions indirectly influencing physiology through resources and diet ($\beta = 0.67$) (Fig. 5b).

Discussion

The long-term decrease in *Mytilus* isotope composition was equally influenced by physiological status and diet (Fig. 5a). Individual mussel condition and growth were directly linked to diet with indirect links to abiotic conditions (Fig. 5b). Finally, population biomass was best explained by trophic diversity in diet (isotope niche metrics).

Linking diet and *Mytilus* isotope composition

In support of Hypothesis 2, terrestrial organic carbon loading was the most important predictor in the $\delta^{13}\text{C}$ model (Table 3). The increase in land runoff (Fig. 2; Asmala et al. 2019) with a ^{13}C -depleted signal, led to more negative $\delta^{13}\text{C}$ values of suspended organic matter (Rolff and Elmgren 2000) reflected in turn in *Mytilus* lower $\delta^{13}\text{C}$ composition. In contrast to Hypothesis 1, however, the decrease in *Mytilus* $\delta^{15}\text{N}$ was not directly linked to the intensity of N₂-fixing cyanobacterial blooms (Table 3). One explanation may be that the largest increase in cyanobacterial blooms occurred in the 1980s (Kahru and Elmgren 2014), that is, before the mussel monitoring was started. Moreover, the ^{15}N -depleted atmospheric nitrogen fixed by cyanobacteria is released from cells as ammonium and also utilized by the rest of the

Table 3. PLSR models results for the 12 *Mytilus* response variable tested. R^2Y is the explanatory coefficient (analogous to the coefficient of determination R^2 in regression analysis), R^2Q is the prediction coefficient, R^2X explained the variance. Models with high predictive capacity according to Lundstedt evaluation criteria (i.e., $R^2Q > 0.4$) are in bold. White cells are predictors with positive influence on the response variable, shaded gray cells with negative influence. Predictors are ranked by importance based on absolute value of regression coefficient (in italics). See Methods section and Table 1 for a description of the predictors and Table S1 for rationale in including predictors in initial models.

Response variable	Model evaluation parameters				Predictors (reg. coefficients)								
	R^2Y	R^2Q	R^2X		1	2	3	4	5	6	7	8	9
$\delta^{15}N$	Diet/sources	0.7	0.6	0.7	N%	Bm : Ab _{tot5m}	DIN	Meso _{tot}	Lag $\delta^{15}N$				
					-0.44	0.32	0.21	0.19	0.18				
$\delta^{13}C$	Diet/sources	0.6	0.3	0.6	TOC _{terr}	Temp _{spr}	Bm : Ab _{tot5m}	Cyano _{tot}	DIN	Meso _{tot}			
					-0.22	-0.18	0.09	-0.08	-0.07	0.04			Cyano _{sum%} -0.03
SEA _B	Trophic niche of population	0.6	0.5	0.8	C : N	CI	Cyano _{sum%}	N : P					
					0.47	-0.28	-0.26	-0.16					
CI	Condition	0.5	0.2	0.7	SEA _B	Temp _{sum_max}	Total N _{sum}	Cyano _{sum}					
					-0.004	0.003	-0.003	-0.002					
C : N	Condition	0.7	0.5	0.7	SEA _B	Total N _{spr}	Temp _{sum}	Total N _{sum}	Temp _{sum_max}				
					0.34	-0.17	0.15	0.12	0.11				
N%	Condition	0.7	0.4	0.6	SEA _B	TOC _{terr}	Temp _{sum}	Temp _{sum_max}	Total N _{sum}	PC1 _{spr}			
					-0.46	0.22	-0.17	-0.13	-0.10	0.07			PC1 _{sum} -0.05
Bm : Ab _{tot5m}	Mean size	0.7	0.5	0.6	Lag Bm : PC2 _{sum}	PC2 _{sum}	Meso _{spr}	Meso _{sum}	Total N _{sum}	TOC _{terr}			Cyano _{sum}
					Ab _{tot5m}								
					0.005	0.004	0.004	0.001	-0.0008	0.0006			PC1 _{sum} -0.0003
N : P	Growth status	0.7	0.3	0.5	SEA _B	Temp _{spr}	Salinity _{sum}	Cyano _{sum}	Temp _{win}	PC1 _{spr}			Temp _{sum_max} PC1 _{sum} -0.11
					-1.36	1.22	1.07	-0.65	0.61	-0.29			Temp _{sum} 0.15
C : P	Growth status	0.5	0.1	0.7	Cyano _{sum}	PC1 _{spr}	Salinity _{sum}	Temp _{win}	Temp _{spr}	Bloom _{sum}			Total P _{sum} N : P _{pelag_sum} 1.02
					-4.07	-3.71	3.11	3.00	2.48	1.84			
P%	Growth status	0.6	0.2	0.6	PC1 _{spr}	Cyano _{sum}	Salinity _{sun}	Diatom _{spr}	Temp _{win}	Temp _{spr}			
					0.02	0.02	-0.02	0.01	-0.01	-0.01			
Biomass _{tot5m}	Population	0.5	0.1	0.5	dN	Meso _{spr}	Salinity _{spr}	PC2 _{sum}	TOC _{terr}	Total N _{sum}			Cyano _{sum}
					-3.85	2.72	2.35	0.92	-0.67	-0.46			N : P _{pelag_sum} 0.30
Abundance _{tot5m}	Population	0.5	0.0	0.5	dN	Lag Ab _{tot5m}	Meso _{spr}	Temp _{sum_max}	Meso _{sum}	Cyano _{sum}			PC1 _{sum} 18
					-1.27	1.22	-0.72	-0.61	-0.60	38			Temp _{sum} 20
													PC1 _{spr} 11

Bloom, total phytoplankton community biovolume; Bm : Ab_{tot5m}, ratio biomass to abundance of total *Mytilus* population at 5 m at Isskären; Cyano, N₂-fixing cyanobacteria; DIN, dissolved inorganic nitrogen; lag, values of the previous year for the given variable; Meso, *Mesodinium rubrum*; PC1/PC2, changes in phytoplankton community; pelag: Pelagic suspended material; win, winter; spr, spring; sum, summer; sum_max, summer maximum; temp, temperature; TOC_{terr}, terrestrial total organic carbon loadings; tot, total productive period; tot5m: *Mytilus* total population at 5 m at Isskären.

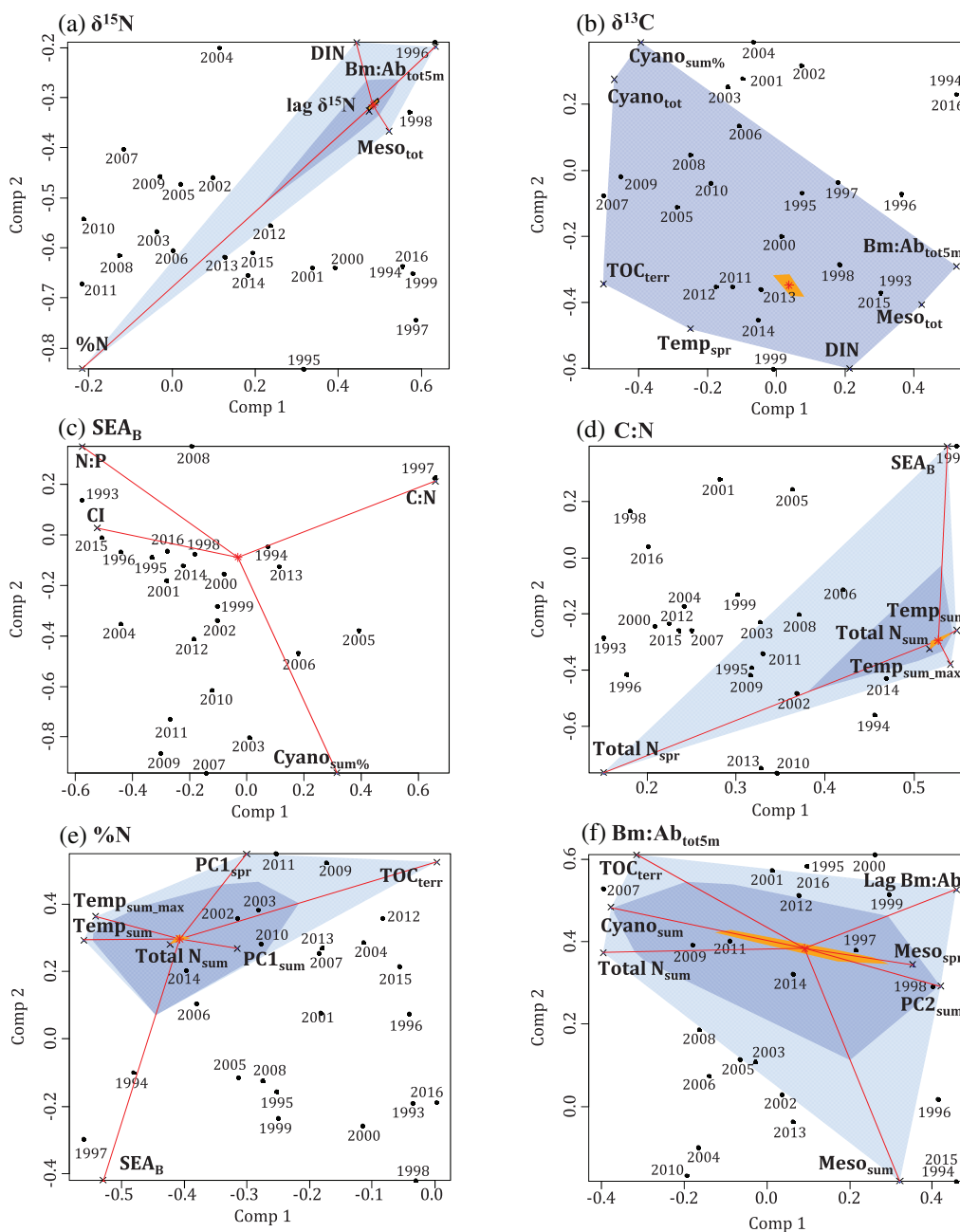


Fig 4. Bagplots illustrating PLSR models with predictive capacity (R^2Q) > 0.3: **(a)** $\delta^{15}\text{N}$, **(b)** $\delta^{13}\text{C}$, **(c)** Bayesian standard ellipse area (SEA_B), **(d)** C : N, **(e)** N%, **(f)** biomass : abundance ratio (Bm : Ab_{tot5m}) of *Mytilus* as response variables and showing significant predictors selected in final models (cf. Table 3). The structure of multivariate data is projected on a bivariate space (Comp 1 and Comp 2) and represented by the shaded areas in the bagplot: Light blue is the “fence” (magnification factor = 3), blue is the “bag” corresponding to 50% of the data set, orange indicates the bivariate median. Bagplots help visualizing the spread, correlation, skewness and tails of the data. Model (c) SEA_B had too few predictors selected to show fence and bag. Models with R^2Q < 0.3 are shown in Supporting Information (Fig. S4) for the response variables **(g)** condition index (CI), **(h)** N : P, **(i)** C : P, **(j)** P%, **(k)** Biomass_{tot5m}, and **(l)** Abundance_{tot5m}. Cyano: N₂-fixing cyanobacteria; DIN, dissolved inorganic nitrogen; Lag: Values of the previous year for the given variable; Meso, *Mesodinium rubrum*; PC1 and PC2, changes in phytoplankton community; spr, spring; sum, summer; sum_max, summer maximum; TOC_{terr}, terrestrial total organic carbon loading; Temp, temperature; tot, total productive period; tot5m, *Mytilus* total population at 5 m at Isskären.

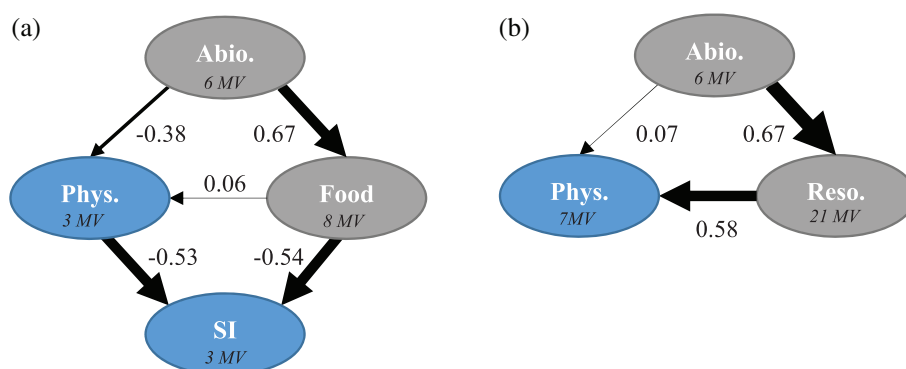


Fig 5. Summary SEM-PLS model of the processes leading to the observed variability in (a) stable isotope composition and (b) condition and growth status of *Mytilus*. Ellipses are latent variables that are reflected by measured variables (MV, see Supporting Information and Table S5 for details). Latent variables in gray are environmental biotic and abiotic proxies (Abio., abiotic; Reso., resources; food), in blue are blue mussel proxies (Supporting Information: isotope composition; $n = 23$, includes lag; Phys., physiology; $n = 24$). Arrows indicate the direction of direct link and the strength of the relation between the latent variables. Path coefficients (β values) are given between each latent variable.

plankton community (Ploug et al. 2011). This results in a lower phytoplankton $\delta^{15}\text{N}$ baseline during summer, confounding a direct relationship with cyanobacterial bloom intensity. However, the decreasing biomass of the ciliate *M. rubrum* was an important food-related predictor for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ decrease (Fig. 2; Tables 3 and S1). *M. rubrum* is mixotrophic, therefore its trophic position is higher than strictly autotrophic phytoplankton, explaining its enriched ^{15}N values (and more depleted ^{13}C) (Brutemark and Granéli 2011). Surprisingly, cyanobacteria was an important predictor of *Mytilus* $\delta^{13}\text{C}$ signature (Table 3), indicating a potential utilization of this food source by blue mussels. N_2 -fixing cyanobacteria $\delta^{13}\text{C}$ signature is less distinct, but generally exhibits a more depleted signal than other phytoplankton (Rolff 2000). Moreover, the higher the cyanobacterial bloom biomass the smaller the isotopic niche (Table 3). Cyanobacterial blooms are known to be associated with depleted values in deposit-feeders and are additionally associated with a large niche, presumably through increased dietary diversity from decomposing filaments and attached microorganisms in the sediment (e.g., Karlson et al. 2015). For sessile suspension-feeders it is possible that feeding is more uniform among individuals resulting in the observed small isotope niche. Alternatively, a small niche not only indicates dietary diversity but also better physiological status in the population (Karlson et al. 2018), which is supported by the negative correlation between niche size and condition index for *Mytilus* (Table 2).

Linking physiology and *Mytilus* isotope composition

Physiological processes (see predictors included in models Table S1) influenced *Mytilus* isotope composition, especially in the $\delta^{15}\text{N}$ model, which reduces its value as a trophic tracer. Osmoregulation in the low-saline Baltic Sea is a nitrogen demanding process (Tedengren and Kautsky 1987), suggesting that $\delta^{15}\text{N}$ could be affected by the high nitrogen excretion.

Accordingly, $\text{N}\%$ was the highest ranked predictor for the $\delta^{15}\text{N}$ model (but not included in any other models, Table 3). Furthermore, the lag-predictor (i.e., effect of previous years $\delta^{15}\text{N}$ value) was included for $\delta^{15}\text{N}$ model (not needed in $\delta^{13}\text{C}$ model), indicating a slow tissue N turnover in *Mytilus* (Smaal and Vonck 1997). Finally, the mean size of mussels was found to be the secondly ranked predictor for $\delta^{15}\text{N}$, possibly related to age structure in the population (Minagawa and Wada 1984). *Mytilus* $\delta^{13}\text{C}$ can also be affected by physiological processes; Higher $\delta^{13}\text{C}$ values have been associated with thermal stress due to higher respiration, causing accumulation of heavy ^{13}C (and ^{15}N) in the tissue (Ek et al. 2015). In our study, higher spring water temperature (i.e., causing higher metabolism) were, however, associated with lower $\delta^{13}\text{C}$ values. An alternative explanation is that the earlier onset of the spring bloom along with warmer spring water (Kahru et al. 2016; Hjerne et al. 2019) leads to a reduced winter starvation period for *Mytilus* with beneficial effects on lipid content and consequently lower $\delta^{13}\text{C}$ values (i.e., lipids are depleted, Post et al. 2007). Our results demonstrate the importance of including individual physiology when interpreting isotope for assessing long-term change.

Linking the abiotic environment and *Mytilus* isotope composition

Changes in the biogeochemical environment influence isotope composition of *Mytilus*, as shown by the positive association of DIN and $\delta^{15}\text{N}$ (Table 3). In the Baltic Sea, land-based nutrient loading has decreased over the past decades (Elmgren et al. 2015); a trend mirrored in the dissolved organic nitrogen concentrations in the coastal zone (DIN; Fig. 2). However, changes in internal denitrification potential due to increased volume of hypoxic waters since the 1990s (Jäntti and Hietanen 2012), may also explain the DIN decrease. The eutrophication status of the Baltic Sea might result in a generally ^{15}N -enriched isotope baseline (e.g., Savage and

Elmgren 2004), and decreasing $\delta^{15}\text{N}$ thus reflecting eutrophication mitigation. However, this pattern could be counteracted by increased nitrogen fixation by cyanobacteria (Rolff 2000; Karlson et al. 2015) and combustion of fossil fuel and synthetic nitrogen fertilizers (Bateman and Kelly 2007; Walters et al. 2015). Eutrophic status can also be reflected in more enriched $\delta^{13}\text{C}$ values of particulate material (Oczkowski et al. 2018), therefore, an alternative explanation for DIN being an important predictor for the $\delta^{13}\text{C}$ model. However, the dynamics of the carbonate system is salinity dependent (i.e., dissolved inorganic carbon $\delta^{13}\text{C}$ increases with salinity; Fry 2002) meaning that one should be cautious when interpreting $\delta^{13}\text{C}$ as an eutrophication indicator in brackish waters.

Mytilus condition and growth data

In support of Hypothesis 3, the main drivers of *Mytilus* individual condition and growth were food quantity (i.e., phytoplankton availability during spring and summer) and quality (i.e., increase in N_2 -fixing cyanobacteria and terrestrial organic carbon) as well as higher water temperature and lower salinity (Table 3). Total N in the water, which reflects both organic particulate (e.g., fresh phytoplankton cells) and dissolved (e.g., free bioavailable components resulting from bloom degradation) material, predicted *Mytilus* condition and mean size. Consumers can be limited by both energy (i.e., C), N and P, but the stoichiometrical requirements vary with season and biological processes (Smaal and Vonck 1997). In spring, *Mytilus* N requirement is high due to reproductive investment (Smaal and Vonck 1997), and the spring bloom (i.e., diatoms, considered as a good food source for invertebrates, Karlson et al. 2018) may satisfy this need. In summer, the negative link between water N and *Mytilus* N might have reflected both a higher need for P (i.e., requirements for rapid postspawning growth) and the use of accumulated N (blue mussels retain N better than C and P, Smaal and Vonck 1997) when bioavailable N becomes limiting. Compared to the stoichiometrical models, the Condition Index model had a low predictive capacity (Table 3) which may be due to the influence of the reproductive cycle on this index (i.e., prespawning vs. postspawning) or to differences in spawning timing (Pierścieniak et al. 2010).

The increase in N_2 -fixing cyanobacteria was associated with low condition *Mytilus* but high growth status (Table 3). *Mytilus* might take advantage of the high P and N content in cyanobacteria during the summer bloom (Walve and Larsson 2007) directly or indirectly as a supplemental food source to invest in somatic growth (e.g., shown experimentally for other marine invertebrates, Karlson et al. 2015). Similarly, terrestrial organic material was an important predictor for better condition of *Mytilus* (TOC_{terr} , Table 3). Terrestrial organic material consists not only of suspended particles and additional nutrients, but also of associated microorganisms (e.g., Liénart et al. 2020) that can be used as supplementary food by

consumers when phytoplankton availability is low (e.g., Lefebvre et al. 2009).

Finally, environmental conditions directly impact the physiological status of *Mytilus* (Table 3). Particularly in the Baltic Sea, the low salinity reduces the scope for growth and affects respiration rate (Tedengren and Kautsky 1986). Accordingly, salinity was selected as final predictor in models for growth. High temperature was also associated with low growth rate and low body condition (Table 3). High temperature has been shown to induce physiological stress (Hiebenthal et al. 2013), to reduce *Mytilus* body condition and growth and to increase mortality, particularly when high temperatures last for several weeks (e.g., recurrent heat stress conditions; Seuront et al. 2019).

Mytilus population data

The models for *Mytilus* population abundance and biomass had lower predictive capacity compared to other models (Table 3), thus relationships should be interpreted with caution. Still, as expected (Hypothesis 4, see also Table S1), the highest-ranked predictors for decreasing population biomass at 5 m were diversity of diet (the isotopic niche metric $d\text{N}$) and quality of specific food items (terrestrial organic carbon, *M. rubrum*, total N, Table 3) together with lower salinity and high summer maximum temperatures. Predicted decreases in surface salinity in the Baltic Sea (Meier et al. 2012) would restrict both the distribution and growth of *Mytilus* (Westerbom et al. 2019). We note that N_2 -fixing cyanobacteria and terrestrial organic carbon were also included in both population biomass and abundance models, however, the direction of association (positive or negative) for these two predictors differed between population and individual condition models. Experimental studies on the direct influence of these two potential food sources on *Mytilus* individual growth and condition are therefore needed.

Over the entire region (comparing with other stations at 5 m depth, Fig. S1), *Mytilus* biomass decreased (Fig. 3; Table S4), while, abundance increased (Fig. 3). The pattern was slightly different at 10 m depth, where *Mytilus* biomass was more stable over time for all the stations but with a similar trend of increasing abundance (Fig. 3, including 14 m at Isskären; Fig. S3; Table S4). Our results support the role of temperature and salinity for population level data (similar to Franz et al. 2019; Westerbom et al. 2019) but additionally highlight the indirect link of climate change through changes in food quality (cyanobacterial blooms and terrestrial inputs).

The low predictive capacity of *Mytilus* population biomass and abundance models would likely be improved by adding predictors related to recruitment or to top-down effects (e.g., predation). In other parts of the Baltic Sea, such as coastal areas of Lithuania and Gulf of Finland, altered predation pressure from fish (e.g., increased abundance of the invasive round goby which fed mainly on blue mussels, Karlson et al. 2007), roach (e.g., Lappalainen et al. 2001) or diving

birds (e.g., Öst and Kilpi 1998) have been shown to reduce mussel populations (e.g., Skabeikis et al. 2019). To what extent *Mytilus* biomass decline could affect predators, in turn, is unknown. Since Baltic *Mytilus* is a keystone species providing habitat for many other species, decreasing biomass and/or abundance is likely to affect local biodiversity and community structure (Koivisto and Westerborn 2010) and thus the entire ecosystem functioning (Attard et al. 2020).

Conclusions

This study, although necessarily correlative in nature, links altered climate and eutrophication induced changes in food sources with changes in isotope composition, individual condition, and growth status and even population biomass and abundance of *Mytilus*, a keystone species in the Baltic Sea. Decreasing trends in *Mytilus* stable isotope composition were equally linked to its diet, especially for $\delta^{13}\text{C}$ (i.e., increase in N_2 -fixing cyanobacteria and terrestrial organic material from land runoffs) and physiology, especially for $\delta^{15}\text{N}$ (Hypotheses 1 and 2). Individual *Mytilus* condition and growth status, even though no time trends were detected, were associated to food quality and indirectly to abiotic factors (Hypothesis 3). Finally, the decrease in *Mytilus* population biomass was mainly related to diet trophic diversity as well as salinity decrease and temperature increase (Hypothesis 4). These findings highlight the importance of a holistic view of possible stressors and their direct and indirect links, from individual to population levels, when assessing the organismal effects from long-term environmental change.

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