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Carbon and nitrogen stable isotopes within the Swedish national monitoring of contaminants in marine biota

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<p>Summary This report gives an overview of spatial variation in carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes in different fish species in the Baltic Sea. The study also tries to investigate if differences in contaminants between sites and among species can be partly explained by nitrogen stable isotopes. Moreover, as a first attempt to understand the spatial trends in terms of biogeochemistry we look at the relationship between stable isotopes in fish and salinity as well as between stable isotopes and the ratio of total nitrogen to total phosphorus. The results in this study shows that there were some clear difference between the Baltic Proper and the West Coast for cod, eelpout, and herring in $\delta^{15}\text{N}$ and the results also showed clear spatial variations in $\delta^{13}\text{C}$ with more negative values in the most northern parts of the Baltic Sea. In addition, different clusters of herring was seen, which coincided with the different regions in the Baltic – Bothnian Bay, Bothnian Sea and Baltic Proper. The observed geographical patterns in stable isotope ratios indicate that regional processes set the foodweb baseline of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Significant differences in the concentration of CB-153 between herring and cod disappeared when the two species were adjusted for $\delta^{15}\text{N}$. The data in this report is limited to one year. Additional data will tell if stable isotopes can be confidently used as an important co-variable that can explain some of the between-year variation in contaminant concentrations, hence making samples among stations and species more comparable and increase the statistical power to detect trends and geographical differences.</p>	

Introduction

The Swedish National Monitoring Programme for contaminants (SNMPC) in biota, collects samples (fish, blue mussel, and guillemot eggs) from different locations along the Swedish coastline on a yearly basis (Fig. 1). Different contaminants (e.g., heavy metals, PAH, chlorinated, brominated, and perfluorinated compounds) are analysed and several biological variables, such as length, weight, condition, and age, are recorded for each specimen.

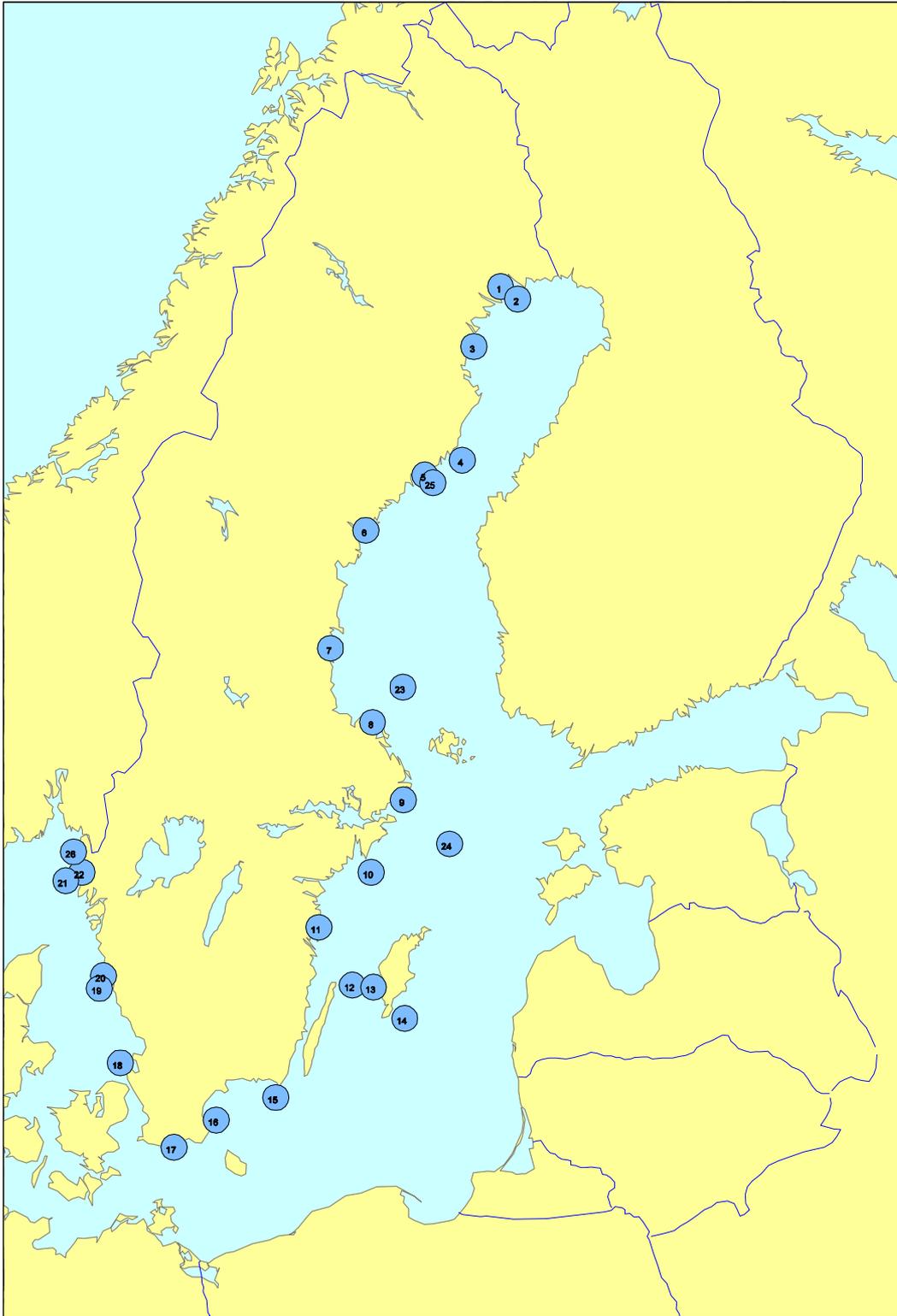
The main purpose with the monitoring is to continuously follow temporal trends of contaminants, to determine whether measures taken to reduce contaminant concentrations have had an effect, and to indicate large scale spatial differences.

Measured concentrations in biota give a picture of the contaminant load in the environment. However, there are several biological and ecological variables that may affect the concentrations. Within the monitoring programme a few biological variables are measured and they can then be used for adjusting the concentrations. Stable isotope analysis is a good way of describing different mechanisms in the ecosystem. More knowledge about these can give a better understanding in the evaluation of the contaminant load. The most commonly studied elements are nitrogen (N) and carbon (C). The ratio of the isotopes $^{15}\text{N}/^{14}\text{N}$ (hereafter referred to as $\delta^{15}\text{N}$) can be used to estimate the trophic position in a food web, that is because $\delta^{15}\text{N}$ of a consumer is enriched by 3-4‰ relative to its diet (Peterson and Fry, 1987). The ratio of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$) can instead provide information about the feeding source and carbon flow pathway in the system (Peterson and Fry, 1987, Post, 2002). In aquatic environments $\delta^{13}\text{C}$ can be useful for distinguishing between benthic production and pelagic production, because $\delta^{13}\text{C}$ is less negative in the benthic compared to the pelagic food web (France, 1995).

The information from $\delta^{15}\text{N}$ analysis can also be of use in status evaluation of contaminants based on Environmental Quality Standards (EQS) that are derived within EU to protect freshwater and marine ecosystems, as well as human health from adverse effects of chemicals. EQSs for 11 of the substances included in the Directive 2013/39/EU (EC, 2014), dealing with priority substances, are set in biota, where either top predators or humans have been considered as the most sensitive protection goal.

The guidance document no. 32 on biota monitoring (the implementation of EQS_{biota}) states that the biota standard should be applied in the most important link in the food chain, identified as the trophic level where concentrations peak. The recommendation for contaminants subjected to biomagnification is thus to use the average trophic level of top predator prey tissue, which have been identified as 3.5 and 4.5 for freshwater and marine environments respectively. A trophic level of 4 is recommended when human fish consumption is considered. Existing monitoring programmes, e.g., SNMPC, are designed to use species on lower trophic levels. The recommendation is to adjust the monitoring data to represent appropriate trophic level before comparing to EQS_{biota}. This can be done by using the trophic level of the monitored samples, preferably calculated from $\delta^{15}\text{N}$ data, together with trophic magnification factors (EC, 2014).

The aim of this study was to get a picture of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope profiles in fish collected within the SNMPC and to investigate possible spatial differences and dissimilarities among species. Additionally, possible correlations between the isotope profile and analyzed contaminant concentrations were also of interest to study.



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Fig. 1. Sampling sites within the National Swedish Marine Monitoring Programme; 1) Rånefjärden, 2) Harufjärden, 3) Kinnbäcksfjärden, 4) Holmöarna, 5) Örefjärden, 6) Gaviksfjärden, 7) Långvindsfjärden, 8) Ångskärsklubb, 9) Lagnö, 10) Landsort, 11) Kvädöfjärden, 12) Byxelkrok, 13) St. Karlsö, 14) SE Gotland, 15) Utlängan, 16) V. Hanöbukten, 17) Abbekås, 18) Kullen, 19) Fladen, 20) Nidingen, 21) Väderöarna, 22) Fjällbacka, 23) Bothnian Sea offshore site, 24) Baltic Proper offshore site, 25) Bonden, 26) Tjärnö.

Method

Collection of samples

Fish (herring *Clupea harengus*, perch *Perca fluviatilis*, cod *Gadus morhua*, and eelpout *Zoarces viviparus*) were collected at different sites along the Swedish coastline (Fig. 1). For each specimen, body weight, body length, sex, age, reproductive stage, state of nutrition, liver weight, and sample weight were recorded. Thereafter muscle samples were taken for stable isotope analyses. For some species and sites individual samples were used while for others pooled samples were used (see Table 1 for more information). The muscle samples were always taken from the middle dorsal muscle layer and the epidermis and subcutaneous fat tissue were carefully removed. For more information about sampling sites and collection of specimens, see Bignert et al. (Bignert et al., 2015). Lipid concentrations in muscle samples from the same individuals are available for herring, perch and eelpout from other analyses.

Table 1. An overview of how many samples that were used for each species and site. Where pooled samples were used, the no of specimens in each pool is presented in column four.

Species	Site	No of samples	No of specimens in each pool
Herring	Harufjärden	12	
	Utlången, spring	2	12
	Hanöbukten	2	12
	Kullen	2	12
	Väderöarna	12	
	Utlången, autumn	12	
	Kinnbäcksfjärden	2	12
	Ångskärsklubb	12	
	Långvindsfjärden	2	12
	Holmöarna	2	12
	Abbekås	2	12
	Fladen	12	
	Rånefjärden	2	12
	Byxelkrok	2	12
	Lagnö	2	12
	Gaviksfjärden	2	12
	Bothnian sea offshore	2	12
	Baltic proper offshore	2	12
	Landsort	12	
Perch	Holmöarna	10	
	Kvädöfjärden	10	
	Örefjärden	2	10
Cod	SE Gotland	10	
	Fladen	10	
Eelpout	Kvädöfjärden	2	6
	Fjällbacka	2	6

Preparation of samples and analyses of C and N stable isotopes

Frozen fish muscle samples were delivered from the Swedish Museum of Natural History in acid-cleaned and pre-weighed plastic vials. Pre-treatment, carried out by the Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University. The samples were freeze-dried for about one week and weighed before and after drying to check that the lyophilisation was successful and to enable calculation of the dry matter (%).

Freeze-dried and pulverized tissue samples were placed into 5x8 mm tin capsules (Säntis Analytical AG) and weighed. The target weight for dual-isotope analysis of biological samples was 1 ± 0.2 mg. The filled tin capsules were formed into small spheres using clean metal tools and placed into 96-well trays that were stored dry before being shipped to the Stable Isotope Facility, UC Davis, California. Sample forms with sample type, weight and tray position were sent electronically to the analysis lab. At least one blank sample (empty tin capsule) was placed in each tray during sample preparation.

Total carbon, total nitrogen and the isotope ratios $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were determined by EA-IRMS (Elemental Analysis – Isotope Ratio Mass Spectrometry) using a PDZ Europa ANCA-GSL elemental analyser in tandem with a PDZ Europa 20-20 isotope ratio mass spectrometer. Laboratory standards that are calibrated to certified reference materials and of similar composition as the samples, as well as blanks, are repeatedly analysed within the sample run.

Delta values $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are reported in ‰ relative to international standards V-PDB (Vienna PeeDee Belemnite) for carbon and Air for nitrogen. Laboratory long-term standard deviation is 0.2 ‰ for ^{13}C and 0.3 ‰ for ^{15}N .

Statistical methods

Before statistical analyses were performed, the $\delta^{13}\text{C}$ values were lipid normalised since we used different species with different fat percentage and that might otherwise influence the results. The C:N ratio have been shown to highly correlate with fat percentage (Post et al., 2007) and since we lack fat percentage for some of our samples, the C:N ratio was used instead.

The normalisation was conducted according to Post et al. (Post et al., 2007) using the equation;

$$\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N}$$

where C and N are the amount of C and N respectively.

Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between sites were tested in the four different species (herring, perch, eelpout, and cod) separately. In herring, the data was not normally distributed and no transformations proved efficient. Therefore the non-parametric Kruskal Wallis and Mann Whitney U test was used for comparing the different sites. For comparing the different sites in perch, one-way Anova and Fishers post-hoc test was used for $\delta^{13}\text{C}$ while Kruskal Wallis and Mann Whitney U test was used for $\delta^{15}\text{N}$, since the data was not normally distributed and no transformations proved efficient. In eelpout and cod, where only two sites were used, t-test was used to compare the two sites for each species. The significant level was set to $\alpha=0.05$.

Hotelling's 95% confidence ellipses for the centre of gravity for each group was also calculated and plotted for $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ (i.e. the ellipse in which 95% of all mean values of equally sized samples from the same populations is expected to fall within).

A Hotelling's T2 test including $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was carried out to check for significant differences between groups (i.e. regions). The significant level was set to $\alpha=0.05$.

Multiple regression analyses were used to study the relation between contaminant concentrations and $\delta^{15}\text{N}$ together with total length and muscle dry weight percentages. Residuals+100 from the mean values at each site for all the variables were used.

As a preliminary investigation of the potential influence of abiotic drivers, stable isotope ratios in fish and local water quality (salinity and nutrients) were compared. To get a representative measure of ambient conditions for each fish population we identified 1-3 of the nearest water monitoring stations and calculated the average concentrations at these stations using a subset of data from years 2012-2013. Anoxic layers were excluded from the calculations. Water chemistry data were retrieved from the marine monitoring database at SMHI

(<http://www.smhi.se/klimatdata/oceanografi/havsmiljodata/marina-miljoovervakningsdata>).

Results

A significant correlation ($p < 0.05$, $R^2 = 0.66$) between C:N ratio and fat percentage for herring and perch was found. This confirms Post et al. (2007) results with a high correlation between fat% and C:N ratio and supports the use of C:N ratio to normalise the $\delta^{13}\text{C}$ for the effect of lipid content.

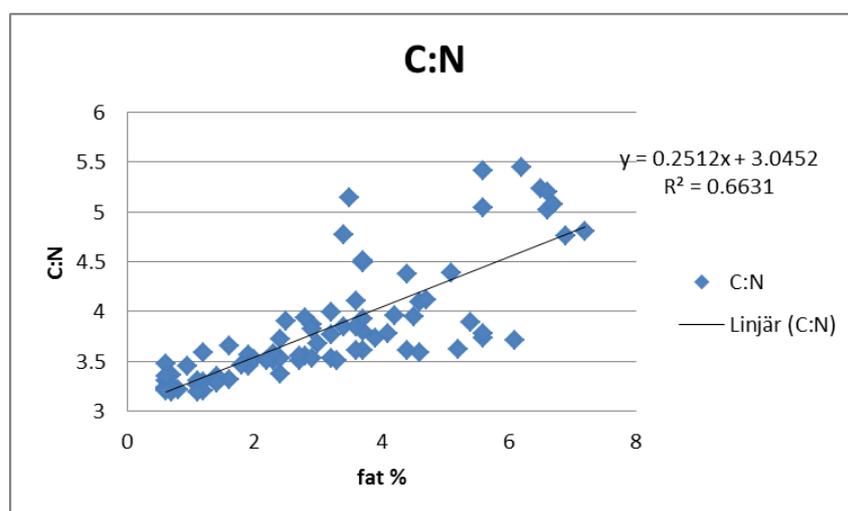


Fig. 2. A correlation between C:N and fat percentage for herring and perch.

Spatial variation

In herring, $\delta^{15}\text{N}$ was generally highest on the Swedish west coast and lowest in the Bothnian Bay and Bothnian Sea. Väderöarna on the west coast, had significantly higher $\delta^{15}\text{N}$ compared to Harufjärden ($p = 0.0005$), Ängskärsklubb ($p = 0.0000$), Långvindsfjärden ($p = 0.0021$), Gaviksfjärden ($p = 0.026$) and Landsort ($p = 0.015$). Moreover, Fladen, also on the west coast, had significantly higher $\delta^{15}\text{N}$ compared to Harufjärden ($p = 0.023$) and Ängskärsklubb ($p = 0.0001$) (Fig. 3).

For lipid normalised $\delta^{13}\text{C}$, Harufjärden in the Bothnian Bay had significantly lower $\delta^{13}\text{C}$ (more negative values) compared to Väderöarna ($p = 0.0000$) and Fladen ($p = 0.0000$) on the Swedish west coast but also compared to Utlängan autumn ($p = 0.0026$) and Landsort ($p = 0.0003$). Moreover, Väderöarna had significantly higher (less negative values) compared to Ängskärsklubb ($p = 0.0009$) and Rånefjärden ($p = 0.028$) (Fig. 4).

$\delta^{15}\text{N}$ in perch did not differ between the three sites (Fig. 5) while lipid normalised $\delta^{13}\text{C}$ was significantly higher (less negative) in Kvädöfjärden and Holmöarna compared to Örefjärden

($p=0.0009$ and $p=0.017$ respectively). In addition, Kvädöfjärden had significantly higher (less negative) $\delta^{13}\text{C}$ compared to Holmöarna ($p=0.033$) (Fig. 6).

In cod, both $\delta^{15}\text{N}$ and lipid normalised $\delta^{13}\text{C}$ were significantly higher in Fladen compared to SE Gotland ($p<0.0000$ for both) (Fig. 7, 8).

In eelpout, $\delta^{15}\text{N}$ was significantly higher from Fjällbacka on the west coast compared to Kvädöfjärden in the Baltic proper (Fig. 9), while there was no difference in lipid normalised $\delta^{13}\text{C}$ (Fig. 10).

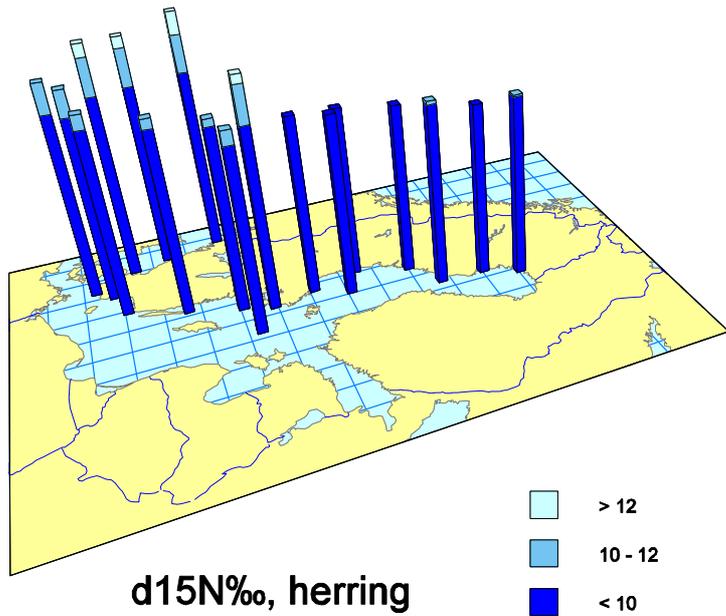


Fig. 3. Spatial variation in $\delta^{15}\text{N}$ for herring.

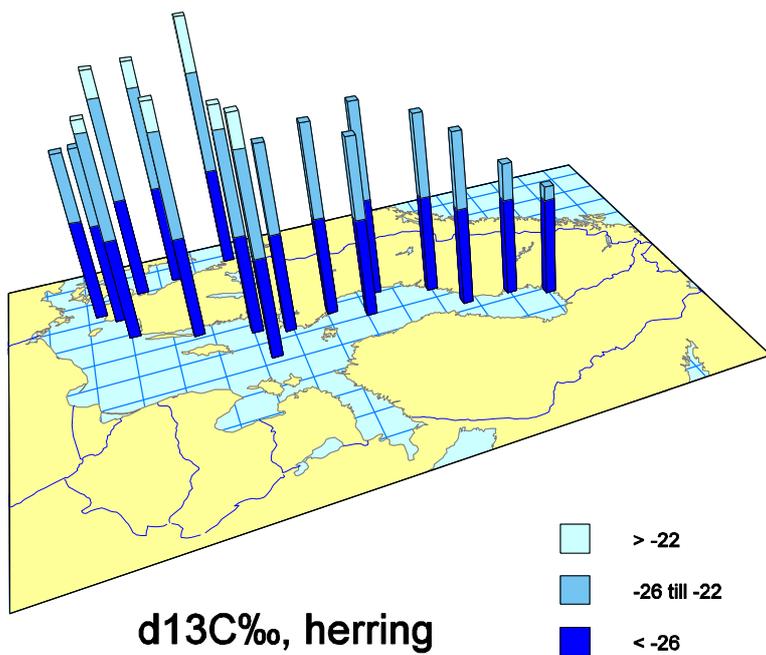


Fig. 4. Spatial variation in $\delta^{13}\text{C}$ for herring.

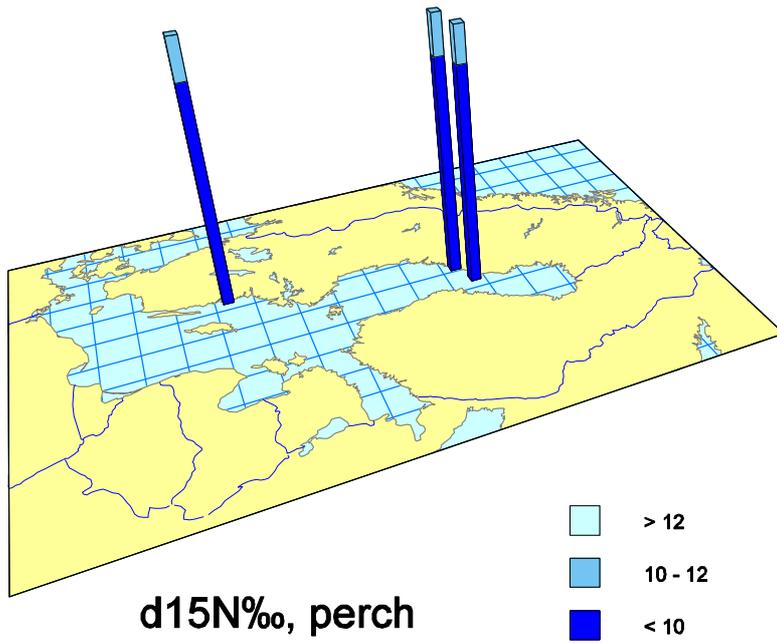


Fig. 5. Spatial variation in $\delta^{15}\text{N}$ for perch.

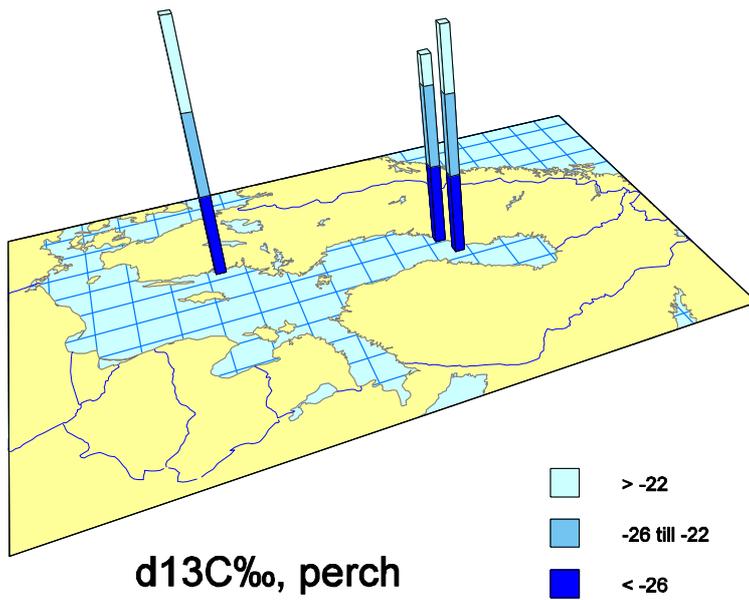


Fig. 6. Spatial variation in $\delta^{13}\text{C}$ for perch.

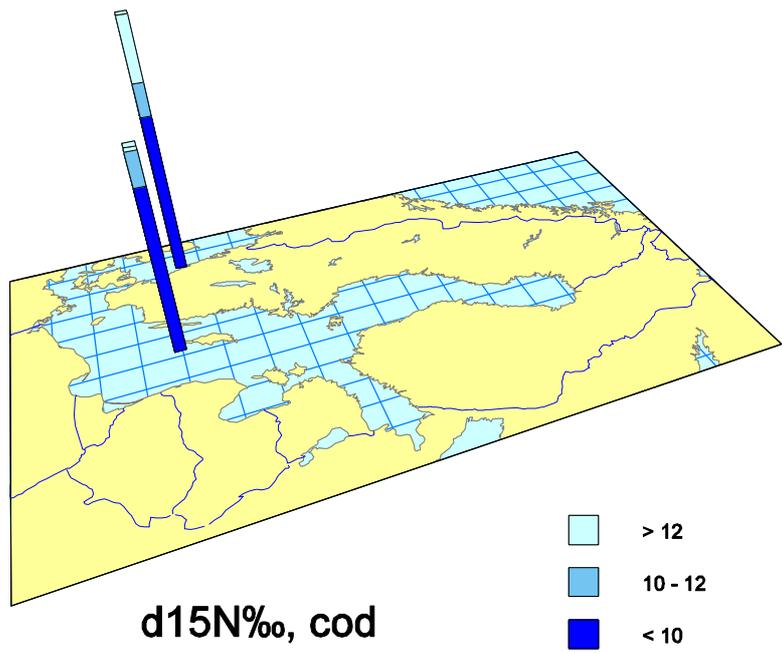


Fig. 7. Spatial variation in $\delta^{15}\text{N}$ for cod.

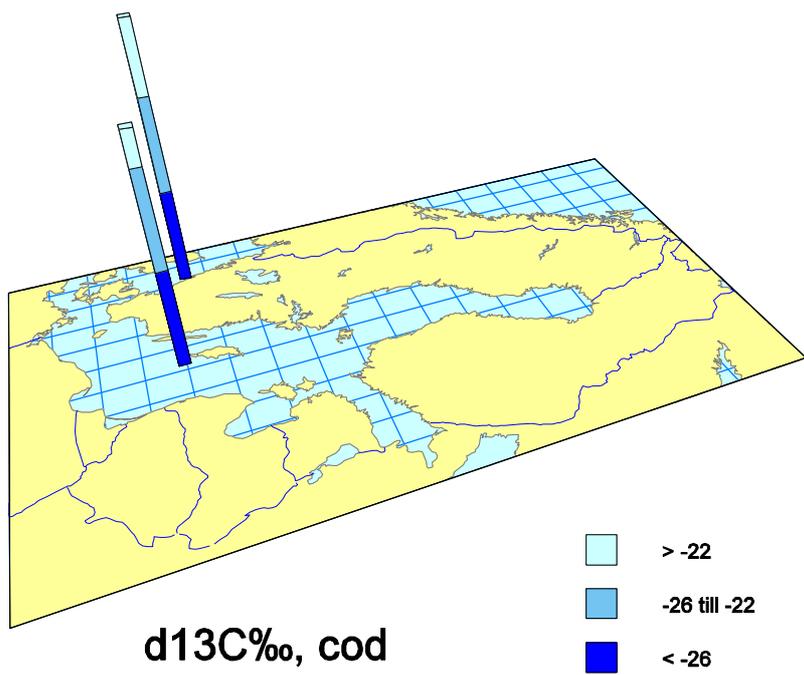


Fig. 8. Spatial variation in $\delta^{13}\text{C}$ for cod.

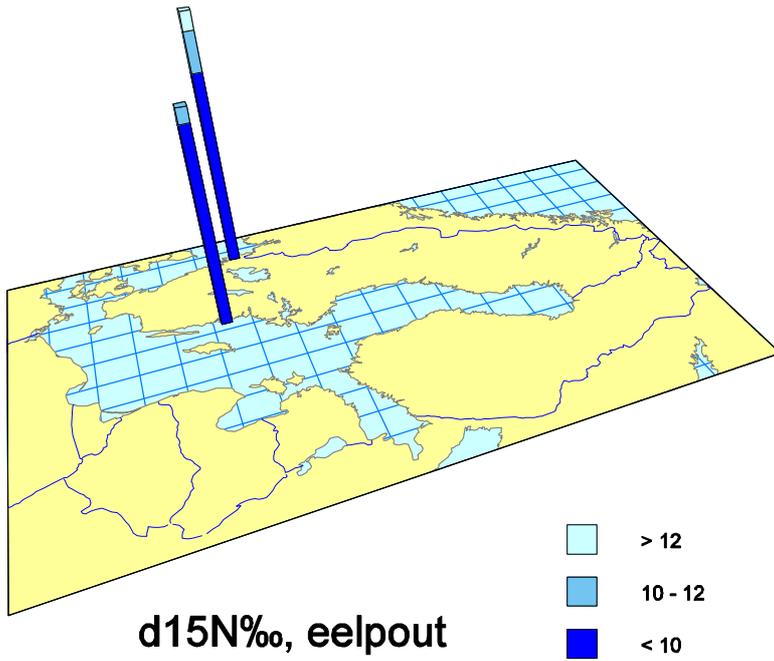


Fig. 9. Spatial variation in $\delta^{15}\text{N}$ for eelpout.

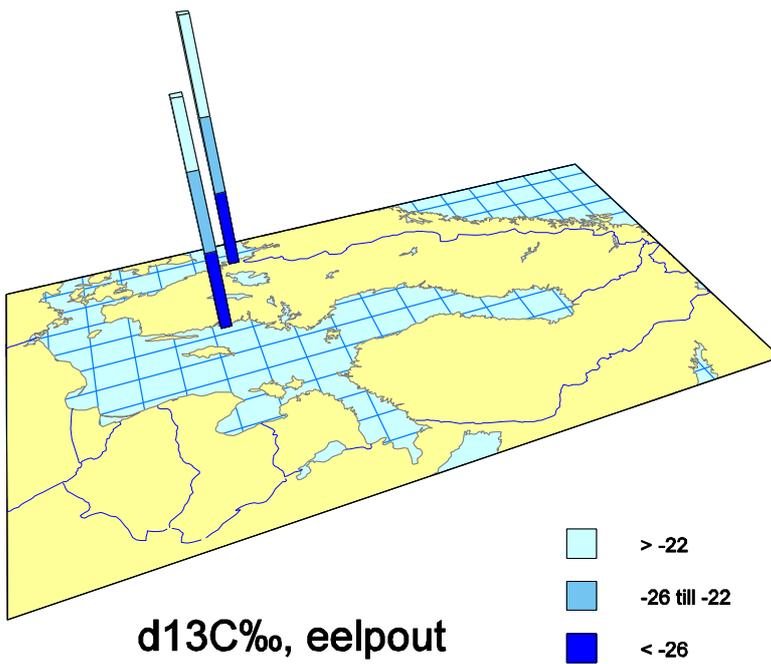


Fig. 10. Spatial variations in $\delta^{13}\text{C}$ for eelpout.

A plot of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ clearly grouped both species and sites (Fig. 11). Perch, Baltic Sea herring, west coast herring, Baltic proper cod and west coast cod were significantly different from each other (Fig. 11). Eelpout also seemed to follow the same pattern between the Baltic Proper and the west coast but due to only two pools in each group they were excluded from the statistical analysis. A plot of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ for herring (Fig. 12) clearly shows clusters, which coincide with different regions along the Swedish coast, i.e. Bothnian bay, Bothnian sea, Baltic proper, and the west coast. All regions were significantly different from each other. Moreover, the effect of using lipid normalised $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ (Fig. 13) can be seen in comparison with figure 12. The clusters are even more pronounced and the ellipses are between 7 and 47% smaller depending on region when the lipid normalised $\delta^{13}\text{C}$ values are used. Figure 14 shows a more detailed plot of $\delta^{15}\text{N}$ vs. lipid normalised $\delta^{13}\text{C}$ where all the herring stations are shown. This figure shows that the distances between two pooled samples, from the same site, are smaller than the longest distance between individual samples within one site. Thus, reducing the risk of extreme values influencing the mean value when using pooled samples.

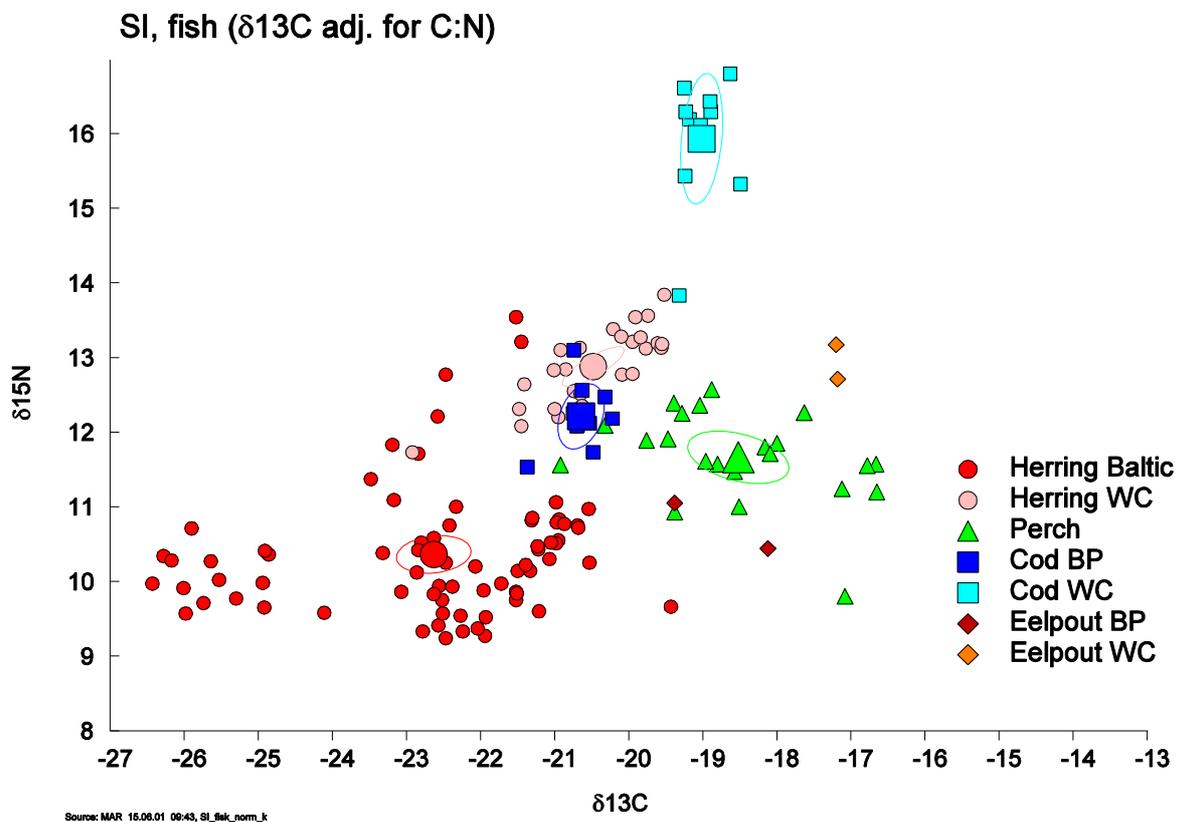


Fig. 11. $\delta^{15}\text{N}$ vs. lipid normalised $\delta^{13}\text{C}$ for the four fish species divided into east and west coast of Sweden. Larger symbols show the mean position for that group. Baltic means the whole Baltic Sea, WC=west coast, BP=Baltic Proper. Hotelling's 95% confidence ellipses for the centre of gravity for each group were also calculated and plotted.

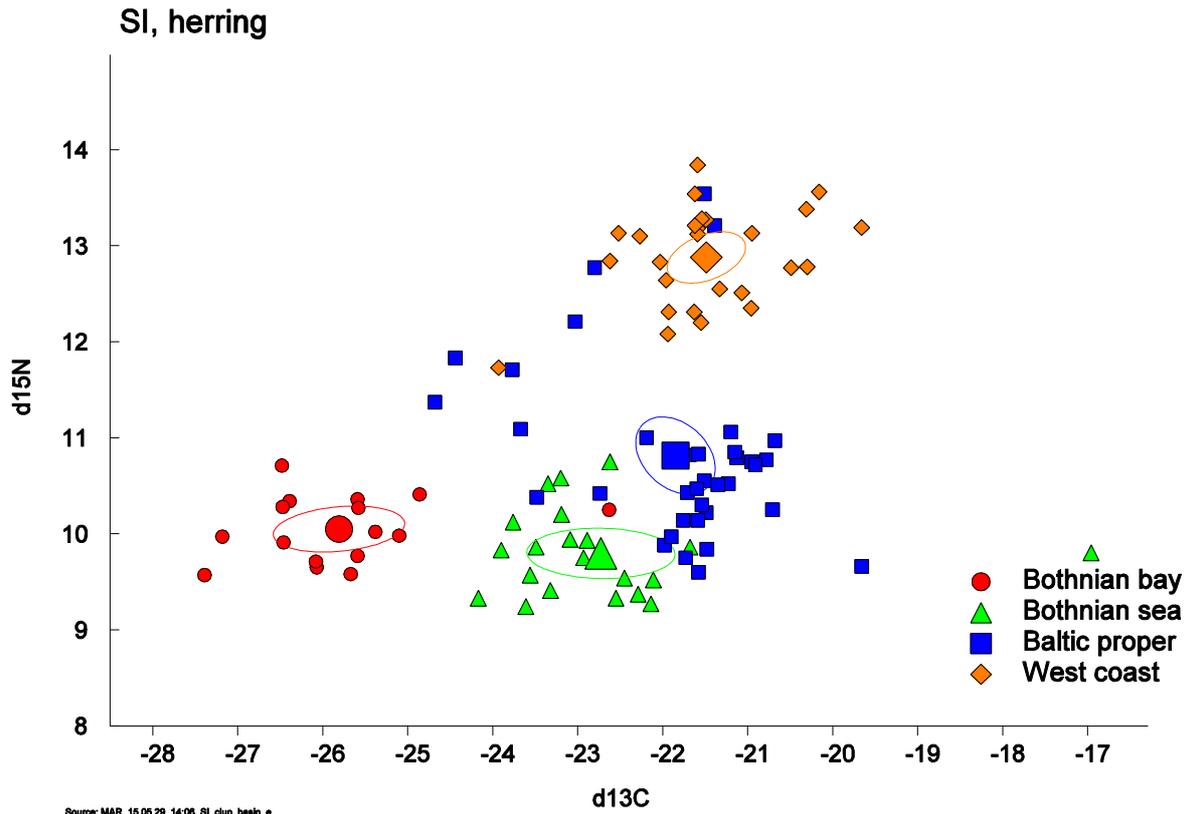


Fig. 12. A plot of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ in herring divided into four regions along the coast. Hotelling's 95% confidence ellipses for the centre of gravity for each group were also calculated and plotted.

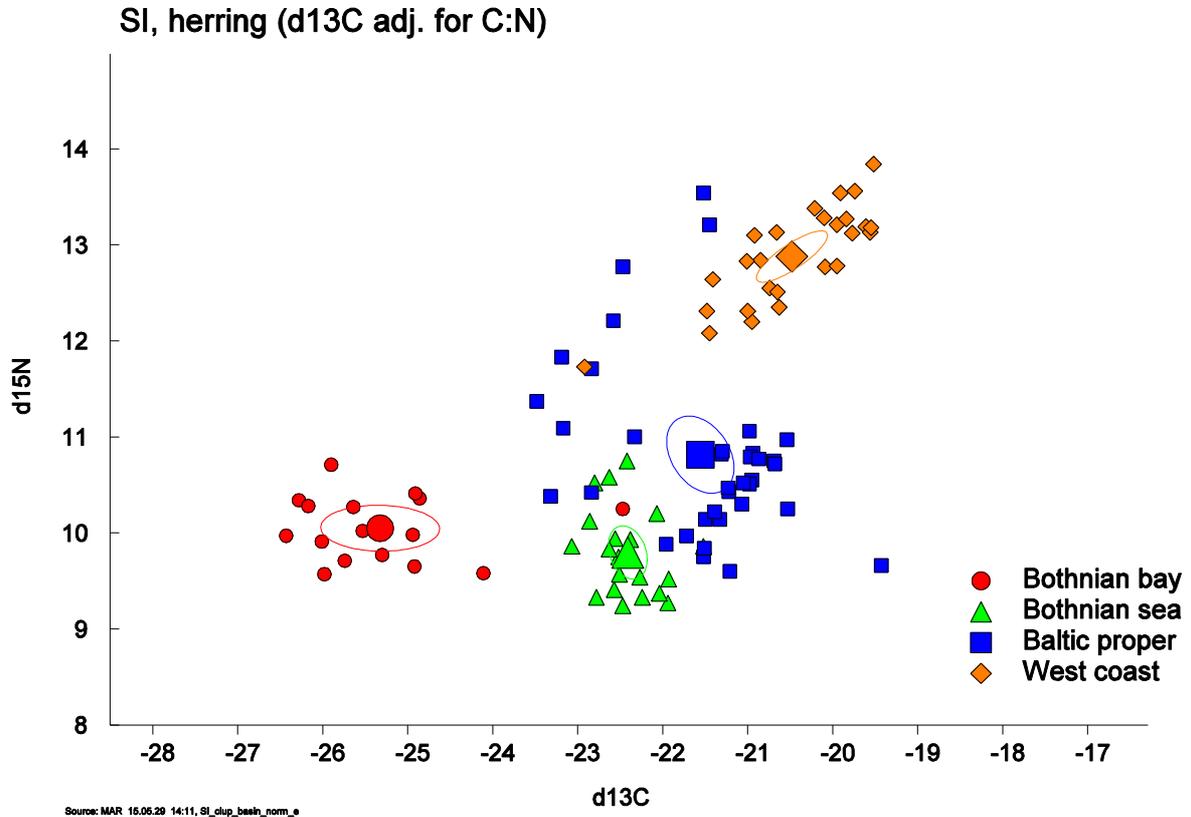


Fig. 13. A plot of $\delta^{15}\text{N}$ vs. lipid normalised $\delta^{13}\text{C}$ in herring divided into four regions along the coast. Hotelling's 95% confidence ellipses for the centre of gravity for each group were also calculated and plotted.

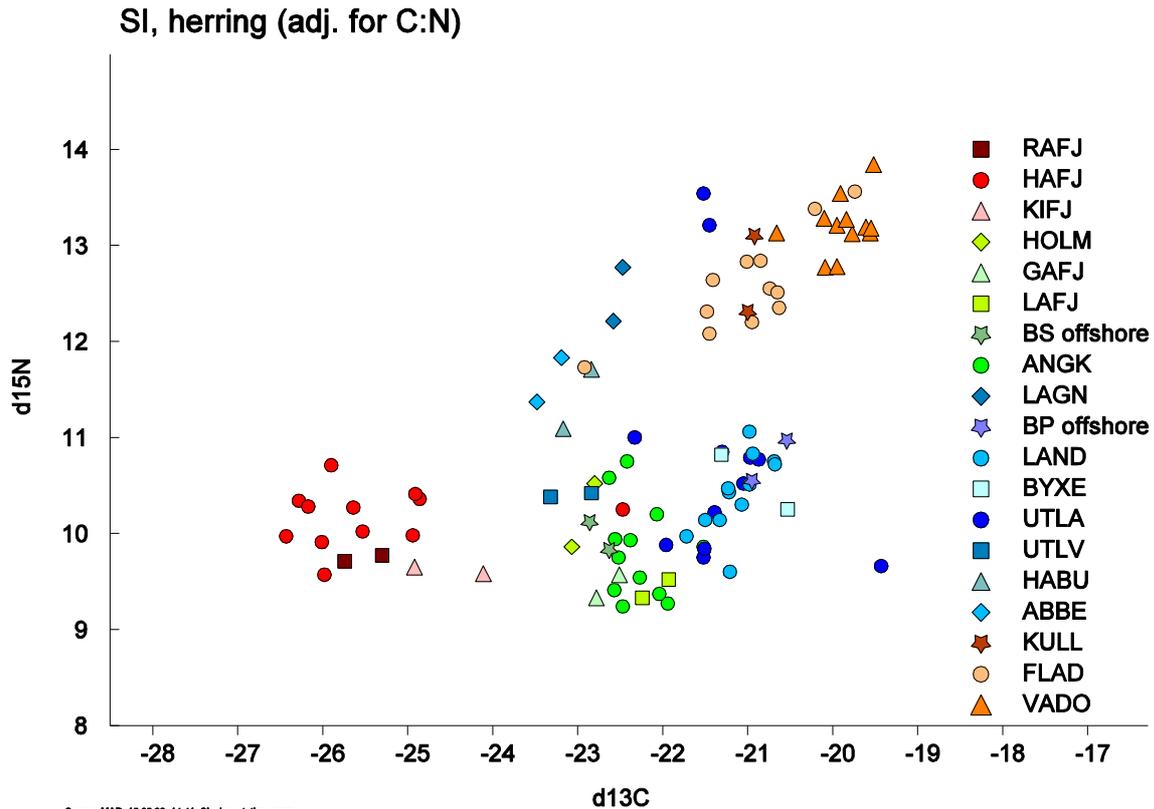


Fig. 14. A plot of $\delta^{15}\text{N}$ vs. lipid normalised $\delta^{13}\text{C}$ in herring divided into all stations used. RAFJ=Rånefjärden, HAFJ=Harufjärden, KIFJ=Kinnbäcksfjärden, HOLM=Holmöarna, GAFJ=Gaviksfjärden, LAFJ=Långvindsfjärden, BS offshore=Bothnian Sea offshore site, ANGK=Ängskärsklubb, LAGN=Lagnö, BP offshore=Baltic Proper offshore site, LAND=Landsort, BYXE=Byxelkrok, UTLA=Utlängan (autumn), UTLV=Utlängan (spring), HABU=Hanöbukten, ABBE=Abbekås, KULL=Kullen, FLAD=Fladen, VADO=Väderöarna.

The observed geographical patterns in stable isotope ratios indicate that regional processes set the foodweb baseline of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. These may be very complex, but for $\delta^{13}\text{C}$ they probably involve the mixing of terrestrial and marine carbon sources; and for $\delta^{15}\text{N}$ the nitrogen sources (atmosphere, rivers, sewage, manure) and transformation processes (N-fixation, denitrification, trophic transfer) would be important drivers. As a first attempt to understand the spatial trends in terms of biogeochemistry we look at the relationship between stable isotopes in fish and salinity as well as between stable isotopes and the ratio of total nitrogen to total phosphorus. The salinity gradient represents the mixing of marine water and freshwater inputs to coastal areas. The aqueous N:P-ratio is indicative of what nutrient that limits primary production, which in turn may relate to the prevalence of nitrogen fixation or losses by denitrification. The N:P ratio may also be affected by local pollution.

The herring $\delta^{13}\text{C}$ increases from about -26‰, indicative of influence of light terrestrial carbon sources, in the low-salinity Bothnian Bay to about -20‰ on the west coast (Fig. 15). There is considerable variability in the salinity range 5-10‰ where we find many stations. The variation may result from differences in the degradation of organic matter, but may also reflect dietary differences among populations or migration across sharp coastal salinity gradients. The general shape of the relationship suggests a steep mixing curve that levels off at a salinity of about 10‰. There are some southern stations Abbekås and Hanöbukten that appear to fall below the perceived mixing line. Nitrogen isotopes also exhibit a positive but less steep relationship with salinity.

Low N:P ratios are found on the west coast and in the southern Baltic Sea suggesting nitrogen limiting conditions. The N:P ratio increases northward with some local exceptions. At the lowest N:P, mean $\delta^{15}\text{N}$ in herring varies between 11-13‰, but as N:P increases $\delta^{15}\text{N}$ drops steeply to 9-10‰ (Fig. 15). Lagnö in the Stockholm archipelago is an outlier with $\delta^{15}\text{N}$ on the same level as the low N:P or high salinity stations. One explanation may be influence from high N input from Mälaren and the city of Stockholm that in combination with low-oxygen areas promotes denitrification, a process that results in outgassing of isotopically light N_2 and thus accumulation of heavier dissolved inorganic nitrogen available for uptake by microorganisms and trophic transfer. The two southern stations Hanöbukten and Abbekås also show elevated $\delta^{15}\text{N}$ (Fig. 15), possibly related to nutrient load from agriculture in this coastal area.

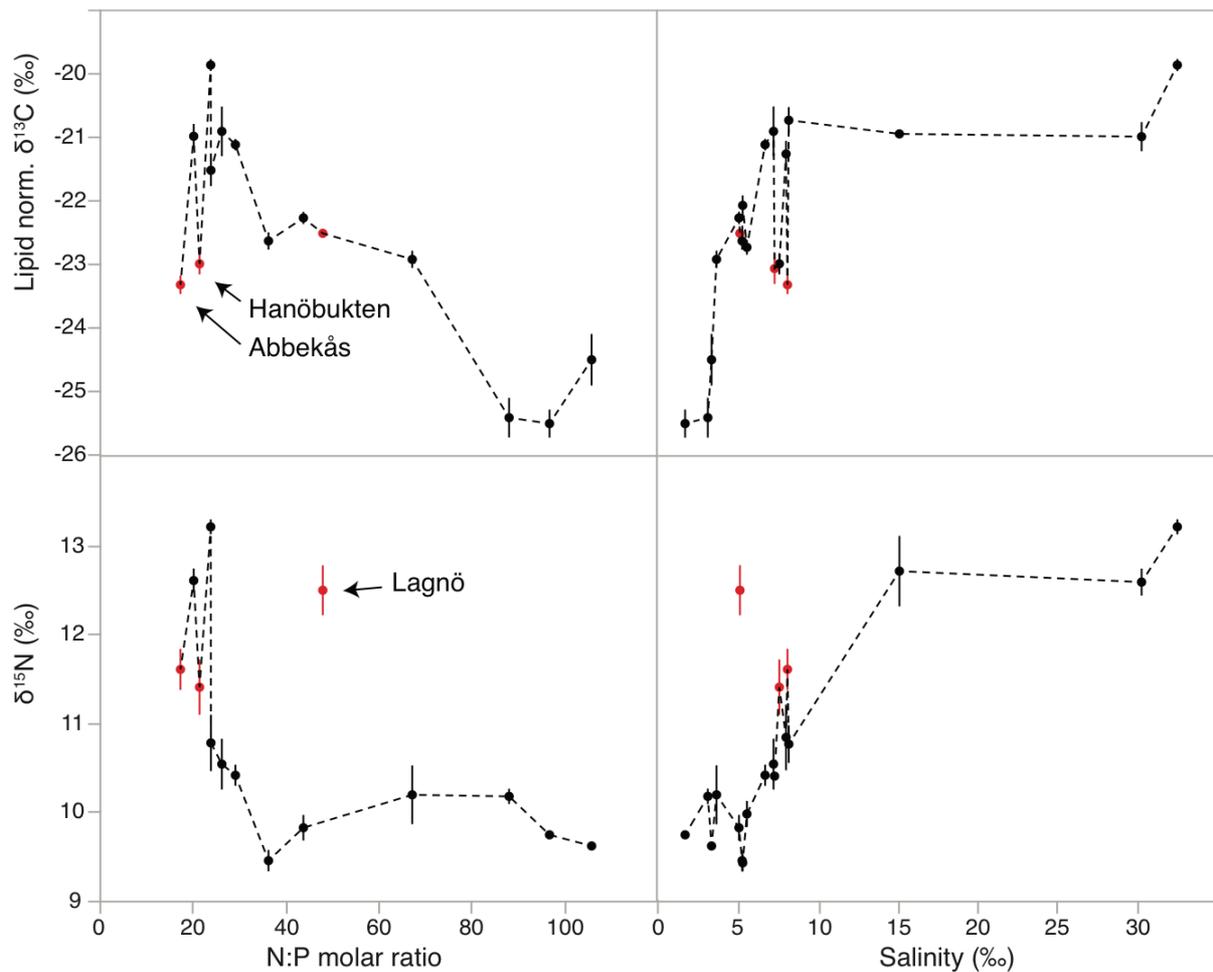
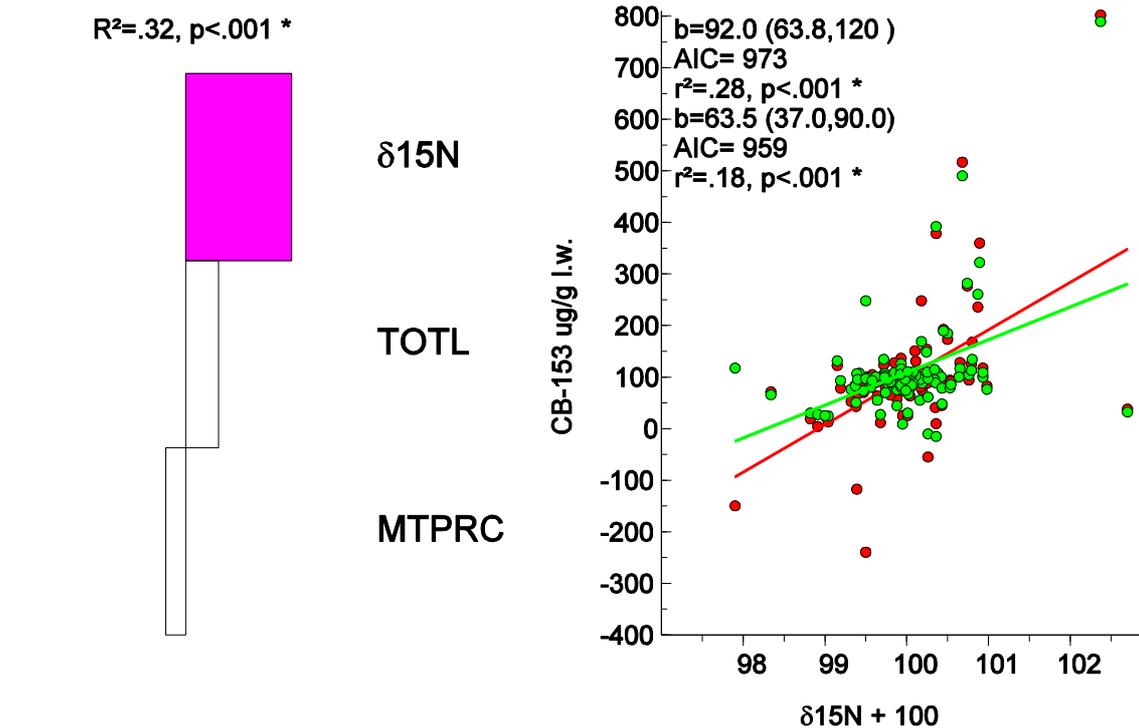


Fig. 15. Relationships between stable isotope ratios – lipid-normalised $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in herring, mean \pm SE – and salinity (right) or the total N to total P ratio (left). The fish were collected in autumn 2013 at different monitoring stations in Swedish coastal waters; water chemistry is represented by the mean of concentrations reported from nearby stations during 2012-2013.

$\delta^{15}\text{N}$ as a co-variable, explaining part of the variation in contaminant concentration

Multiple regression analyses were carried out for each contaminant as the dependent variable and $\delta^{15}\text{N}$ together with total length, muscle dry weight percentages and for herring also lipid content as independent variables. The relation between the contaminant concentrations and $\delta^{15}\text{N}$ were further evaluated with all co-variables kept constant (exemplified for CB-153, all fish species, in Fig. 16) (Table 2). For CB-153 (all fish species), the $\delta^{15}\text{N}$ was the most important and the only significant co-variable (purple box left panel and red line in right panel, Fig.16). Keeping length and muscle dry weight constant decreased the slope somewhat (green line, Fig.16) but also decreased the residual variance at the same time (c.f. the AIC for unadjusted and adjusted data). Subsequently, we can conclude that for some contaminants, the $\delta^{15}\text{N}$ significantly explains some of the variation (at least 18 % in the example below, less for the other contaminants, see Table 2) even when adjusted for other important co-variables.

CB-153 in all species vs $\delta^{15}\text{N}$ etc



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Fig 16. Left panel shows the size and significance (purple $p < .001$, white $p = \text{N.S.}$) of the beta-coefficients for the multiple regression analysis of CB-153 vs the residuals+100 of the included co-variables ($\delta^{15}\text{N}$, total length (TOTL), dry weight in muscle (%) (MTPRC)). Right panel shows CB-153 residuals vs residuals of $\delta^{15}\text{N}$ (red dots) and CB-153 vs $\delta^{15}\text{N}$, when total length and dry weight in muscle (%) are kept constant (green dots). AIC= Akaike information criterion.

Table 2. Partial r^2 , (contaminant vs $\delta^{15}\text{N}$ when abovementioned co-variables are kept constant) p-values and significant co-variables for residuals in herring and in all fish species for different contaminants. Totl=total length, F%=lipid content, MD%=muscle dry percentage.

	Herring		Significant co-variables	All fish		Significant co-variables
	r^2	p		r^2	p	
Hg	0.12	0.004	$\delta^{15}\text{N}$.14	0.001	$\delta^{15}\text{N}$
DDE	0.04	0.075	totl	0.09	0.001	$\delta^{15}\text{N}$, totl, MD%
HCB	0.01	NS	F%	0.00	NS	totl, MD%
CB- ¹⁵³	0.24	0.001	$\delta^{15}\text{N}$	0.18	0.001	$\delta^{15}\text{N}$,
BD-47	0.05	0.058	F%, totl, MD%	0.09	0.016	$\delta^{15}\text{N}$, MD%
HBCD	0.01	NS	totl	0.00	NS	totl, MD%

The geometric mean concentration of CB-153 (ng/g lipid weight) was significantly higher in cod than in herring at Fladen at the Swedish west coast. Higher $\delta^{15}\text{N}$ in cod compared to herring indicates a higher trophic level for cod. In Fig 17, CB-153 concentrations in herring and cod were adjusted i.e. estimated at constant $\delta^{15}\text{N}$ (=the average $\delta^{15}\text{N}$ of the two species). The significant difference in concentration disappears and the geometric mean concentration in the $\delta^{15}\text{N}$ adjusted herring becomes similar to $\delta^{15}\text{N}$ -adjusted cod.

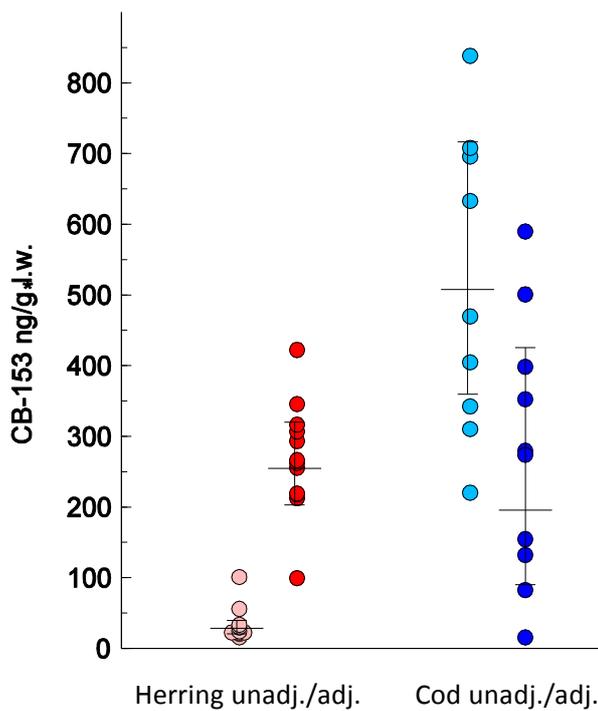


Figure 17. Unadjusted CB-153 concentrations in herring (pink) and cod (blue) were adjusted i.e. estimated at constant $\delta^{15}\text{N}$, red and dark blue respectively. The significant difference in concentration disappears and the geometric mean concentration in the $\delta^{15}\text{N}$ adjusted herring becomes similar to $\delta^{15}\text{N}$ -adjusted cod (dark red and blue dots).

Discussion

This study was a first attempt to get an overview of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in fish collected within the Swedish Environmental Monitoring Programme. There were some clear differences between Baltic proper and the west coast for cod, eelpout and herring $\delta^{15}\text{N}$, and the results also showed clear spatial variations in $\delta^{13}\text{C}$ with more negative values in the most northern parts of the Baltic Sea.

Several reasons can be suggested to explain the differences in $\delta^{15}\text{N}$ seen between the East and West Coast. The differences could be a result of higher species diversity on the West Coast, possibly leading to a longer food chain and therefore higher $\delta^{15}\text{N}$ values in the same species compared to the East Coast. Another explanation could be that in the Baltic proper there are cyanobacteria blooms which in turn cause reduced $\delta^{15}\text{N}$ in both primary producers and primary and secondary consumers (Nordström et al., 2009, Karlson et al., 2014, Lesutiene et al., 2014). The $\delta^{15}\text{N}$ in the baseline in the Baltic and the West Coast can also differ. Thus, in order to be able to evaluate stable isotope data properly, and the differences between areas, there is a need for using a baseline organism, which the data can be compared to (Post, 2002). Otherwise it is difficult to know if a variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among sites or species is due to changes in the food web structure and carbon flow, or if it is due to a variation in the baseline. Our observation of a somewhat predictable relationship between $\delta^{15}\text{N}$ of herring, a species with a diet that is assumed to be trophically narrow, with salinity and nutrient ratios suggests that there indeed is a variation in the baseline. With an established baseline it would also be possible to calculate the trophic position within a food web (Post et al., 2000), which in turn can be useful for, for example, comparisons of contaminant concentrations from different sites or in different species (Fig. 17) taking into account differences due to possible bioaccumulation and biomagnification. This is particularly important considering the European Commission Guidance document suggesting normalisation to a specific trophic level in status evaluation (EC, 2014).

Since there was a clear difference between our basins it would be a good idea to collect baseline organisms from these four areas. Zooplankton and bivalves are commonly used as baseline organisms in stable isotope analyses (Matthews and Mazumder, 2005, Solomon et al., 2008, Kopp et al., 2015). Blue mussel has been suggested as a good baseline organism (Post, 2002) and the EC guidance document no. 32 also suggests the use of molluscs as a baseline organism for stable isotope analyses (EC, 2014). Blue mussel is a primary consumer and it belongs to the trophic position 2 (Post et al., 2000, Post, 2002). Blue mussel is already collected within the Swedish Environmental Monitoring Programme, making it an obvious choice. However, blue mussel is collected from the west coast and the Baltic proper, but not from the Bothnian Sea or Bothnian bay. Hence, we lack specimens from the north of Sweden that could work as a baseline organism. In addition, the benthic amphipod *Monoporeia affinis*, which is collected in the national and regional monitoring for other purposes, could also be an option or a complement to use as a baseline organism for the Baltic Sea.

In this study, carbon and nitrogen have been analysed. However, in addition, sulphur stable isotopes have been suggested as a good complement to carbon stable isotopes (Peterson and Fry, 1987, Croisetiére et al., 2009). That is because sulphur isotopes can distinguish between food webs that are based on sedimentary detritus and living algae (i.e. feeding in the water column) and this can be important for understanding the flow of both nutrients and contaminants, since sediment can be a sink. Sulphur isotopes has recently also been used to estimate residency in estuarine fish (Fry and Chumchal, 2011).

The data in this report is limited to one year. Additional data will tell if stable isotopes can be confidently used as an important co-variable that can explain some of the between-year variation in contaminant concentrations, hence making samples among stations and species more comparable and increase the statistical power to detect trends and geographical differences. Because of the relatively low cost in comparison with most of the analysed contaminants within the national program in biological samples, we would encourage the analyses of stable isotopes in coming years and to re-evaluate their potential benefits as soon as more data is available. A cost-effective way to reach that point sooner would be retrospective analyses of preserved samples from previous years. There are freeze-dried samples of fish muscle and blue mussel collected and prepared within SNMPC during six consecutive years (2007-2012) before the current batch of samples.

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