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Migratory patterns and cyanotoxin concentrations of pikeperch (*Sander lucioperca*) in the coastal waters of the Baltic Sea

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We used stable isotopes of sulfur (δ^{34}S) and carbon (δ^{13}C) to distinguish resident and migratory pikeperch (*Sander lucioperca*) among individuals captured from a coastal freshwater lagoon (Curonian Lagoon) and adjacent Baltic Sea waters. We found that non-migratory pikeperch collected from the lagoon had negative δ^{34}S ratios (–1.84‰ to –0.17‰), whereas migrating individuals exhibited higher and more variable δ^{34}S values (4.4‰ to 18.5‰). Our findings suggest that S isotopes may be a valuable tool for assessing migratory habits in the brackish waters. We also compared cyanotoxin concentrations among the resident individuals of Curonian Lagoon and the migratory individuals in or returning from the Baltic Sea. No difference in toxin levels was observed among the resident and migratory pikeperch, although toxin concentrations were frequently close to or exceeded the recommended concentrations for safe long-term human consumption.

Introduction

Large-bodied fishes are ecologically important components of food webs and have high societal value as commercial and recreational fisheries (Rogers *et al.* 2010, Ljunggren *et al.* 2010, Bergström *et al.* 2015). Knowledge of migratory habits is an important component of fishery management as it relates to the ability to acquire resources for growth and reproduction (Saulamo and Thoresson 2005). The pikeperch is a migratory predator and valuable commercial fish occurring in coastal waters of the Baltic Sea (Lehtonen *et al.* 1996). Recent declines of pikeperch in the Baltic Sea have been attributed to overfishing (Mustamäki *et al.* 2014).

Pikeperch have a high salinity tolerance and can move long distances in a short time (Fickling and Lee 1985, Brown *et al*. 2001). They spawn in river inlets and shallow bays, using either fresh or brackish water habitats for growth (Skóra 1996, Lappalainen *et al.* 2003). The freshwater

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Curonian Lagoon (SE Baltic Sea) contains a stock of pikeperch that includes migratory and non-migratory individuals (Ložys 2004). The migration of pikeperch from freshwater bays to the sea is attributed to several factors, including avoidance of competition for food (Lehtonen *et al.* 1996) and reduced energy costs for osmotic regulation (Ložys 2004). Our prior work has documented the benefits of migration by showing that migrating pikeperch gained significantly higher fat content and body weight than non-migratory individuals (Ložys 2004). Migratory behavior of pikeperch was studied using otolith microchemical analysis which suggested, that the majority of the population resides in the lagoon, while a small proportion of the population migrates between the lagoon and the Baltic Sea spending on average (± SD) 25% ± 14% of their lifetime in brackish waters (Ložys *et al*. 2017). Lithuanian fisheries data show a recent increase in the annual pikeperch catch to over 100 t in the Curonian lagoon and a simultaneous decline in the Baltic Sea catch from 48 t to 1.5 t since 2002.

Stable isotope analysis (SIA) has been used to infer migratory habits of fish (Clément *et al.* 2014, Hart *et al.* 2015) and has the benefit of providing information on dietary sources, as well as trophic position (Cabana and Rasmussen 1994, Adams and Paperno 2012). Retention of the isotopic signal from the feeding habitat is in part related to the tissue isotopic turnover rate, which is a function of tissue growth and metabolism, the latter being more important in adult individuals (Fry and Arnold 1982, Bosley *et al.* 2002, Dattagupta *et al.* 2004). As a result, isotopic equilibration of tissues after migration is a relatively slow process. The length of time required for 50% equilibration could be 3–4 months in large pikeperch (calculated as a function of body mass according to Vander Zanden *et al.* 2015). The full equilibration takes longer, and therefore it could be expected that individuals frequently shifting between a freshwater and a marine habitat are isotopically differentiated from individuals with greater site fidelity. Sulfur isotope ratios (δ^{34}S) have received less attention in comparison to carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes in studies of migratory fish (Weber *et al.* 2002, Fry and Chumchal 2011, Godbout *et al.* 2010, Swanson *et al.* 2011). This is in part because site specific research is needed to differentiate freshwater and marine local baselines based on sulfur. Sea water sulfates exhibit δ^{34}S values of ~20‰ (Fry and Chumchal 2011), whereas sulfur isotopes in freshwaters are usually depleted in ^{34}S, relative to marine-derived material (Peterson and Fry 1987, MacAvoy *et al.* 2000). The δ^{34}S values of sulfates from different riverine ecosystems (i.e., the freshwater endpoint) can be more variable than in the ocean, but mostly fall in the range between –5% and +15‰ (reviewed by Nehlich 2015).

Cyanobacterial toxins (microcystins and nodularin) affect fish by inducing alterations in many processes, such as growth rate and osmoregulation, increased liver enzyme activities, heart rate, modified behavior, histopathological effects and so on (Malbrouck and Kestemont 2006 and references therein). Their presence in fish tissues poses a concern for fish health, as well as for human exposure via the consumption of fish (Wilson *et al.* 2008, Garcia *et al.* 2010, Poste *et al.* 2011, Acuña *et al.* 2012). A number of studies have reported the presence of nodularin (produced by *Nodularia spumigena)* in seston, as well as biota in the open waters of the Baltic Sea (Sipiä *et al.* 2006, Karjalainen *et al.* 2008, Kankaanpää *et al.* 2009, Mazur-Marzec *et al.* 2013). Our recent studies of the Curonian Lagoon have documented the occurrence of bloom events caused by species that produce microcystins (e.g. *Microcystis* and *Planktothrix* (Bresciani *et al.* 2012, Lesutienė *et al.* 2014) and the presence of microcystins in water, sediments and higher trophic levels (Paldavičienė *et al*. 2009, Paldavičienė *et al.* 2015, Šulčius *et al*. 2015, Bukaveckas *et al.* 2017). These and other studies (e.g. Wood *et al*. 2014) have reported high intra-specific variation in tissue microcystin concentrations, but did not consider whether variation among individuals could be explained in part by migratory habits.

The primary objective of this study was to use δ^{34}S and δ^{13}C isotope ratios to distinguish between migrating and resident pikeperch in the Curonian Lagoon. A secondary objective was to characterize cyanotoxin levels in pikeperch and to determine whether these differed among resident and migratory individuals.

Material and methods

Study area

The Curonian Lagoon is a sub-estuary of the Baltic Sea and the largest lagoon in Europe. It has a surface area of 1584 km^2, and a mean depth of ~3.8 m. It is connected to the Baltic Sea by the narrow Klaipeda strait (Fig. 1) and receives occasional inputs of brackish water (salinity 7 PSU) during wind-driven intrusions. The average salinity in the Klaipeda Strait is 2.5–3, whereas the main body of the lagoon is freshwater (salinity < 0.5 PSU) (Zemlys *et al.* 2013). The lagoon is hypertrophic, as indicated by high chlorophyll *a* and nutrient concentrations. Summer chlorophyll *a* is typically greater than 40 µg l^{-1} (max > 400 µg l^{-1}) and is dominated by cyanobacteria, mainly *Aphanizomenon flos-aquae* (Bresciani *et al.* 2012). The coastal water habitat is affected by the hypereutrophic lagoon waters in the plume area (Jaanus *et al*. 2011). In the plume, maximum chlorophyll *a* values can reach those in the lagoon, reported above, however the variation is high 0–156 µg l^{-1}, whereas outside the plume area, chlorophyll *a* concentrations are low < 3 µg l^{-1} (Vaičiūtė *et al.* 2012).

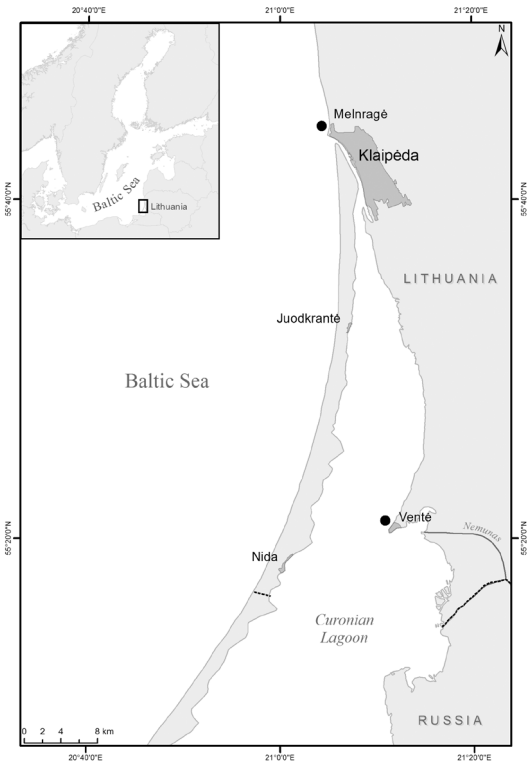

Fig. 1. Map of the study site. Dots indicate fishing areas in the Baltic Sea (close to Melnragė settlement) and Curonian Lagoon (Ventė) from which pikeperch were collected.

Sampling and sample analysis

Commercial fisheries in the Curonian Lagoon target pikeperch above the minimum size limit of 46 cm, which is typically reached by 4–5 years of age (Virbickas *et al*. 1974). Commercial size pikeperch for stable isotope and cyanotoxin analysis were collected in the northern part of the Curonian Lagoon and the coastal area of the Baltic Sea in September–October 2014 (Fig. 1). The fish, a total of 35 individuals, were obtained by commercial fishermen using gillnets and immediately transported to the laboratory. Length and weight were measured. The liver and muscle tissue from the dorsal part of the fish were dissected and dried at 60 °C for 48 h. Nine liver samples for SIA were lost due to incomplete sampling procedures. Bivalves (*Dreissena polymorpha* and *Mytilus edulis*) were collected to provide a baseline for estuarine and marine isotopic end-points, respectively. *Dreissena* were obtained as by-catch from fishing nets. *Mytilus* were collected during earlier studies, in 2012, from hard substrates located ~20 km northwards from the lagoon inlet. More recent efforts to collect this species were hampered by their dramatic decline after invasion by round goby (*Neogobius melanostomus*). Mollusks were washed and placed overnight in filtered water for gut content evacuation. Whole soft tissues of small *Dreissena* (~1 cm) and *Mytilus* (~2 cm) were dissected. All samples were dried at 60 °C for 48 h and ground into a fine powder in an agate mortar. Fish and mussel samples were weighed (0.5–0.7 mg for C and N; 1.2–2.0 mg for S) in tin capsules for SIA.

Carbon and nitrogen isotope compositions, as well as %C and %N, of fish and mollusk samples was determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Ratios of sulfur

isotopes were determined using an Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 IRMS (Sercon Ltd., Cheshire, UK). All analyses were performed at the Stable Isotope Facility, University of California, Davis, CA, USA. The results of isotopic ratios were compared to conventional standards, (i.e., Vienna Peedee Belemnite for carbon, atmospheric N_2 for nitrogen and Vienna Canyon Diablo troilite for sulfur). The results were expressed as δ values: $\delta X = [(R_{sample}/R_{standard}) - 1)] \times 10^3$ (‰), where X = ^{13}C, ^{15}N or ^{34}S, and $R = {}^{13}C/{}^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$, respectively. Repeated analyses of homogeneous material yielded standard deviations of less than 0.08‰ for carbon, 0.2‰ for nitrogen and less than 0.5‰ for sulfur. The long-term reproducibility of $\delta^{34}S$ measurements at the UC Davis Stable Isotope Facility is ±0.4‰. Lipid removal was not performed prior to analysis, in order to not bias the $\delta^{15}N$ values. The fat content in the muscle was low and the C:N ratio (± SD) of 3.2 ± 0.04 was below the recommended limit for aquatic organisms (C:N > 3.5), at which the lipid correction of $\delta^{13}C$ values should be performed (Post *et al.* 2007). In most of the liver samples, the C:N ratio was higher than 7 (on average 7.59 ± 3.03), i.e., outside the interval for which a linear normalization function could be applied for aquatic organisms (Post *et al.* 2007). Therefore, we excluded $\delta^{13}C$ values in the liver for further interpretation.

The cyanotoxins microcystin and nodularin were analyzed by an enzyme-linked immunosorbent assay (ELISA) using the Microcystin-ADDA ELISA microplate kit (Abraxic, USA). The ELISA method does not discriminate between nodularin and microcystin variants. Therefore, the total concentrations of cyanotoxins as a sum of microcystins and nodularin were reported (Karjalainen *et al.* 2008). The ELISA kit has a detection limit of 0.05 µg l^{-1} and was used according to the manufacturers' instructions. For cyanotoxin extraction, dried tissues samples (1.04 ± 0.55 SD g DW) were ground with a mortar and pestle, and extracted in 5 ml 75% methanol in water at +4 °C for 24 h (Garcia *et al.* 2010, Wilson *et al.* 2008). Extracts were centrifuged at 4500 rpm for 15 min. The supernatant was diluted with deionized water at least 15 times. A positive control with a toxin concentration (± SD) of 0.75 ± 0.185 µg l^{-1} and negative control samples supplied in the ELISA kit were used for quality control measures. The absorbance was recorded using a LabSystems Multiscan RC (Thermo Scientific) plate reader at 450 nm. Samples with concentrations above the upper limit of quantitation (5 ppb) were diluted and reanalyzed. All samples, calibration standards and controls were analyzed in duplicate. The mean standard error among duplicate tissue samples was 0.031 µg g^{-1} DM.

A section of pikeperch muscle was removed for fat content analysis (the content of total lipids in muscle) using a modified method described by Svedäng and Wickström (1997). Approximately 35–45 g of bone- and skin-free muscle tissue was homogenized. The proteins of the sample were digested by boiling in hydrochloric acid (4 M) for 1 h. The digested solution was filtered. The fat, remaining on the filter, was dried and extracted with 150 ml of petroleum ether, boiled for 90 min at 150 °C and rinsed for 60 min (Soxtec-method; Gerhardt Soxterm fat extraction system). After extraction, samples were dried at 103 °C for 1 h in a mechanical convection oven and weighed (± 0.001 g). The fat content (%) of the sample was calculated according to the formula: $W = [(M_2 - M_1)/M_0] \times 100$, where M_1 is the mass of the empty extraction beaker, M_2 is the mass of the extraction beaker with fat after drying and M_0 is the weight of the sample at the start of the analysis.

Data analysis and calculations

If not stated otherwise, the results are reported as means with standard deviations (SD). A *t*-test and one-way ANOVA were used to compare various parameters (e.g., stable isotope ratios, toxin levels, fat content) among groups classified according to $\delta^{34}S$ values. Levene's test was used to test the homogeneity of variances prior to ANOVA and regression analysis. Post-hoc Tukey HSD test was used to compare group means. Non-parametric tests (Mann-Whitney *U* and Kruskal-Wallis) were used in cases where variances differed significantly among groups. The Kruskal-Wallis test followed by post-hoc comparisons of mean ranks of all pairs of groups

was used to find significant differences among groups classified according to δ^{34}S values. Because of the size differences between the collected individuals in the lagoon and in the sea, an analysis of covariance (ANCOVA) was performed using body length as a covariate to compare cyanotoxin concentrations and trophic position between migrating and sedentary individuals. Fulton's condition factor (K) was calculated using a function $K = 100 \times (W \times L^{-3})$, where W is the total body wet weight (g), L is the total length (cm).

To estimate the trophic position (TP) of sedentary and migrating pikeperch, we used the R package *tRophicPosition* 0.7.2 (https://cran.r-project.org/web/packages/tRophicPosition/index.html). This package accounts for variation in baseline δ^{15}N among sampling sites when running one or two-baseline Bayesian mixing models to calculate the TP of the consumer. We assumed a nitrogen fractionation of 3.4‰ ± 0.98‰ (Post 2002). Instead of carbon, as suggested in the original method description (Quezada-Romegialli *et al*. 2017), we used sulfur isotope values with zero fractionation (+0.5‰ ± 0.56‰, as reported by McCutchan *et al.* 2003) to run a two-baseline ('freshwater' and 'marine') model to calculate the TP of migratory individuals. To calculate the TP of each individual and, we used δ^{15}N and δ^{34}S linear regression functions of pooled values of freshwater and marine mollusks. The individual TP values of the pikeperch were derived by calculating the difference between the individual δ^{15}N scores in pikeperch and the intercept of the mollusk δ^{15}N to δ^{34}S regression line divided by the trophic fractionation factor (δ^{15}N = 3.4‰; Post 2002). In this calculation, we assume that there is no significant shift in sulfur isotopic ratios between trophic levels.

We compared cyanotoxin levels in individual pikeperch to the safety threshold for human consumption of microcystin-LR, assuming that nodularin is a structurally similar cyanobacterial toxin with similar effects to organisms (Faltermann *et al.* 2016). We used a safety threshold of 0.28 µg g^{-1} DW, based on a consumption rate of 300 g per week for a person with a body mass of 60 kg and a long-term tolerable daily intake of 0.04 µg microcystin-LR per kg body weight (WHO 2003).

Results

Stable isotopes in mollusks

Sulfur stable isotope ratios differed by 12.3‰ between freshwater (*Dreissena polymorpha)* and marine (*Mytilus edulis)* baseline consumers (2.8‰ ± 1.0‰ and 15.1‰ ± 1.1‰, respectively; Fig. 2). This difference was larger than that observed for δ^{13}C (*Dreissena* = –31‰ ± 0.2‰, *Mytilus* = –22.7‰ ± 0.7‰) and δ^{15}N (*Dreissena* = 10.2‰ ± 0.3‰, *Mytilus* = 7.6‰ ± 0.6‰; Fig. 3) isotope ratios.

Stable isotopes, size and condition of pikeperch

Frequency histograms of δ^{34}S values in pikeperch muscle showed a bimodal distribution (Fig. 4). Two distinct normal distributions (Kolmogorov-Smirnov test for normality, $p > 0.2$) were distinguished with median values of 0.7‰ and 15 ‰, and no observations between 2‰ and 4‰. Individuals were classified as 'migratory' if their δ^{34}S signal was higher than 4‰ and 'sedentary' if the values were lower than 2‰ (Fig. 4). Therefore, seven individuals (33%) from the fish collected in the lagoon were identified as 'migratory' (Figs. 2 and 3). Migratory individuals where further divided in to two groups: 'transient' with intermediate δ^{34}S values 4–14‰ and 'long-term' migratory with a δ^{34}S values higher than 14‰.

Among the sedentary individuals, δ^{34}S values in the muscle were lower than in *Dreissena* (0.9‰ *vs*. 2.8‰, respectively; *t*-test: $t_{15} = 4.16$, $p < 0.001$). 'Long-term' migratory individuals contained higher δ^{34}S values in the muscle than *Mytilus* (16.7‰ ± 1.2‰ *vs*. 15.1‰ ± 1.1‰; *t*-test: $t_{16} = –2.88$, $p < 0.05$). In general, sulfur stable isotope values in the muscle of pikeperch were lower than in the liver (Fig. 3). δ^{34}S values in the liver tissues of sedentary individuals did not differ significantly from that in *Dreissena* (2.2‰ in pikeperch liver (n = 13) *vs*. 2.8‰ in *Dreissena* (n = 7); *U*-test: $Z = –0.87$, $p = 0.38$), while 'long-term' migratory individuals contained significantly more ^{34}S-enriched liver tissues than baseline consumer tissues (17.8‰ ± 1.7‰ *vs*. 15.1‰ ± 1.1‰; *t*-test: $t_{11} = –3.47$, $p < 0.01$).

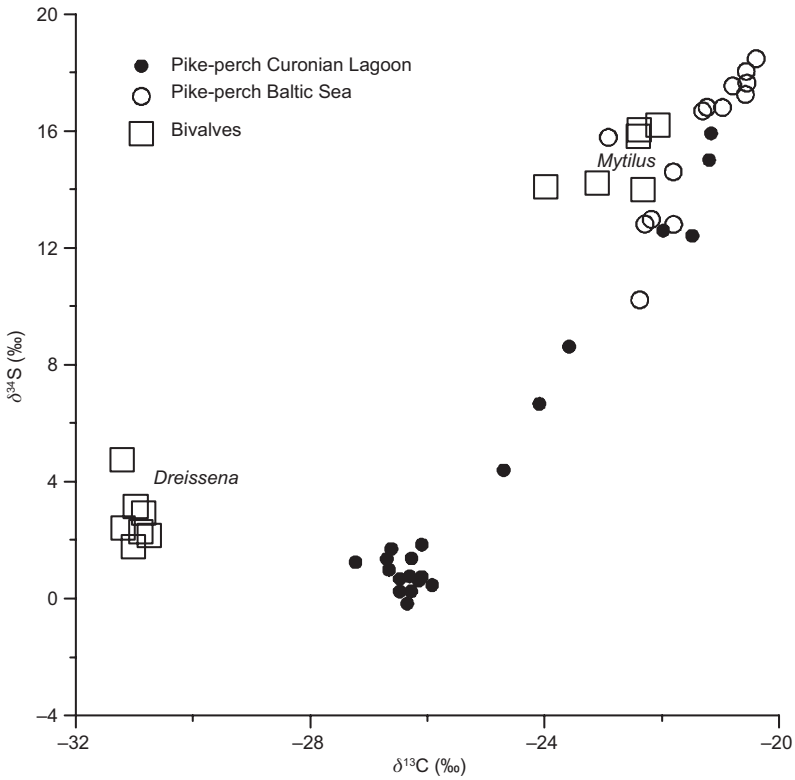

Fig. 2. Stable $\delta^{13}C$ and $\delta^{34}S$ isotope bi-plot showing values of pikeperch and mollusks *Dreissena polymorpha* collected in the Curonian Lagoon and *Mytilus edulis* collected in the Baltic Sea.

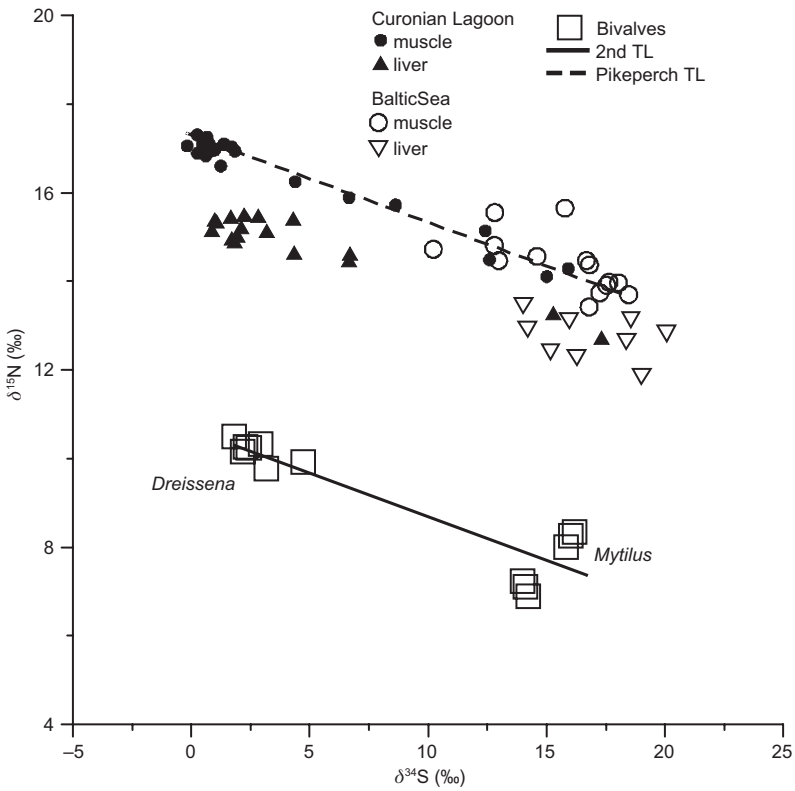

Fig. 3. Stable $\delta^{34}S$ and $\delta^{15}N$ isotopes bi-plot for pikeperch and bivalves. The 2nd trophic level (TL) line shows a regression of $\delta^{15}N$ on $\delta^{34}S$ values in mollusks ($\delta^{15}N = -0.197 \times \delta^{34}S + 10.66$, $F_{1,11} = 56.56$, $p < 0.05$, $R^2 = 0.82$); the 4th TL line is a regression for pikeperch data with the fitted intercept (17.3 ± 0.06, df = 34, $p < 0.05$) for the mollusk regression.

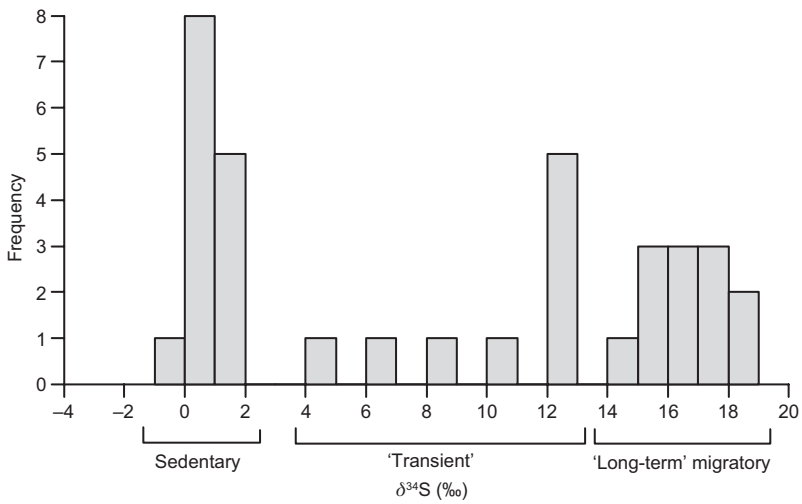

Fig. 4. Frequency histogram of δ^{34}S values in pikeperch. Lines show division between three groups: 'sedentary', 'transient' and 'long-term' migratory.

The body length, weight, condition factor and fat content of muscle tissue was significantly lower in sedentary than in migrating individuals (Table 1 and Fig. 5). 'Long-term' migratory fish contained the most enriched δ^{34}S values, and also exhibited greater body length, body weight, condition factor and fat content in the muscle (Fig. 5). The C:N ratio in the liver (a proxy for fat content) varied significantly among groups, and was highest in the 'transient' individuals containing intermediate δ^{34}S values (Fig. 5).

Trophic position estimates of pikeperch

The adjusted intercept of the linear relationship between δ^{34}S and δ^{15}N values in pikeperch muscle (17.3‰ ± 0.1‰) was higher than that for mollusks (10.7‰ ± 0.3‰; Fig. 3). This difference (6.6‰) corresponds to the expected enrichment for two trophic levels; similar to the result obtained using Bayesian models. The mean estimated trophic position of sedentary pikeperch was 4.02 ± 0.09 (mode 4.01, single 'freshwater' baseline Bayesian model based on δ^{34}S) and for migratory pikeperch was 3.98 ± 0.15 (mode 4.01, two baseline 'freshwater' and 'marine' Bayesian model based on δ^{34}S). We used the linear function of the relationship between δ^{34}S and δ^{15}N signatures in mollusks to calculate individual trophic position values of pikeperch. Individual estimates of trophic positions did not

Table 1. Stable isotope values in the muscle and individual characteristics (total length, weight, fat content in the muscle, Fulton's condition factor (K), estimated trophic level and the total concentrations of cyanotoxins in the muscle or liver tissues (microcystins and/or nodularin, μg g^{-1} DW) of pikeperch identified as 'sedentary', 'transient' and 'long-term' migratory. For comparison, the results of ANOVA (F) and non-parametric Kruskal-Wallis test (H), are provided; n = sample size.

	Sedentary (n = 14)	'Transient' (n = 9)	'Long-term' migratory (n = 12)	F	H	p
δ^{34}S (‰)	0.86 ± 0.59	10.39 ± 3.17	16.71 ± 1.19	–	29.90*	< 0.0001
δ^{13}C (‰)	–26.57 ± 0.33	–22.85 ± 1.13	–21.27 ± 0.73	–	28.42*	< 0.0001
δ^{15}N (‰)	17.01 ± 0.18	15.23 ± 0.65	14.18 ± 0.57	–	28.25*	< 0.001
Total length (cm)	40.2 ± 3.7	43.18 ± 2.11	45.74 ± 3.05	9.88	–	< 0.001
Weight (g)	545 ± 146	744 ± 105	951 ± 224	–	19.18*	< 0.001
Fat_{muscle} (%)	0.45 ± 0.18	0.67 ± 0.26	0.90 ± 0.50	–	8.23*	< 0.05
Fulton's K	0.82 ± 0.06	0.92 ± 0.06	0.99 ± 0.16	7.58	–	< 0.01
Trophic level	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.2	0.59	–	n.s
$Cyanotoxins_{muscle}$	0.18 ± 0.06	0.26 ± 0.10	0.16 ± 0.09	3.57	–	< 0.05
$Cyanotoxins_{liver}$	0.44 ± 0.11	0.62 ± 0.24	0.63 ± 0.39	–	2.05*	n.s.

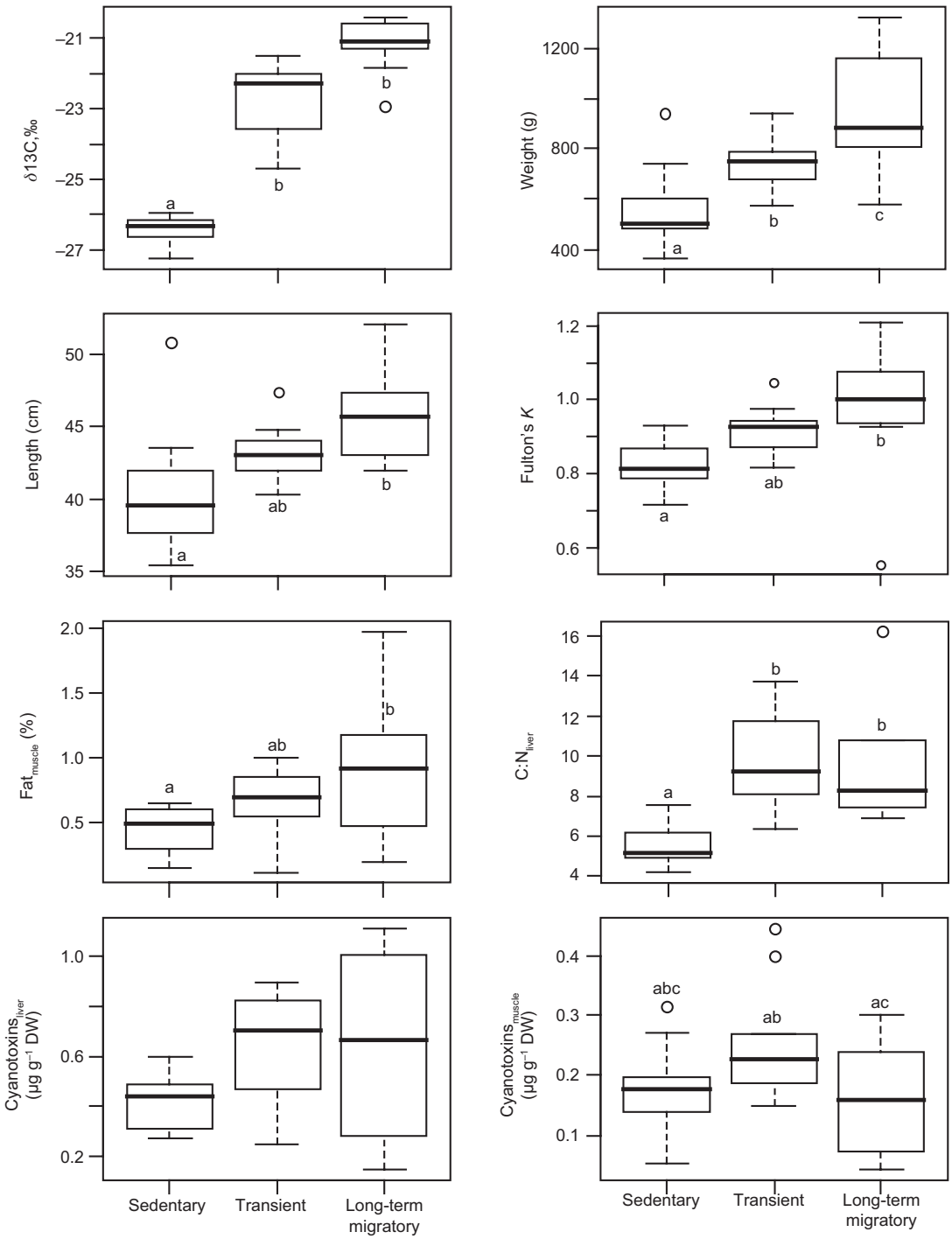

Fig. 5. Box-and-whiskers plots of $\delta^{13}C$ (‰) values, total length (cm), weight (g), body condition index (Fulton's *K*), fat content (%) in the muscle, C:N ratio in the liver and cyanotoxin concentrations (microcystins and/or nodularin, μg g^{-1} DW) in pikeperch groups defined by the $\delta^{34}S$ values. The line within each box represents the median, box boundaries are 25th and 75th percentiles, whiskers are minimum and maximum values, excluding outliers (indicated with circles). Significant differences between the groups (post-hoc Tukey HSD test's, $p < 0.05$, following ANOVA or multiple comparisons of mean ranks following Kruskal-Wallis test results) are denoted with non-matching letters.

differ significantly among sedentary and migratory individuals, and there was no significant relationship between the trophic position and body length (ANCOVA: contingent effect F = 1.36, df = 1, p = 0.25; length effect F = 0.26, df = 1, p = 0.61).

Cyanobacterial toxins

Cyanotoxins were detected in all liver and muscle samples of pikeperch. Muscle concentrations varied by an order of magnitude among individuals (0.04 to 0.45 μg g^{-1} DW). Cyanotoxin concentrations in liver ranged from 0.15 to 1.12 μg g^{-1} DW and were significantly higher relative to muscle tissues (means = 0.55 ± 0.27 μg g^{-1} DW *vs*. 0.19 ± 0.09 μg g^{-1} DW, respectively; Wilcoxon matched pair test: Z = 5.09, n = 35, $p < 0.001$). Liver concentrations did not differ significantly among sedentary and migratory individuals (Table 1). In the muscle of 'transient' migratory individuals concentrations were significantly higher than in the 'long-term' migratory individuals (Tukey HSD post-hoc test, $p < 0.05$), however, neither one of the migratory pikeperch groups differed from the sedentary pikeperch (Fig. 5).

There was no significant correlation ($p > 0.05$) between trophic position and cyanotoxin concentrations in muscle or liver tissues. Muscle cyanotoxin concentrations tended to be lower in the larger individuals (Fig. 6). However, ANCOVA revealed only marginally significant body length effect and confirmed significant contingent group effect on log-transformed toxin values in the muscle (length effect F = 2.9, df = 1, p = 0.1, contingent (sedentary, 'transient' and 'long-term' migratory) effect F = 3.9, df = 1, p = 0.03). The tolerable threshold value for human consumption (i.e. 0.28 μg g^{-1} DW) was exceeded in the muscle tissues of five individuals (14% of all individuals), including four migratory and one sedentary (Fig. 6).

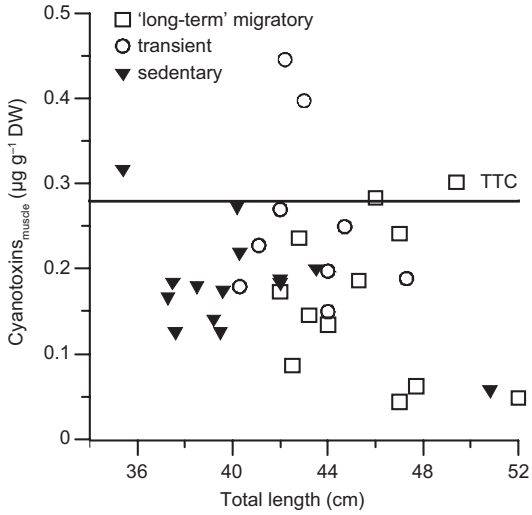

Fig. 6. Variation of cyanotoxin concentrations (microcystins and/or nodularin, μg g^{-1} DW) in relation to the body length in the muscle tissues of pikeperch.

Discussion

This is the first study to demonstrate the utility of using sulfur stable isotopes to identify migrating fish in the coastal waters of the Baltic Sea. Our results from the Curonian Lagoon showed that sedentary pikeperch exhibited a consistently low δ^{34}S signal (from –1.84‰ to –0.17‰). In the sea, pikeperch had enriched and more variable δ^{34}S values ranging from 10.2‰ to 18.5‰. There are two likely sources for this variation: differentiation in prey isotopic values or incomplete equilibration to the marine diet of some migrated individuals.

According to our prior studies, benthic and pelagic fishes have significantly different δ^{34}S values (13.4‰ and 18.5‰, respectively) in the Lithuanian coastal area of the Baltic Sea (Morkūnė *et al*. 2016). However, pikeperch feed mainly on gregarious, pelagic fishes (Kottelat and Freyhof 2007), such as herring (*Clupea harengus*) and sandeels (*Ammodytes tobianus*) in Lithuanian coastal areas (Ložys 2002). Therefore, variation in isotopic composition in relation to prey type, i.e., pelagic *vs*. benthic, is less probable. Some variation of δ^{34}S values in pelagic prey could occur because of freshwater discharge in the coastal waters. The highest observed distance of the plume is 45 km, most frequently 10 km northwards and 6 km westwards (Vaičiūtė 2012). By comparison, reported migration distances of the pikeperch in the Baltic are mostly less than 18 km, although movements

over 100 km have been observed (Saulamo and Neuman 2002 and references therein). In our opinion, the observed pattern of increasing $\delta^{34}S$ and $\delta^{13}C$ values of migrating individuals approaching the marine pelagic end-point ($\delta^{34}S$ ~18‰, $\delta^{13}C$ ~–21‰) was probably a result of exponential isotopic change following a diet shift (Hesslein *et al.* 1993). A significant number of migrating individuals (> 40%), even those collected in the lagoon, were close to full equilibration to the marine pelagic end-point ($\delta^{34}S$ = 16‰–20‰). It could be also deduced, that these individuals were solely dependent on a pelagic fish diet from outside the freshwater plume area for a rather significant time period, considering that large pikeperch can reach only 50% of equilibration to its diet in 3–4 months (calculated according to Vander Zanden *et al.* 2015). These findings on extensive use of marine habitat as feeding location based on SIA contrasts to lifetime otolith microchemistry results, which show only 25% ± 14% (maximum of 62%) of brackish residence for migrating individuals (Ložys *et al.* 2017). Based on these results it could be hypothesized, that migrating pikeperch only uses the freshwater habitat for overwintering, but not feeding and growth.

The $\delta^{15}N$ and $\delta^{34}S$ analysis suggests a high degree of consistency in the trophic position of pikeperch across estuarine and coastal habitats, as well as between individuals of different size. The TP of ~4 estimates are comparable to those reported in earlier studies conducted in the Curonian Lagoon TP = 4.1 ± 0.2 (Rakauskas *et al.* 2013) and other ecosystems (e.g. 4.2 ± 0.1, cf. Kopp *et al.* 2009). The advantage of sulfur isotopes in comparison to carbon isotopes for calculation of trophic position and identification of recent habitat use is that the former do not require use of fractionation factors, which makes the method less sensitive to error. When using local baselines based on mollusks, it is important to consider, that the stock of pikeperch in Lithuanian coastal waters may contain a mixed population, originating from various inland waters (Vistula, Curonian Lagoon, and Gulf of Riga) connected to the SE Baltic Sea (Ložys *et al.* 2017). However, we still have only limited knowledge on variation in δ34S values at the base of the coastal food web, as the applicability of the method has not been widely tested in the Baltic Sea (but see Mittermayr *et al.* 2014, Morkūnė *et al.* 2018).

The pikeperch cyanotoxin levels measured in this study (median 0.18 μg g^{-1} DW) were relatively high, frequently close to the recommended concentrations for safe long-term human consumption (≤ 0.28 μg g^{-1} DW). The median cyanotoxin concentration in the liver (0.48 μg g^{-1} DW) of pikeperch was even higher, than concentrations estimated in the liver tissues of various fish species previously recorded in the Curonian Lagoon, including planktivores (Bukaveckas *et al*. 2017). In the Baltic Sea flounder (*Platichthys flesus*) nodularin content is recorded usually below 0.2 μg g^{-1} DW in the muscle and up to 2.23 μg g^{-1} DW in the liver (Sipiä *et al.* 2006, Kankaanpää *et al.* 2009, Mazur-Marzec *et al.* 2013). This is a surprising result, because predatory fish are generally expected to exhibit lower concentrations of cyanotoxins due to biodilution in the food chain (Ibelings and Chorus 2007, Kozlowsky-Suzuki *et al.* 2012, Bukaveckas *et al*. 2017). We found no significant differences in cyanotoxin concentrations between sedentary and migrating pikeperch, but as in prior studies, we show that algal toxin concentrations are quite variable among individuals within a population. This is an important issue because human health risks are often assessed based on average toxin concentrations for a population, but high variability means that some individuals can exceed the threshold, even if the population mean does not. For the Baltic region, further study using techniques suitable to differentiate microcystin and nodularin (e.g., liquid chromatography-mass spectrometry) may be helpful for resolving the linkage between migration and exposure of pikeperch, and to better understand the factors that give rise to variable toxin levels among individuals within a population.

Conclusions

Differences in $\delta^{34}S$ values between freshwater (Curonian Lagoon) and brackish water (Baltic Sea) end-points were an effective means for distinguishing migrating and resident contingents of pikeperch. Application of this method

to other migratory fish species in the Baltic Sea may allow us to better understand the factors constraining population size, and to assess the stocks of interrelated management units. In line with the prior research, migratory pikeperch had better condition than sedentary individuals, indicating a growth benefit of migration. Relatively high levels of cyanotoxins, approaching recommended concentrations for safe long-term human consumption, indicates the need for monitoring algal toxins in these waters to better understand exposure risks and consequences for fish health.

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References

Acuña S., Baxa D. & Teh S. 2012. Sublethal dietary effects of microcystin producing *Microcystis* on threadfin shad, *Dorosoma petenense*. *Toxicon* 60: 1191–1202.

Adams D.H. & Paperno R. 2012. Stable isotopes and mercury in a model estuarine fish: Multibasin comparisons with water quality, community structure, and available prey base. *Sci. Total Environ.* 414: 445–455.

Bergström L., Heikinheimo O., Svirgsden R., Kruze E., Ložys L., Lappalainen A., Saks L., Minde A., Dainys J., Jakubavičiūtė E., Ådjers K. & Olsson J. 2015. Long term changes in the status of coastal fish in the Baltic Sea. *Estuar. Coast. Shelf. Sci.* 169: 74–84.

Bosley K.L., Witting D.A., Chambers R.C. & Wainright S.C. 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Mar. Ecol. Prog. Ser.* 236: 233–240.

Bresciani M., Giardino C., Stroppiana D., Pilkaitytė R., Zilius M., Bartoli M. & Razinkovas A. 2012. Retrospective analysis of spatial and temporal variability of chlorophyll-*a* in the Curonian Lagoon. *J. Coastal Cons.* 16: 511–519.

Brown J.A., Moore W.M. & Quabius E.S. 2001. Physiological effects of saline waters on zander. *J. Fish Biol.* 59: 1544–1556.

Bukaveckas P.A., Lesutiene J., Gasiunaite Z.R., Lozys L., Olenina I., Pilkaityte R., Putys Z., Tassone S. & Wood J.D. 2017. Microcystin in aquatic food webs of the Baltic and Chesapeake Bay regions. *Estuar. Coast. Shelf Sci.* 191: 50–59.

Cabana G. & Rasmussen J.B. 1994. Modeling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372: 255–257.

Clément M., Chiasson A.G., Veinott G. & Cairns D.K. 2014. What otolith microchemistry and stable isotope analysis reveal and conceal about anguillid eel movements across salinity boundaries. *Oecologia* 175: 1143–1153.

Dattagupta S., Berquist D.C., Szalai E.B., Macko S.A. & Fisher C.R. 2004. Tissue carbon, nitrogen, and sulphur stable isotope turnover in transplanted *Bathymodiolus childressi* mussels: relation to growth and physiological condition. *Limnol. Oceanogr.* 49: 1144–1151.

Faltermann S., Grundler V., Gademann K., Pernthaler J. & Fent K. 2016. Comparative effects of nodularin and microcystin-LR in zebrafish: 2. Uptake and molecular effects in eleuthero-embryos and adult liver with focus on endoplasmic reticulum stress. *Aquat. Toxicol.* 171: 77–87.

Fickling N.J. & Lee R.L.G. 1985. A study of the movements of the zander, *Lucioperca lucioperca* L., population of two lowland fisheries. *Aquacult. Fish. Manage.* 16: 377–393.

Fry B. & Arnold C.K. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* 54: 200–204.

Fry B. & Chumchal M.M. 2011. Sulfur stable isotope indicators of residency in estuarine fish. *Limnol. Oceanogr.* 56: 1563–1576.

Garcia A.C., Bargu S., Dash P., Rabalais N.N., Sutor M., Morrison W. & Walker N.D. 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9: 134–143.

Godbout L., Trudel M., Irvine J.R., Wood C.C., Grove M.J., Schmitt A.K. & McKeegan K.D. 2010. Sulfur isotopes in otoliths allow discrimination of anadromous and non-anadromous ecotypes of sockeye salmon (*Oncorhynchus nerka*). *Environ. Biol. Fish*. 89: 521–532.

Hart L.M., Bond M.H., May-McNally S.L., Miller J.A. & Quinn T.P. 2015. Use of otolith microchemistry and stable isotopes to investigate the ecology and anadromous migrations of Northern Dolly Varden from the Egegik River, Bristol Bay, Alaska. *Environ. Biol. Fish*. 98: 1633–1643.

Hesslein R.H., Hallard K.A. & Ramlal P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Can. J. Fish. Aquat. Sci.* 50: 2071–2076.

Ibelings B.W. & Chorus I. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health. *Environ. Pollut*. 150: 177–192.

Jaanus A., Andersson A., Olenina I., Toming K. & Kaljurand K. 2011. Changes in phytoplankton communities along a north–south gradient in the Baltic Sea between 1990 and 2008. *Boreal Env. Res.* 16 (suppl. A): 191–208.

Kankaanpää H., Sjövall O., Huttunen M., Olin M., Karlsson K., Hyvärinen K., Sneitz L., Häärkönen J., Sipiä V.O. & Meriluoto J.A. 2009. Production and sedimentation of peptide toxins nodularin-R and microcystin-LR in the northern Baltic Sea. *Environ. Pollut.* 157: 1301–1309.

Karjalainen M., Pääkkönen J.-P., Peltonen H., Sipiä V., Valtonen T. & Viitasalo M. 2008. Nodularin concentrations in Baltic Sea zooplankton and fish during a cyanobacte-

rial bloom. *Mar. Biol.* 155: 483–491.

Kopp D., Cucherousset J., Syväranta J., Martino A., Céréghino R. & Santoul F. 2009. Trophic ecology of the pikeperch (*Sander lucioperca*) in its introduced areas: a stable isotope approach in southwestern France. *CR Biol.* 332: 741–746.

Kottelat M. & Freyhof J. 2007. *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin.

Kozlowsky-Suzuki B., Wilson A.E. & Ferrão-Filho A.S. 2012. Biomagnification or biodilution of microcystins in aquatic foodwebs? Meta-analyses of laboratory and field studies. *Harmful Algae* 18: 47–55.

Lappalainen J., Dorner H. & Wysujack K. 2003. Reproduction biology of pikeperch (*Sander lucioperca* (L.)) — a review. *Ecol. Freshw. Fish* 12: 95–106.

Lehtonen H., Hansson S. & Winkler H. 1996. Biology and exploitation of pikeperch, *Stizostedion lucioperca* (L.), in the Baltic Sea area. *Ann. Zool. Fennici* 33: 525–535.

Lesutienė J., Bukaveckas P.A., Gasiūnaitė Z.R., Pilkaitytė R. & Razinkovas-Baziukas A. 2014. Tracing the isotopic signal of a cyanobacteria Bloom through the food web of a Baltic Sea coastal lagoon. *Estuar. Coast. Shelf Sci.* 138: 47–56.

Ložys L. 2002. Peculiarities of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) ecology in the Curonian Lagoon and the coastal zone of the Baltic Sea. Ph.D. thesis, Vilnius University.

Ložys L. 2004. The growth of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea. *Hydrobiologia* 514: 105–113.

Ložys L., Shiao J.-C., Iizuka Y., Minde A., Pūtys Ž., Jakubavičiūtė E., Dainys J., Gorfine H. & Tzeng W.-N., 2017. Habitat use and migratory behaviour of pikeperch *Sander lucioperca* in Lithuanian and Latvian waters as inferred from otolith Sr:Ca ratios. *Estuar. Coast. Shelf Sci.* 198: 43–52.

Ljunggren L., Sandström A., Bergström U., Mattila J., Lappalainen A., Johansson G., Sundblad G., Casini M., Kaljuste O. & Eriksson B.K. 2010. Recruitment failure of coastal predatory fish in the Baltic Sea coincident with an offshore ecosystem regime shift. *ICES Mar. Sci.* 67: 1587–1595.

MacAvoy S.E., Macko S.A., McIninch S.P. & Garman G.C. 2000. Marine nutrient contributions to freshwater apex predators. *Oecologia* 122: 568–573.

Malbrouck C. & Kestemont P. 2006. Effects of microcystins on fish. *Environ. Toxicol. Chem.* 25: 72–86.

Mazur-Marzec H., Sutryk K., Kobos J., Hebel A., Hohlfeld N., Błaszczyk A., Toruńska A., Kaczkowska M.J., Łysiak-Pastuszak E., Kraśniewski W. & Jasser I. 2013. Occurrence of cyanobacteria and cyanotoxin in the Southern Baltic Proper. Filamentous cyanobacteria versus single-celled picocyanobacteria. *Hydrobiologia* 701: 235–252.

McCutchan J.H., Lewis W.M., Kendall C. & McGrath C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–390.

Mittermayr A., Fox S.E. & Sommer U. 2014. Temporal variation in stable isotope composition (δ^{13}C, δ^{15}N and δ^{34}S) of a temperate *Zostera marina* food web. *Mar. Ecol. Prog. Ser.* 505: 95–105.

Morkūnė R., Lesutienė J., Barisevičiūtė R., Morkūnas J. & Gasiūnaitė Z.R. 2016. Food sources of wintering piscivorous waterbirds in coastal waters: a triple stable isotope approach for the southeastern Baltic Sea. *Estuar. Coast. Shelf Sci.* 171: 41–50.

Morkūnė R., Lesutienė J., Morkūnas J. & Barisevičiūtė R. 2018. Triple stable isotope analysis to estimate the diet of the Velvet Scoter (*Melanitta fusca*) in the Baltic Sea. *PeerJ* 6, e5128, doi 10.7717/peerj.5128.

Mustamäki N., Bergström U., Ådjers K., Sevastik A. & Mattila J. 2014. Pikeperch (*Sander lucioperca* (L.)) in decline: high mortality of three populations in the northern Baltic Sea. *Ambio* 43: 325–336.

Nehlich O. 2015. The application of sulphur isotope analyses in archaeological research: a review. *Earth Sci. Rev.* 142: 1–17.

Paldavičienė A., Mazur-Marzec H. & Razinkovas A. 2009. Toxic cyanobacteria blooms in the Lithuanian part of the Curonian Lagoon. *Oceanologia* 51: 203–216.

Paldavičienė A., Zaiko A., Mazur-Marzec H. & Razinkovas-Baziukas A. 2015. Bioaccumulation of microcystins in invasive bivalves: a case study from the boreal lagoon ecosystem. *Oceanologia* 57: 93–101.

Peterson B.J. & Fry B. 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* 18: 293–320.

Post D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718.

Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J. & Montaña C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179–189.

Poste A.E., Hecky R.E. & Guildford S.J. 2011. Evaluating Microcystin exposure risk through fish consumption. *Environ. Sci. Technol.* 45: 5806–5811.

Rakauskas V., Pūtys Ž., Dainys J., Lesutienė J., Ložys L. & Arbačiauskas K. 2013. Increasing population of the invader round goby *Neogobius melanostomus* (Actinopterigii: Perciformes: Gobiidae), and its trophic role in the Curonian Lagoon, SE Baltic Sea. *Acta Ichthyol. Piscat.* 43: 95–108.

R Development Core Team 2017. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rogers S.I., Casini M., Cur P., Heat M., Irigoe X., Kuos H., Scheida M., Sko H., Stergio K., Trenkel V.M., Wikner J. & Yunev O. 2010. *Marine Strategy Framework Directive, Task Group 4 Report, Food Webs*. European Commission Joint Research Centre, Ispra, Italy and International Council for the Exploration of the Sea, Copenhagen, Denmark. [Available at http://ec.europa.eu/environment/marine/pdf/4-Task-group-4.pdf].

Saulamo K. & Neuman E. 2002. *Local management of Baltic fish stocks — significance of migrations*. Swedish Board of Fisheries, Göteborg. [Available at https://

www.havochvatten.se/download/18.64f5b3211343cffddb2800019472/1348912829954/finfo2002_9.pdf].

Saulamo K. & Thoresson G. 2005. Management of pikeperch migrating over management areas in a Baltic archipelago area. *Ambio* 34: 120–124.

Sipiä V.O., Sjovall O., Valtonen T., Barnaby D.L., Codd G.A., Metcalf J.S., Kilpi M., Mustonen O. & Meriluoto J.A. 2006. Analysis of nodularin-R in eider (*Somateria mollissima*), roach (*Rutilus rutilus* L.), and flounder (*Platichthys flesus* L.) liver and muscle samples from the western Gulf of Finland, northern Baltic Sea. *Environ. Toxicol. Chem.* 25: 2834–2839.

Skóra K.E. 1996. A comparison of changes in the composition of fish catches in the Polish lagoons in 1960–1989. In: *Proceedings of Polish–Swedish Symposium on Baltic coastal fisheries resources and management, Gdynia, 2–3 April 1996*, Sea Fisheries Institute, Gdynia, pp. 225–241.

Svedäng H. & Wickström H. 1997. Low fat contents in female silver eels: indications of insufficient energetic stores for migration and gonadal development. *J. Fish Biol.* 50: 475–486.

Swanson H.K., Kidd K.A. & Reist J.D. 2011. Quantifying importance of marine prey in the diets of two partially anadromous fishes. *Can. J. Fish. Aquat. Sci.* 68: 2020–2028.

Šulčius S., Pilkaitytė R., Mazur-Marzec H., Kasperovičienė J., Ezhova E., Błaszczyk A. & Paškauskas R. 2015. Increased risk of exposure to microcystins in the scum of the filamentous cyanobacterium *Aphanizomenon flos-aquae* accumulated on the western shoreline of the Curonian Lagoon. *Mar. Pollut. Bull.* 99: 264–270.

Vaičiūtė, 2012. *Distribution patterns of optically active components and phytoplankton in the estuarine plume in the south-eastern Baltic Sea*. Ph.D. thesis, Klaipėda University.

Vaičiūtė D., Bresciani M., Bučas M. 2012. Validation of MERIS bio-optical products with in situ data in the turbid Lithuanian Baltic Sea coastal waters. *J. Appl. Remote Sens.* 6, 063568-1, doi.org/10.1117/1.JRS.6.063568

Vander Zanden M.J., Clayton M.K., Moody E.K., Solomon C.T. & Weidel B.C. 2015. Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PloS ONE* 10(1): e0116182, doi.org/10.1371/journal.pone.0116182.

Virbickas J., Gerulaitis A., Misiūnienė D. & Sinevičienė D. 1974. *Biology and fishery of the pikeperch in the water bodies of Lithuania*. State Publ. House "Mintis", Vilnius.

Weber P.K., Hutcheon I.D., McKeegan K.D. & Ingram B.L. 2002. Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Can. J. Fish. Aquat. Sci.* 59: 587–591.

Wilson A.E., Gossiaux D.C., Höök T.O., Berry J.P., Landrum P.F., Dyble J. & Guildford S.J. 2008. Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (*Perca flavescens*). *Can. J. Fish. Aquat. Sci.* 65: 1487–1497.

Wood J.D., Franklin R.B., Garman G.C., McInich S.P., Porter A.J. & Bukaveckas P.A. 2014. Exposure to the cyanotoxin microcystin arising from inter-specific differences in feeding habits among fish and shellfish from the James River Estuary, Virginia. *Environ. Sci. Technol.* 48: 5194–5202.

WHO 2003. *Guidelines for safe recreational water environments; coastal and fresh eaters*, vol. 1. World Health Organisation, Geneva.

Zemlys P., Ferrarin C., Umgiesser G., Gulbinskas S. & Bellafiore D. 2013. Investigation of saline water intrusions into the Curonian Lagoon (Lithuania) and two-layer flow in the Klaipeda Strait using finite element hydrodynamic model. *Ocean. Sci.* 9: 573–584.