Evaluation of the Dietary Composition of Eurasian Perch (Perca Fluviatilis) in an Interconnected River-lake-gulf Aquatic System as a Supplementary Tool for the Interpretation of Measured Mercury (Hg) Concentrations (One-Year Study).

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Research

Keywords: Mercury bioaccumulation in fish, biomagnification, analysis of nitrogen (15N) and carbon (13C) stable isotopes, dietary composition, fresh and brackish water linked system, environmental modelling, Eurasian Perch (Perca fluviatilis), The Gulf of Riga

Posted Date: March 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-292980/v1

Version of Record: A version of this preprint was published at Environmental Sciences Europe on July 8th, 2021. See the published version at https://doi.org/10.1186/s12302-021-00523-w.

Abstract

Background

Eurasian Perch (*Perca fluviatilis*) is one of ecologically significant fish species in the Baltic Sea and has been recognized as a suitable organism to measure concentrations of hazardous substances that characterize levels of local pollution (e.g. heavy metals or persistent organic pollutants). However, the ability of the species to inhabit a wide range of feeding grounds raises concerns about the adequacy of monitoring data in relation to the representativeness of measured levels of hazardous substances at specific locations. Accounting of the migratory characteristics of this species can shed light on the origin of the analyzed specimens and thus trace the pollution uptake chain. Perch samples and potential perch prey were collected at three remote stations in a fully interlinked system river– lake–coastal waters of the Gulf of Riga. Mercury (Hg) concentration and stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were measured in each sampled item. The perch data were divided into three subgroups associated with specific feeding grounds and one mixed group. A Bayesian mixing model was implemented to quantify the feeding preferences of each group, and based on the results, influence of each food source on Hg uptake by perch was modelled by means of Gaussian GAM model.

Results

Calculated stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ demonstrated clear evidence that perch specimens migrate between the sampling stations. Substantial proportion of specimens sampled in river and lake stations had isotopic signals consistent with feeding in the gulf. The group of perch associated with feeding in river grounds exhibited the highest Hg concentrations with mean value of 209 μg kg^{-1} wet weight. The food item *C. harengus membras* and Crustacea showed significant mitigating effect on Hg concentration. The rest of the food items were not significant at the $\alpha = 0.05$ level, although had different directions of the influence and slope values.

Conclusions

The study clearly showed that the high mobility of perch along associated aquatic systems has noticeable effect. Therefore, trophic position and isotopic signatures, along with identification of the food sources, can serve as important supplementary tools for more accurate data interpretation of Hg accumulation.

Background

The global mercury (Hg) cycle is dominated by anthropogenic and natural emissions of gaseous substances of Hg to the atmosphere [19]. Simulatously, the wide use of mercury and mercury containing products [4] has resulted in more than 3000 localized mercury contaminated sites worldwide [15]. Furthermore, the improperly disposed industrial, household and medical products as well as pesticides used in the past have created legacy pollution sources. Although the sources are not classifiable as mercury contaminated sites, they are impacting environmental quality of geographically localized water basins they are discharging into.

In order to address local pollution sources and so to improve environmental quality, various programs, like river basin management plans in European Union, are being developed and implemented. To assess effectiveness of implemented measures, pollution levels and their trends are usually analysed in frame of environmental monitoring programs. Consequently, environmental research employs a variety of scientific methods [8, 23] in quantitative and qualitative analyses of the mercury in aquatic systems, including selection of a matrix for analysis such as water, sediments and biological material. Eurasian Perch (*Perca fluviatilis*) is one of the ecologically significant fish species

proposed by HELCOM as a biological matrix for environmental studies in the Baltic Sea, along with Baltic herring (*Clupea harengus membras*), cod (*Gadus morhua*) and eelpout (*Zoarces viviparus*) [7]. *Perca fluviatilis* is omnivorous in the first years of life, although the adults mostly follow a piscivorous diet [11]. The species occupy high trophic position; therefore, significant levels of the hazardous substances are commonly found in their tissues. The perch is widespread in freshwater and brackish water ecosystems, but usually are not considered to be an anadromous fish. It has been found as a suitable organism to measure concentrations of hazardous substances characterizing levels of local pollution. At the same time, it has been put forward by Järv [12] that the home-range migration (average 20 km, maximum observed 180 km) is a common behavioural feature for perch. The salt tolerance of perch and relatively low water salinity of the Baltic Sea and the Gulf of Riga allows this species to move from inland lakes and rivers to coastal waters. Generally, it has been assumed that once the feeding grounds in the coastal waters have been reached the specimens become reasonably stationary, consequently they can be used as a representative biological organism to characterize level of pollution in the specific area. However, the high mobility of perch, the ability of the species to inhabit a wide range of feeding grounds [12, 11], in addition to relatively high variability of measured concentrations raises concerns about adequacy of monitoring data regarding to the representability of measured levels of hazardous substances at specific locations. These concerns are most pronounced in cases where different water bodies form interlinked network fully in range of perch migration distance, like the system river-lake-marine coastal waters. Consideration of migratory characteristics of this species can shed light on the origin of the analyzed samples and thus trace the chain of pollution uptake, thus filling knowledge gaps on pollution distribution in interconnected aquatic systems.

Stable isotopes of nitrogen (N) and carbon (C) in soft tissues of fish are commonly used to study food webs and migration in aquatic ecosystems [27, 17, 10]. Carbon stable isotope ratios $^{13}C/^{12}C$ in marine and fresh water systems have been used to determine the movements of migratory species between coastal and pelagic ecosystems based on changes in dietary preferences during the migration [21,29], which is possible due to clear isotopic differences between ^{13}C depleted freshwater and ^{13}C enriched marine food webs. Change in nitrogen isotope ratios $^{15}N/^{14}N$ has been used to distinguish trophic levels in freshwater and marine environments [20, 25].

By employing $^{13}C/^{12}C$ and $^{15}N/^{14}N$ stable isotope analysis in combination with perch stomach content analysis, the aim of this study was to determine how fundamentally different food bases affect the uptake of mercury from the food chains, as well as whether the perch, caught at a particular location, are representative for this location, or rather for the entire interconnected coastal-freshwater aquatic system.

Materials And Methods

Study site description

For this study the fully interlinked system, river Daugava – lake Ķīšezers – coastal area of the Gulf of Riga (Baltic Sea) was chosen (Figure 1). The river Daugava sampling site (Station 1) was selected on the last section of the river between Riga Hydroelectric Power Plant (HEPP) and the estuary Daugavgriva, circa 5 km downstream to the HEPP and well upstream of the channel connecting lake Ķīšezers and river Daugava. The site can be characterized by rocky sediment type and rapid stream velocity. For the sampling Station 2 a lagoon type lake – Ķīšezers was selected. The lake is connected to river Daugava by a natural channel. It is rich with aquatic vegetation and represents a stagnant water pool. The sampling Station 3 was located in the Gulf of Riga, near the mouth of Daugava. The location represents brackish water coastal ecosystem with significant amount of detritus originating from adjacent rivers.

Sampling and pre-treatment

The sampling campaigns took place in April and August 2017. Perch (*Perca fluviatilis*) and other fish species were collected by means of scientific nets: gill nets “Nordic” and nylon gill nets. Benthic organisms were sampled by Wildco Petite Ponar Grab, while zooplankton and crayfish by bag seine net and two-ring drop net, respectively. For suspended particular matter (SPM) surface water samples (up to 5 L) were taken from each sampling site by pre-cleaned plastic bottle. Collected surface water samples were vacuum filtered for at least 30 minutes on pre-combusted (at 450°C for 2 h) 24 mm diameter Whatman GF/F filters to collect sufficient amounts of material.

Length and weight of the whole fish was determined immediately after sampling by measuring board (accuracy to ±0.1 cm) and technical scale KERN FCE3K1N (accuracy to ±1 g). Thereafter, dorsal muscles were extracted. The dorsal muscles were placed into plastic container and frozen at temperature -18 °C. The samples of muscle tissues, zooplankton and benthic organisms were dried in vacuum freeze dryer until sample weight loss stopped and then homogenized by knife mill or agate pestle. Plastic containers with dried tissue samples were stored in desiccator in dark at room temperature (+ 20 °C) until further analyses.

Analytical methods

The concentration of mercury (Hg) in dorsal muscles of fish and other organisms was determined in laboratory at Latvian Institute of Aquatic Ecology (Daugavpils University) according to US EPA method 7473 “Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" [30] using “Teledyne Leeman labs” direct Hg analyser “Hydra IIc” (Mason, Ohio, USA). Calibration curves were generated using mercury working standard solutions (0.01, 0.1, 0.5, 1.0, 2.0 mg L^{-1}) varying volume 20 µL and 50 µL in sampling boats. Hg working standards were made diluting Hg stock solution 1000 mg Hg L-1 (Roti®Star Hg standards for AAS in 10% HNO_3, traceable to SRM from NIST, CARL ROTH, Karlsruhe, Germany). Hg calibration curve was constructed by plotting the absorbance versus Hg weights 0.2 to 100 ng loaded in sampling boats. The regression coefficient from the initial calibration curves averaged r = 0.9995 (n=7). The analyses of reference material of mussel tissue ERM-CE278k and aquatic plant ERM-BCR060 (both certified by Institute for Reference Materials and Measurements at Joint Research Centre, European Commission, Geel, Belgium) were performed for calibration verification at the start and to prove accuracy of determination at the end of every batch of 20 samples (Table 1). Our results were in good agreement with the certified value given for the reference materials as well as our laboratory takes part regularly and successfully in the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) studies for quality assurance. Method blank (50 µL of deionized water) and random sample duplicates were also run during each batch. Method blank was less than 25% of the lowest detected Hg content in sample (0.20 ng) and was considered acceptable. Relative percent difference between sample duplicate analyses was 1.5 ± 2% (n = 22).

Table 1. Quality assurance with biota reference material ERM-CE278k and ERM-BB422 within current study.

Reference material	Certified value ±S.D., mg kg^{-1}	Mean of determined value ± S.D., mg kg^{-1}	n	Recovery range, %
ERM-CE278k	0.071 ± 0.007	0.080 ± 0.005	18	103 – 126
ERM-BCR060	0.34 ± 0.04	0.36 ± 0.02	6	100 - 123

Stomach content of the perch was assessed by means of stomach content analysis, using dissection method suggested by Manko [18].

Analysis of stable isotopes

Analysis of stable isotopes and calculation of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios was performed for all caught organisms. Prior to the stable isotope analyses 2 mg per sample of dried tissues were wrapped in a tin cup and analysed in the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of Latvia by elemental analyser (EuroEA-3024, EuroVector S.p.A, Italy) coupled with continuous flow stable isotope ratio mass spectrometer (Nu-HORIZON, Nu Instruments Ltd., UK). Isotope ratios were reported relative to Vienna Pee Dee Belemnite with a lithium carbonate anchor (VPDB-LSVEC) for $\delta^{13}C$ and to atmospheric nitrogen (AIR) for $\delta^{15}N$. Stable isotope ratios were denoted as parts per thousand (‰) deviation from the standard, as follows:

$$\delta X\ (‰) = (Rsample / Rstandard - 1) \times 1000,$$

where X is ^{13}C or ^{15}N and the R ratio is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

An internal standard sample (glutamic acid) was used to check reproducibility of the stable isotope ratio determination. The pooled standard deviations were 0.14‰ (n = 121) for $\delta^{13}C$ and 0.21‰ (n = 121) for $\delta^{15}N$. Reference material L-glutamic acid USGS-40 (Reston Stable Isotope Laboratory of the U.S. Geological Survey, Reston, Virginia, NIST®RM 8573) was used to check accuracy of the stable isotope ratio determination where stable carbon isotopic and nitrogen isotopic compositions with combined uncertainties are $\delta^{13}C_{VPDB\text{-}LSVEC} = -26.39 \pm 0.04‰$ and $\delta^{15}N_{AIR} = -4.52 \pm 0.06‰$ (Qi et al. 2003). Our results for reference standard USGS-40 were $\delta^{13}C = -26.38$, (SD= ±0.03‰, n=20) and $\delta^{15}N = -4.54$ (SD= ±0.08‰, n=20).

Data analyses and statistical assessment

Ward's minimum variance Clustering analysis was performed for identification of perch sub-groups with agglomeration of objects based on variables $\delta^{13}C$, $\delta^{15}N$ and sampling location. The aim of the analysis was to split spatially sub-groups of perch with consideration of sampling location and geographical markers provided by the signals of stable isotope ratios. Optimal number of clusters was selected according to the Silhouette Widths method [3].

Bayesian mixing model SIAR was chosen for quantification of common diet in the computed sub-groups of perch, based on $\delta^{13}C$ and $\delta^{15}N$ ratios [24]. Trophic discrimination factor was approximated for each station separately, based on $\delta^{13}C$ and $\delta^{15}N$ ratios and trophic levels found in the literature (Table S1, Additional file 1) of every organism sampled. The estimated trophic discrimination factors were 3.37±1.27 ($\delta^{15}N$) and 0.36±1.00 ($\delta^{13}C$) for Station 1, 3.92±1.31 ($\delta^{15}N$) and 1.04±1.52 ($\delta^{13}C$) for Station 2, 3.32±1.14 ($\delta^{15}N$) and 0.74±1.21 ($\delta^{13}C$) for Station 3. Sources for the model were selected based on results of the stomach content analysis and the list of real-life sampled organisms at the study areas.

Biomagnification factor (BMF) was calculated only from species included into station-specific diet of perch and perch itself. The food web BMF was computed from parameter *b* or slope of the following equation [22, 5]:

$$\log(Hg) = a + b \times \delta^{15}N,$$

where $BMF = 10^b$

Analysis of covariance (ANCOVA) was implemented to compare obtained biomagnification regression curves. During the analysis interactions between assigned groups (from cluster analysis) and $\delta^{15}N$ were evaluated to understand either the focus on specific diet shows significant difference in slopes of Hg biomagnification. Two regression models were compared: M1 – LOG concentration of mercury estimation from independent variable $\delta^{15}N$ and independent

factor Group; M2 – LOG concentration of mercury estimation from interrelated variable $\delta^{15}N$ and factor Group. Group (the dataset was divided onto sub-groups, based on results of the clustering analysis mentioned above) as independent factor was considered for identification of differences between intercepts of the biomagnification regressions. For the analysis selected value of significance level *α* was 0.05.

Smoothing function of Generalized Additive Models (GAM) was used to cover slightly non-linear relationship of LOG-transformed mercury concentration in perch dorsal muscles and length of specimens, thus allowing more sensitive evaluation of effect of dietary preferences. Data exploration protocol recommended by Zuur et al. [34] was applied before modelling process. The obtained models were validated according to the guide suggested by Zuur & Ieno [35], including check of homogeneity, independence, influential observations, normality and fit of estimated values. Akaike Information Criterion (AIC) [33] was applied to compare the obtained GAMs and determine the best fit for the data, thus identify feeding sources and other concomitant factors impacting mercury accumulation in consumer tissues. Due to collinearity of some variables, such as Crustacea and *Neogobius melanostomus* (correlation coefficient -0.8), Crustacea and *Neomysis integer* (correlation coefficient -0.7), *Gymnocephalus cernua* and *N. integer* (correlation coefficient -0.7), *G.cernua* and *N.melanostomus* (correlation coefficient -0.7), *N.melanostomus* and *N.integer* (correlation coefficient -0.7) three different models (A, B and C) were performed. Each of the models includes combination of non-collinear variables, and the three models together contain all the food items selected as sources for SIAR model mentioned above. *Ammodytes tobianus* was excluded from the models because of high covariance with *C. harengus membras* (correlation coefficient 1.0), thus further it may be considered, that the species have similar effect on mercury uptake. The following three models were selected:

$Hg\ concentration_{ij} \sim Gaussian(\mu_{ij})$

$E(Hg\ concentration_{ij}) = \mu_{ij}$

Model A: eqn (1)

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Crustacea_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ \ juvenile_{ij} + G.cernua_{ij} + O.limosus_{ij} + \ C.harengus_{ij} + s(Length)_{ij} \ + fSeason$$

Model B: eqn (2)

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ juvenile_{ij} + O.limosus_{ij} + \ C.harengus_{ij} + N.melanostomus_{ij} + s(Length)_{ij} \ + fSeason$$

Model C: eqn (3)

$$\log(\mu_{ij}) = Intercept + \delta^{15}N_{ij} + Chironomidae\ larva_{ij} + P.\ fluviatilis\ juvenile_{ij} + O.limosus_{ij} + \ C.harengus_{ij} + N.integer_{ij} + s(Length)_{ij} \ + fSeason$$

$a_i \sim N(0, \sigma^2_{Nest})$

The Model C, with the most negative slope coefficient demonstrated by significant food item *C. harengus membras*, was selected as an example for visualisation of modelling results. Wilcoxon rank sum exact test were implemented to test differences between distribution and in the rank sums comparing mercury concentrations in perch tissues estimated from the Gaussian GAM model. The model was simulated for the scenarios with the maximum and minimum contribution ratio of *C. harengus membras* and continuously ranged from the maximum to minimum

consumption (contribution) ratios of the other food items. The range limits at specific sampling stations were same as computed by the SIAR model mentioned above. The visualisation example can be found in Additional file 2.

Data exploration, artworks, and statistical analyses were performed using R software for Windows, release 4.0.3.

Results

Mercury (Hg) concentrations and stable isotope analysis

Hg concentrations measured in the dorsal muscles of perch varied notably in all three stations (Table 2), thus mean concentrations and standard deviations (± SD) were 188.2±42.0, 154.2±71.3 and 110.8±65.1 μg kg^{-1} of wet weight in Station 1, 2 and 3, respectively. The ranges of nitrogen stable isotope ratios ($\delta^{15}N$) measured in perch were obviously similar (between approximately 14 and 18 $\delta^{15}N$ ‰) at all three sites. At the same time, stable isotope ratios of carbon ($\delta^{13}C$), which can be used as an indicator of distance between the feeding ground and marine environments, showed wide ranges (approximately 12 ‰ units) for perch individuals caught in Stations 1 and 2, covering also isotopic signals associated with the coastal sampling station, and relatively narrow ranges (approximately 4 ‰ units) for the individuals from Station 3. Similarly, to perch, also ruffe (*Gymnocephalus cernua*) and roach (*Rutilus rutilus*) exhibited high variations of Hg concentration and stable isotope ratios.

Table 2. List of collected organisms and measured mercury (Hg) concentration, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios.

Species	Type	Hg µg kg^{-1} dw (Min÷Max)	Hg µg kg^{-1} ww (Min÷Max)	$\delta^{13}C$±SD ‰ (Min÷Max)	$\delta^{15}N$±SD ‰ (Min÷Max)
Station 1					
SPM	-	38.5±18.2 (18.5÷54.3)	NA	-31.5±0.8 (-32.3÷-30.6)	4.2±3.0 (1.6÷7.6)
Amphipoda	Crustacean	57.7±0	NA	-27.9±0 (-27.9÷-27.9)	10.3±0 (10.3÷10.3)
Hydrachna	Arachnid	NA	NA	-33.4±0.1 (-33.5÷-33.3)	17.0±1.6 (12.0÷18.8)
Ephemeroptera	Insect	NA	NA	-33.3±0.0 (-33.3÷-33.3)	13.4±0.1 (13.3÷13.5)
Trichoptera	Insect	NA	NA	-31.4±0 (-31.4÷-31.4)	13.2±0.2 (13.0÷13.4)
Dreissena polymorpha	Bivalva	141.0±0	15.6±0	-32.3±0.7 (-33.0÷-31.6)	12.3±0.3 (12.0÷12.6)
Oligochaeta	Annelida	272.0±0	NA	-29.8±0.0 (-29.8÷-29.8)	13.8±0.0 (13.8÷13.8)
Chironomidae larva	Insect	253.0±0	2.3±0	-29.7±0.0 (-29.8÷-29.7)	13.4±0.0 (13.4÷13.4)
Orconectes limosus	Crustacean (crayfish)	223.0±0	33.8±0	-30.8±0.2 (-30.9÷-30.6)	15.0±0.1 (14.9÷15.0)
Gymnocephalus cernua	Fish	1539.0±516.2.0 (1904.0÷1174.0)	264.0±100.9 (182.8÷377.1)	-28.5±2.4 (-30.2÷-23.9)	17.4±0.9 (15.9÷18.5)
Alburnus alburnus	Fish	504.0±0	91.0±0	-30.2±0.5 (-30.8÷-29.7)	15.6±0.2 (15.4÷15.8)
Vimba vimba	Fish	NA	NA	-28.6±0.0 (-28.6÷-28.6)	18.1±0.0 (18.1÷18.1)
Rutilus rutilus	Fish	825.0±0	166±9.8 (159÷173)	-30.7±0.6 (-31.6÷-29.6)	16.1±0.5 (15.4÷17)
Silurus glanis	Fish	1003.0±0	187.8±0	-30.6±0.0 (-30.7÷-30.6)	17.9±0.0 (17.8÷17.9)
Sander lucioperca	Fish	1082.0±0	220.1±0	-30.1±0.0 (-30.1÷-30.1)	19.0±0.0 (18.9÷19.0)
Perca fulviatilis	Fish	1035.8±218.0 (456.0÷1308.0)	188.2±42.0 (82.2÷249.6)	-26.6±3.7 (-32.5÷-20.7)	17.3±1.1 (13.6÷18.8)
Station 2					
SPM	-	52.8±22.7 (76.1÷30.6)	NA	-31.4±0.6 (-32.1÷-30.9)	4.6±2.8 (2.6÷7.8)
Amphipoda	Crustacean	10.1±0	NA	-26.1±1.0 (-26.8÷-25.4)	6.9±0.7 (6.4÷7.4)
Dreissena polymorpha	Bivalva	60.7±0	6.6±0	-31.5±0.1 (-31.5÷-31.4)	10.1±0.1 (10÷10.2)

Oligochaeta	Annelid	162.0±0	NA	-31.6±0 (-31.6÷-31.6)	10.6±0.1 (10.5÷10.6)
Chironomidae larva	Insect	145.0±0	NA	-35.6±0 (-35.6÷-35.6)	8.3±1.0 (8.1÷9.8)
Vimba vimba juv.	Fish	61.0±0	25.4±0	-27.4±0 (-27.4÷-27.4)	12.0±0 (12.0÷12.0)
Perca fulviatilis juv.	Fish	45.5±0	21.9±0	-28.7±0 (-28.7÷-28.7)	10.8±0 (10.8÷10.8)
Leuciscus cephalus juv.	Fish	36.9±0	15.6±0	-27.4±0 (-27.4÷-27.4)	11.8±0 (11.8÷11.8)
Rutilus rutilus juv.	Fish	47.4±0	23.1±0	-28.5±0 (-28.5÷-28.5)	10.9±0 (10.9÷10.9)
Gymnocephalus cernua	Fish	487.0±0	92.4±0	-29.6±0 (-29.6÷-29.6)	16.6±0.1 (16.5÷16.6)
Alburnus alburnus	Fish	427/0±0	85.1±0	-31.4±0.1 (-31.5÷-31.3)	14.8±0.1 (14.7÷14.8)
Sander lucioperca	Fish	887.1±502.8 (359.5÷1603.0)	175.0±97.8 (70.0÷337.5)	-28.9±2.1 (-31.1÷-24.5)	17.7±0.2 (17.3÷18.0)
Rutilus. rutilus	Fish	559.3±155.3 (364÷719)	112.6±33.4 (70.6÷146.9)	-29.67±0.8 (-58.2)	14.72±0.21 (14.4÷15.1)
Perca fulviatilis	Fish	813.3±367.4 (289.0÷1752.0)	154.2±71.3 (57.3÷351.2)	-24.8±3.4 (-32.9÷-20.9)	16.6±0.7 (14.4÷18.1)
Station 3					
SPM	-	14.4±14.5 (2.58÷42.7)	NA	-26.5±1.8 (-28.9÷-24.2)	6.0±1.5 (4.2÷9.1)
Neomysis integer	Crustacean	28.8±0	NA	-22.6±0 (-22.6÷-22.6)	11.4±0 (11.4÷11.4)
Crangon crangon	Crustacean	300.7±0	NA	-19.6±0 (-19.6÷-19.6)	12.9±0 (12.9÷12.9)
Saduria entomon	Crustacean	103±0	20.4	-16.9±1.4 (-18.3÷-15.6)	12.8±3.6 (9.67÷16.0)
Amphipoda	Crustacean	36±0	NA	-20.1±1.5 (-21.0÷-16.7)	10.2±1.6 (6.6÷11.2)
Copepoda	Crustacean	NA	NA	-17.5±3.3 (-19.9÷-15.2)	7.3±0.5 (6.9÷7.7)
Cladocera	Crustacean	NA	NA	-24.3±0.2 (-24.5÷-24.1)	5.6±0.0 (5.6÷5.7)
Polychaeta	Annelid	NA	NA	-21.0±0.1 (-21.1÷-20.9)	14.6±0.1 (14.5÷14.8)
Limecola balthica	Bivalva	NA	NA	-21.7±0.0 (-21.8÷-21.7)	11.2±0.1 (10.9÷11.5)
Gymnocephalus cernua	Fish	343.0±0	68.7±0	-24.5±4.2 (-29.2÷-21.2)	16.1±0.2 (15.7÷16.4)
Neogobius melanostomus	Fish	134.0±8.1	26.7±0.8	-21.0±0.3	15.2±0.7

		(127.0÷141.0)		(-21.3÷-20.4)	(14.6÷16.4)
Ammodytes tobianus	Fish	NA	NA	-23.3±0.0 (-23.3÷-23.3)	14.2±0.0 (14.1÷14.2)
Zoarces viviparus	Fish	NA	NA	-21.0±0.0 (-21.1÷-21.0)	17.4±0.0 (17.3÷17.4)
Sprattus sprattus balticus	Fish	NA	NA	-22.1±0.1 (-22.1÷-22.0)	14.0±0.0 (14.0÷14.1)
Platichthys. flesus	Fish	193.0±0	37.4±0	-21.9±1.9 (-24.6÷-20.6)	15.5±0.7 (14.6÷16.2)
Clipea harengus membras	Fish	182.8±59.1 (140.0÷320.0)	33.7±12.5 (24.7÷60.2)	-21.2±1.1 (-23.1÷-18.4)	13.5±1.2 (10.2÷15.2)
Osmerus eperlanus	Fish	227.0±87.6 (121.0÷331.0)	51.9±17.5 (29.3÷64.9)	-22.1±1.4 (-24.2÷-20.6)	16.6±1.0 (14.7÷17.6)
Sander lucioperca	Fish	223.0±0	45.5±0	-22.4±0.9 (-23.3÷-21.6)	16.6±0.3 (16.2÷16.9)
Rutilus. rutilus	Fish	363.4±224.2 (179.0÷687.0)	70.0±39.1 (37.8÷126.2)	-26.1±3.6 (-30.2÷-21.1)	15.0±0.6 (13.4÷16.0)
Perca fulviatilis	Fish	579.2±343.5 (210.0÷1794.0)	110.8±65.1 (44.5÷362.7)	-21.7±1.0 (-24.2÷-20.0)	16.6±0.9 (14.9÷18.6)

Table 2. List of collected organisms and measured mercury (Hg) concentration, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios.

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Stomach content analysis

The analysis of stomach content showed that dietary preferences of perch significantly differ between fresh water and brackish water habitats (Figure 2). At sampling Stations 1 and 2, the crustaceans (found in 56% and 42% of the analyzed stomachs, respectively) were the predominant prey. Juvenile perch (22% at Station 1 and 25% at Station 2) and Chironomidae larva (11% at Station 1 and 21% at Station 2) were second favorite prey organisms while *O. limosus* and *G. cernua* were found mainly only in the digestive tract of perch from Station 1. At the same time, *N. integer* was the most preferred prey in Station 3 (found in 78% of stomachs). *N. integer* was also found in 25% of perch stomachs from freshwater Station 2. The *N. melanostomus* was the second most common prey in Station 3, where it was found in 29% of perch stomachs. The *A. tobianus* and *C. harengus membras* were represented only in 12% and 6% of stomachs from Station 3.

Cluster analysis

Scatterplot of the calculated stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ demonstrated clear evidence that perch specimens migrate between the sampling stations (Figure 3). Substantial proportion of specimens sampled in Stations 1 and 2 had isotopic signals consistent with feeding in Station 3 (Figure 3A). Consequently, we divided the dataset into four subgroups, according to the three characteristics: sampling place, stable isotope ratios $\delta^{13}C$, and stable isotope ratios $\delta^{15}N$ related to a trophic position of organism (Figure 3B and 3C).

The division was done as a cluster analysis based on the linear model criterion of least squares. Three of the subgroups were clearly representing respective sampling stations, while the fourth subgroup was well positioned as the mixed group with overlapping isotope ratio signals, which cannot be associated to any of the three sampling stations.

Exploration of the identified groups

The data was re-examined comparing Hg concentrations and distribution of individual's length among the new groups designated via cluster analysis. Group 1 exhibited the highest Hg concentrations (Figure 4A) while lowest mean concentrations of Hg was found in group 3. Opposite to concentration levels, the highest mean length of perch was found in group 3 while the lowest one in group 2. The groups 1 and 4 exhibited the middle values (Figure 4B). Although the calculated bioaccumulation slopes were quite similar among the groups (coefficient values from 0.015 to 0.029), the intercepts differed noticeably (coefficient values from 1.4 for group 3 to 2.1 for group 1), thus indicating high variation of background Hg concentrations (Figure 4C).

Quantification of feeding preferences

Feeding preferences of the assigned subgroups were defined by means of Bayesian mixing model SIAR (Figure 5) based on results of the stomach content analysis of every perch individual representing respective subgroup. The different types of crustaceans, *G. cernua, Chironomidae* larva and *O. limosus* were defined as the main food sources for perch in the Station 1. The feeding base in the Station 2 mainly consisted of crustaceans, *G. cernua,* Chironomidae larva and juvenile perch while the *N. integer, N. melanostomus, A. tobianus*, *C. harengus membras* and crustaceans were main food items of perch in the Station 3. The most preferred prey of the newly formed mixed group was *O. limosus* from Station 1, juvenile perch from the Station 2, and *N. integer* and *N. melanostomus* from the Station 3.

The food items' contribution ratios were extracted from the model and used for the further analysis of influence of dietary preferences. The data tables are published in the supplementary materials.

Biomagnification Factor (BMF)

Biomagnification factor was calculated for specific food chains, reflecting the localized diet of perch for each identified subgroup. The absolute values of the calculated biomagnification factors were quite similar for the station-assigned groups (1.45, 1.40, 1.46, respectively), but substantially higher for the mixed group (1.76).

The biomagnification curve of the mixed group (group 4) was significantly different from the others by a steeper slope (Table 3, M2) and by higher intercept compared to group 3 (Table 3, M1). Meanwhile, groups 1 to 3 had statistically similar slopes, thus indicating similar biomagnification patterns (Table 3, M2). At the same time, significantly lower intercept of group 3, compared to groups 1 and 2 (Table 3, M1) probably denotes again lower Hg background concentrations found at the station.

Table 3 Comparison of biomagnification curves. Bold italic text indicates significant differences between the curves.

	M1: without interaction[1]		M2: with interaction[2]		ANOVA: M1 vs M2[3]	
Compared groups	F value	p-value	F value	p-value	F value	p-value
1. vs 2.	0.009	0.926	0.187	0.669	0.187	0.669
1. vs 3.	**8.569**	**0.005**	0.011	0.916	0.011	0.916
1. vs 4.	0.000	0.990	**13.480**	**<0.001**	**13.484**	**<0.001**
2. vs 3.	**9.943**	**0.002**	0.771	0.383	0.771	0.382
2. vs 4.	0.785	0.381	**10.410**	**0.003**	**10.414**	**0.003**
3. vs 4.	**6.859**	**0.011**	**15.110**	**<0.001**	**15.110**	**<0.001**

[1]The regression model M1 includes independent variable $\delta^{15}N$, independent factor Group and dependent variable LOG mercury concentrations.

[2]The regression model M2 includes interaction between independent variable $\delta^{15}N$ and independent factor Group and dependent variable LOG mercury concentrations.

[3]One-way analysis of variance of the regression models M1 and M2

Influence of dietary preferences

Generalized Additive Modelling was implemented to understand how dietary preferences of perch in different feeding grounds affect the Hg uptake. To avoid covariance of food source variables three validated models with different combination of food items were selected and interpreted (Table 4). The obtained results indicated seasonality (spring and autumn sampling) as a significant factor affecting measured Hg LOG-concentrations, for example, samples collected in spring had higher levels of Hg concentration than the autumn samples (demonstrated by a positive intercept correction for the spring season from 0.106 up to 0.120). The stable isotope ratio $\delta^{15}N$ showed a significant relationship with the Hg concentration, however the positive slope coefficient was only 0.09 in the all three models.

The models let us to establish that the food item *C. harengus membras* had the most significant mitigating effect on Hg concentration, with negative slopes ranging from -0.349 to -0.501. Another food item with significant Hg-lowering properties was Crustacea with a negative slope coefficient of -0.460. The rest of the food items were not significant at the $\alpha = 0.05$ level, although had different directions of the influence and slope values. A highly positive effect was observed for Chironomidae larva (slope values from 0.233 to 0.460). *O. limosus* (slope values from 0.143 to 0.184), perch juvenile (slope values from 0.010 to 0.186) and *G. cernua* (slope value 0.120) were other food items that contributed to the uptake of Hg by perch. *N. melanostomus* and *N. integer* exhibited a neutral influence on Hg concentration measured in consumer perch, indicating slightly positive slope coefficients of 0.080 and 0.023, respectively.

Table 4. Estimated regression parameters (intercept and slope values), standard errors, t-values and P-values for the Gaussian GAM presented in equations 1, 2 and 3.

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Table 4. Estimated regression parameters (intercept and slope values), standard errors, t-values and P-values for the Gaussian GAM presented in eqn (1, 2, 3).

Model A

$\log(\mu_{ij}) =$

$0.536 + 0.092 \times \delta^{15}N_{ij} - 0.460 \times \textit{Crustacea}_{ij} + 0.460 \times \textit{Chironomidae larva}_{ij} + 0.098 \times \text{Perch juvenile}_{ij} + 0.120 \times \textit{G.cernua}_{ij} + 0.142 \times \textit{O.limosus}_{ij} - 0.349 \times \textit{C.harengus}_{ij} + s(\text{Length})_{ij}$ AUTUMN

$0.642 + 0.092 \times \delta^{15}N_{ij} - 0.460 \times \textit{Crustacea}_{ij} + 0.460 \times \textit{Chironomidae larva}_{ij} + 0.098 \times \text{Perch juvenile}_{ij} + 0.120 \times \textit{G.cernua}_{ij} + 0.142 \times \textit{O.limosus}_{ij} - 0.349 \times \textit{C.harengus}_{ij} + s(\text{Length})_{ij}$ SPRING

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.53618	0.31742	1.689	0.095569
SeasonS	0.10594	0.02712	3.907	0.000211
$\delta^{15}N$	0.09255	0.01916	4.83	7.60E-06
Crustacea	-0.4601	0.19332	-2.38	0.019996
Chironomidae larva	0.45997	0.23451	1.961	0.053751
Perch juvinile	0.09828	0.13144	0.748	0.457114
G. cernua	0.11981	0.12699	0.943	0.348636
O. limosus	0.14249	0.13139	1.084	0.281832
C. harengus	-0.34935	0.16865	-2.071	0.041948

Approximate significance of smooth terms:

	edf	**Ref.df**	**F**	**p-value**
s(Length)	2.901	3.649	18.3	<2e-16

R-sq.(adj) = 0.759 Deviance explained = 79.1%

Model B

$\log(\mu_{ij}) =$

$0.514 + 0.090 \times \delta^{15}N_{ij} + 0.299 \times \textit{Chironomidae larva}_{ij} + 0.186 \times \text{Perch juvenile}_{ij} + 0.174 \times \textit{O.limosus}_{ij} - 0.484 \times \textit{C.harengus}_{ij} + 0.079 \times \textit{N.melanostomus}_{ij} + s(\text{Length})_{ij}$ AUTUMN

$0.628 + 0.090 \times \delta^{15}N_{ij} + 0.299 \times \textit{Chironomidae larva}_{ij} + 0.186 \times \text{Perch juvenile}_{ij} + 0.174 \times \textit{O.limosus}_{ij} - 0.484 \times \textit{C.harengus}_{ij} + 0.079 \times \textit{N.melanostomus}_{ij} + s(\text{Length})_{ij}$ SPRING

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.51438	0.32367	1.589	0.11639
SeasonS	0.11453	0.02798	4.093	0.00011
$\delta^{15}N$	0.09064	0.01931	4.694	1.24E-05
Chironomidae larva	0.29922	0.21468	1.394	0.16766
Perch juvinile	0.18633	0.12887	1.446	0.15255
O. limosus	0.17368	0.12915	1.345	0.18291
C. harengus	-0.484	0.16458	-2.941	0.0044
N. melanostomus	0.0792	0.10274	0.771	0.44329
Approximate significance of smooth terms:				
	edf	**Ref.df**	**F**	**p-value**
s(Length)	2.814	3.545	17.62	<2e-16
R-sq.(adj) = 0.745 Deviance explained = 77.5%				

Model C

$\log(\mu_{ij}) =$

$0.529 + 0.091 \times \delta^{15}N_{ij} + 0.233 \times \textit{Chironomidae larva}_{ij} + 0.167 \times \text{Perch juvenile}_{ij} + 0.184 \times \textit{O.limosus}_{ij} - 0.501 \times \textit{C.harengus}_{ij} + 0.023 \times \textit{N.integer}_{ij} + s(\text{Length})_{ij}$ AUTUMN

$0.649 + 0.091 \times \delta^{15}N_{ij} + 0.233 \times \textit{Chironomidae larva}_{ij} + 0.167 \times \text{Perch juvenile}_{ij} + 0.184 \times \textit{O.limosus}_{ij} - 0.501 \times \textit{C.harengus}_{ij} + 0.023 \times \textit{N.integer}_{ij} + s(\text{Length})_{ij}$ SPRING

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.52858	0.34508	1.532	0.1299
SeasonS	0.12052	0.02698	4.467	2.87E-05
$\delta^{15}N$	0.09056	0.01975	4.584	1.87E-05
Chironomidae larva	0.23255	0.24345	0.955	0.3426
Perch juvinile	0.16704	0.15511	1.077	0.2851
O. limosus	0.18396	0.13431	1.37	0.175
C. harengus	-0.50118	0.22139	-2.264	0.0266
N. integer	0.02286	0.13537	0.169	0.8664
Approximate significance of smooth terms:				
	edf	**Ref.df**	**F**	**p-value**
s(Length)	2.747	3.467	19.01	<2e-16
R-sq.(adj) = 0.742 Deviance explained = 77.3%				

Discussion

The combination of stomach content analysis, as a sort of “snap-shot” of the recently consumed prey, with metabolically active tissues (such as muscles) that provide dietary and source information for up to several weeks [9] were instrumental in sorting out to which geographically distinct sampling area each perch specimen should be assigned. Since perch in the Gulf of Riga (Station 3 area) do not have suitable spawning and nursing grounds the specimen group assigned to that area has obviously migrated to the Gulf of Riga from freshwater similarly to that observed elsewhere by Järv [12]. This agrees with behavioural features of perch, like seasonal patterns in their distribution and movement between habitats [28]. The distinct stomach content and isotopic signal characteristic for this group suggests that once migrated to the coastal waters the perch specimens stay there whether as stable kin-related groups as suggested by Gerlach et al. [6] and Semeniuk et al. [28] or as separate individuals. The approach applied in this study enabled us also to identify recent arrivals, e.g., specimens that have been feeding and accumulating Hg in another distinct area than that they were caught in.

As we successfully demonstrated, the perch specimens in freshwater ecosystems (river and lake stations) have substantially higher Hg concentrations. So, with some degree of certainty we can speculate that observed inter-annual differences, from 30 μg kg^{-1} ww in 2019 to 103 μg kg^{-1} ww in 2015, of Hg values obtained within national monitoring program (LIAE database) in coastal waters represented by Station 3, can mostly be explained by different proportion between recent arrivals from adjacent freshwater basins and specimens that have been feeding in area for more extended time period. Furthermore, the seasonal factor produced by all three GAM models, e.g., higher mercury levels were associated with spring sampling, can be clearly related to recent migration from inland waters to the coastal.

Although the concentrations of Hg in specimens representing freshwater ecosystem are substantially higher than in specimens representing marine coastal waters, the subtraction of values measured in recent arrivals from calculation of mean concentration resulted in slight increase of mean concentration in the coastal group. Most likely observed phenomenon is related to significant upward change of median size of perch, and not to the pollution level itself. Therefore, it can be argued, that comparison of concentration means alone is poor pollution assessment approach.

At the same time, the Hg bioaccumulation curves in relation to the individual's length gave more detailed information about the specific uptake tendencies. The results indicate that functional processes responsible for mercury accumulation (for example fish biometrics), Hg bioavailability and chemical composition of Hg substances [32] are quite similar, independently on the origin of specimen or local feeding base. So, the geographical differences in Hg concentration were mainly observed because background concentrations of Hg are substantially higher in the inland water bodies than in the Gulf of Riga. This conclusion is supported by notably higher Hg levels in suspended matter, used as a proxy of phytoplankton, measured in river and lake stations than in the Gulf of Riga. And, as stated by Kehrig [14], Hg enters food web at phytoplankton level and is transferred to higher organisms via trophic transfer.

The general structure of perch diet was quite similar among the studied areas, e.g., mostly several types of crustaceans, *Chironomidae* larvae and small fish. However, *C. harengus membras* presented only in the gulf station exhibited noticeable mercury reduction properties, which explains the substantial differences in the levels of mercury measured in the station-associated groups indicated by the clustering analysis. Moreover, according to the study, the trophic position of prey alone (in our case, $\delta^{15}N$) cannot be associated with the intensity of Hg uptake by consumer. For example, *Chironomidae* larvae ($\delta^{15}N$ 8.3÷13.4) and *Crustacea* ($\delta^{15}N$ 7.0÷12.9) within the comparable maximum consumption ratio exhibited the opposite effects on the estimated mercury concentration in perch tissues, and *N. integer* ($\delta^{15}N$ 11.4) despite the twofold maximum consumption ratio showed a neutral impact. Similarly, higher pollution rates cannot be associated with the trophic position of prey within the same feeding ground, which was well demonstrated by *Chironomidae* larvae and *G. cernua* ($\delta^{15}N$ 15.7÷18.5), where the prey with lower values of nitrogen isotope ratios had stronger correlation with high Hg concentrations estimated from the model. Therefore, the suggestion by Le Croizier et al.[16] to utilize precise determination of the food sources for better facilitation of tracing of metal accumulation requires information on background concentrations at the site is important in our study as well.

The limitation of this study is that we present complete picture only from a single year perspective. We can of course speculate that the site-specific food items defined in this study will influence perch Hg levels at an equal level also during following years. However, the well-known opportunistic feeding behaviour of perch [28] suggests that they will inevitably switch to other taxa if availability of previously consumed taxa becomes limited, or if appears more profitable source of energy, similarly as round goby (*Neogobius melanostomus*, invasive in the Baltic Sea) became a highly preferred prey for perch in recent years [1, 26]. Another weak point to be considered is that the isotopic signal changes faster than the level of accumulated Hg [3, 31]. So, the specimens, that at the onset of feeding period have spent sufficient time in one area to equilibrate Hg concentration with the level characteristic for that area and then

migrates to another area and have time to change isotopic signals before they are caught, might not have sufficient time to adjust also Hg levels. This could be improved by more regular sampling, which would give more precise information about influence of perch mobility on measured mercury concentrations. Also, comparison with another distant Gulf of Riga station insignificantly affected by the large freshwater ecosystems, could be useful adjunction for the further studies.

Conclusion

In general, the study clearly showed that the high mobility of perch along associated aquatic systems has notable effect on the measured mercury levels, which could be an issue for consideration during pollution monitoring events. Jones et al. [13] suggests that, to avoid misinterpretation of spatial and temporal trends, fish biometrics modelling is of high significance when designing any monitoring program focused on seafood safety. In its turn the current study showed that trophic position and isotopic signatures can also serve as important supplementary tools for more accurate data interpretation.

Abbreviations

AAS - Atomic Absorption Spectrophotometry

AIC - Akaike Information Criterion

ANCOVA - Analysis of covariance

BMF - Biomagnification factor

C – carbon

$^{13}C/^{12}C$ carbon stable isotope ratio

GAM – Generalized Additive Model

GF/F – glass fibre filter

HELCOM - The Baltic Marine Environment Protection Commission

HEPP - Hydroelectric Power Plant

Hg – Mercury

LIAE – Latvian Institute of Aquatic Ecology

LOG – logarithmic

N - nitrogen

$^{15}N/^{14}N$ – nitrogen stable isotope ratio

QUASIMEME - Quality Assurance of Information for Marine Environmental Monitoring in Europe

SD – standard deviation

SIAR – package “Stable Isotope Analysis in R”

SPM - suspended particular matter

US EPA - United States Environmental Protection Agency

Declarations

Availability of data and materials

The datasets obtained and analyzed in the current study are available from the corresponding author on reasonable request.

Acknowledgements

The authors would like to thank stuff of Latvian Institute of Aquatic Ecology Viktors Pērkons, Atis Labucis, PhD Māris Skudra and Miks Pipirtis taking care of sampling campaigns, operation of boat and sampling equipment, such as scientific gill nets and Wildco Petite Ponar Grab; Ieva Putna-Nīmane and Anete Fedorovska taking part in zooplankton sampling and pre-treatment; as well as Mintauts Jansons for analysis of mercury concentrations and management of analytical equipment. The authors would also like to thank Professor, Dr.chem. Artūrs Vīksna, Lauma Buša, Māris Bērtiņš from the Laboratory of Analytical Chemistry at Faculty of Chemistry, University of Latvia for analysis of stable isotopes.

Funding

This study has received funding from the State Research Program EVIDEnT – “The value and dynamic of Latvia’s ecosystems under changing climate” and the project “Improving knowledge on the state of the marine environment” financed by European Maritime and Fisheries Fund 2014-2020, Project Nr.: 17-00-F06803-000001.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Supplementary Information

Additional file 1

Additional file 2

Additional file 3

Authors’ contributions

NS: conceptualization, sampling, investigation, sample treatment, data interpretation and visualization, writing of drafted the original version. JA: conceptualization, data interpretation, writing—review and editing; RP: sampling site selection, experimental analysis, writing—review and editing. JT: sampling, experimental analysis, editing and reviewing. All authors read and approved the final manuscript.

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Figures

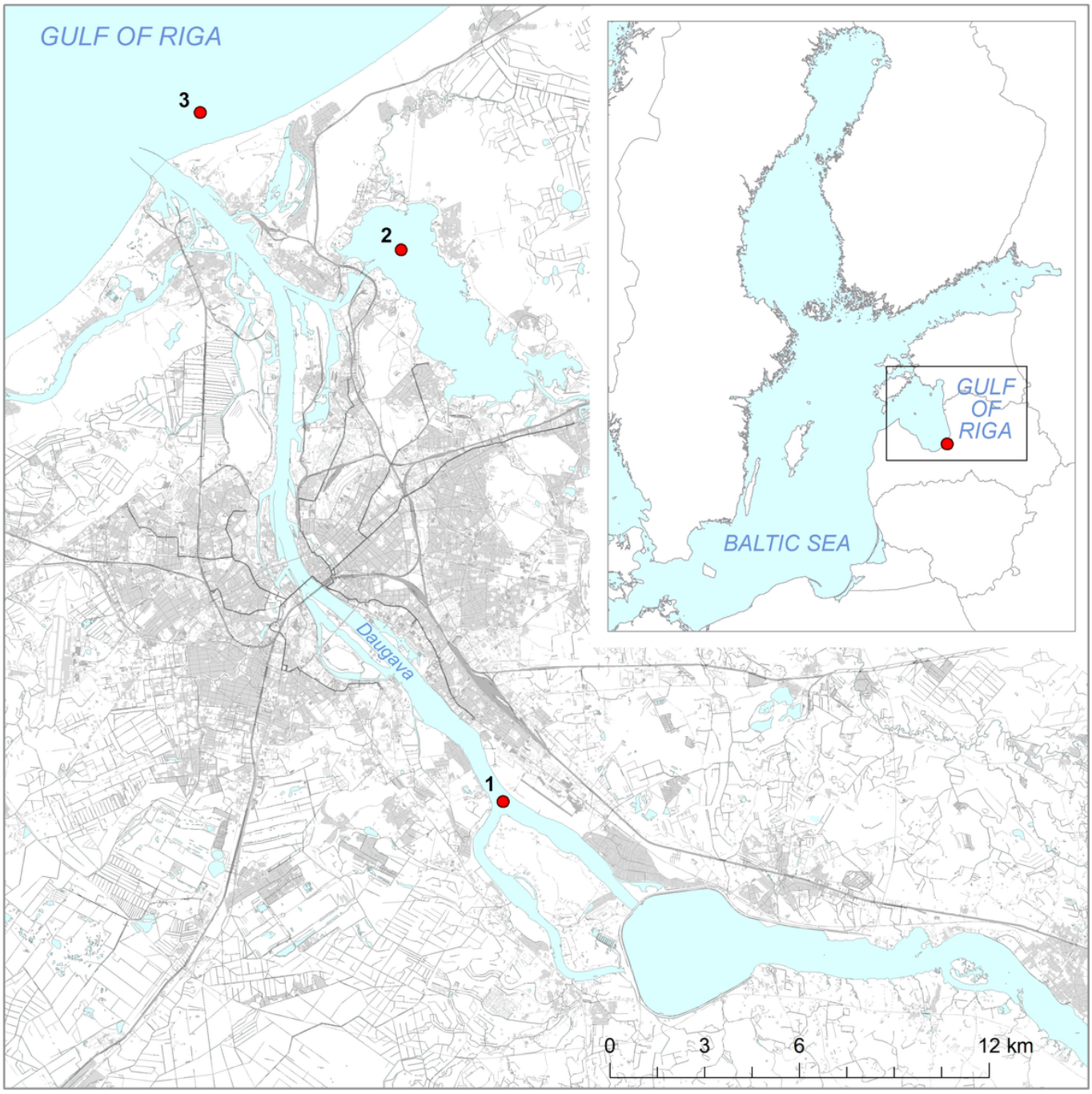

Figure 1

Sampling sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

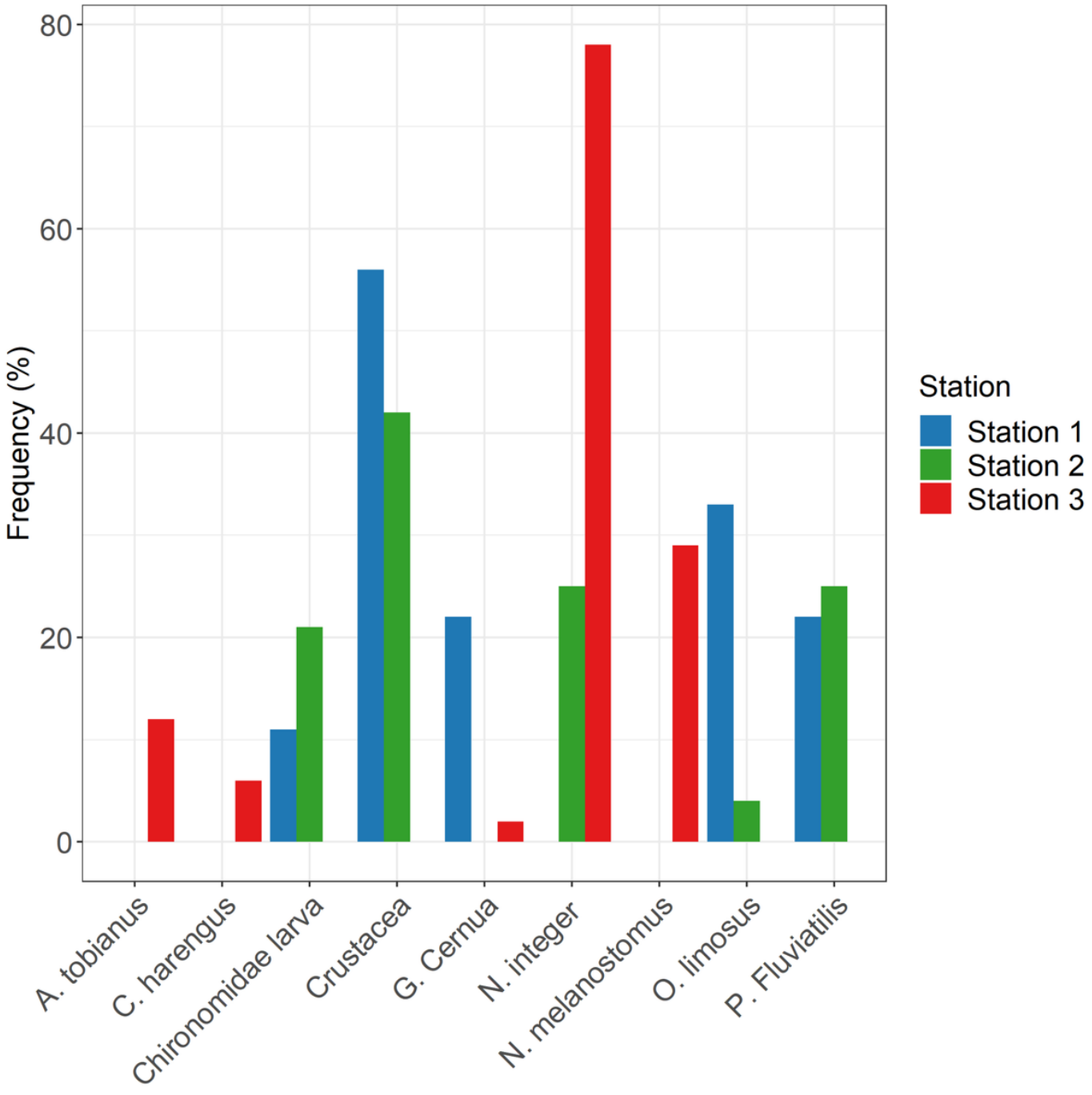

Figure 2

Diet composition of perch based on stomach content analysis.

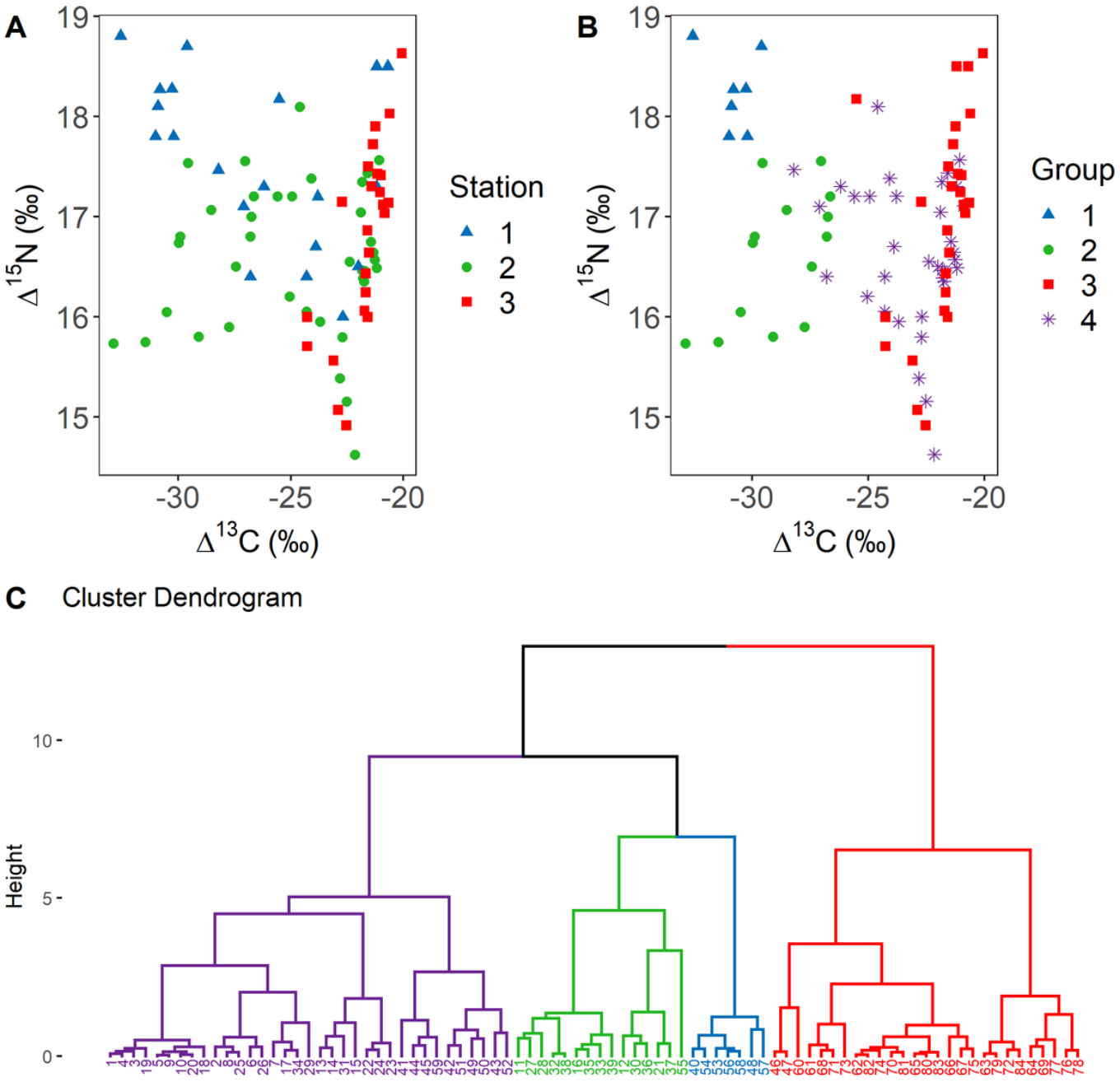

Figure 3

Scatterplots of measured values of stable isotope ratios (δ13C and δ15N). A before and after B clustering analysis; C cluster dendrogram indicating new grouping of the sampled perch, blue – Station 1, green – Station 2, red – Station 3, purple – the mixed group.

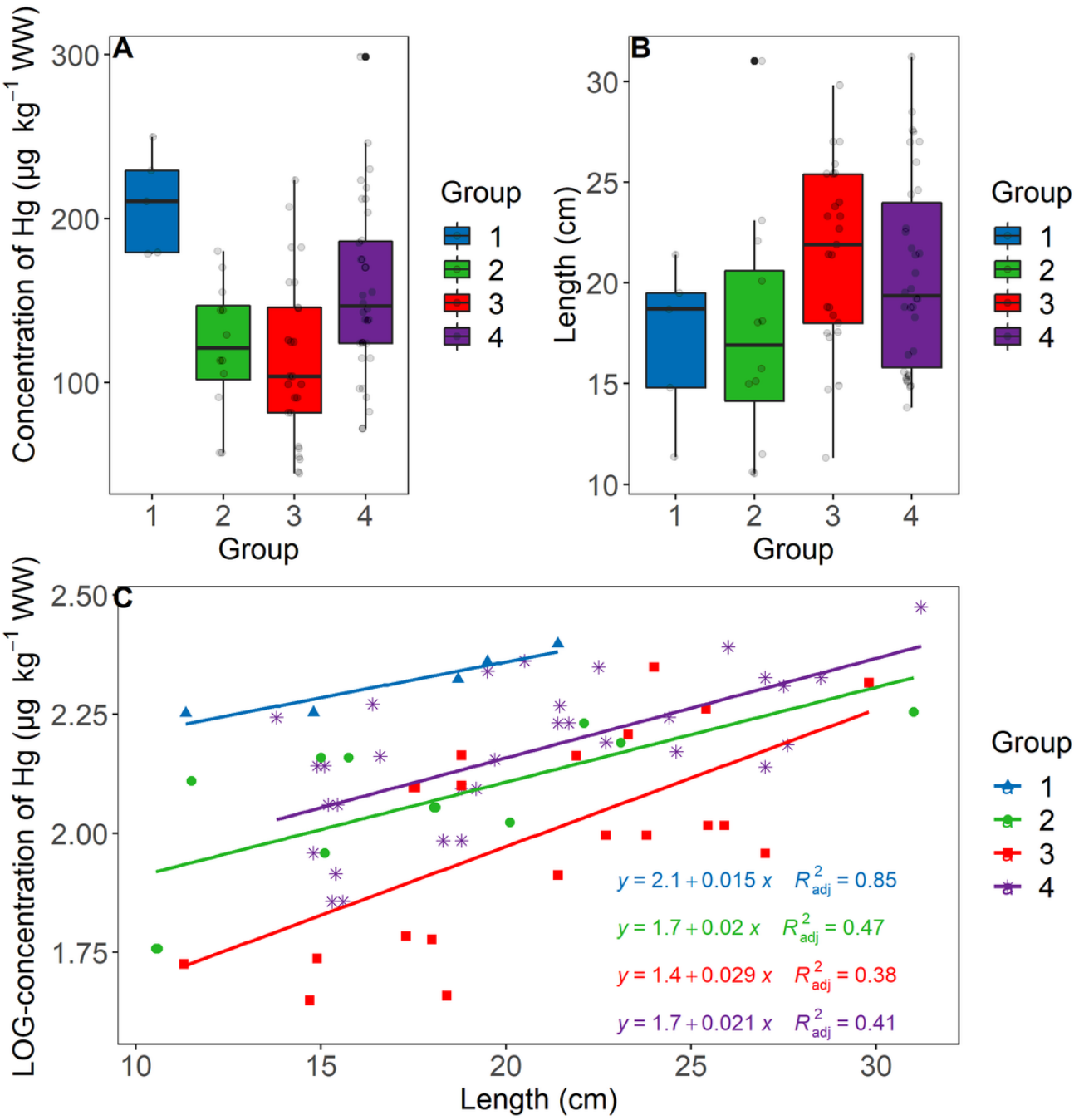

Figure 4

A Variation of Mercury concentrations (µg kg-1 ww) among the four subgroups of perch. B Variation of perch length among the identified four groups. C Mercury bioaccumulation curves expressed as linear equations of LOG-transformed Hg concentration versus perch length.

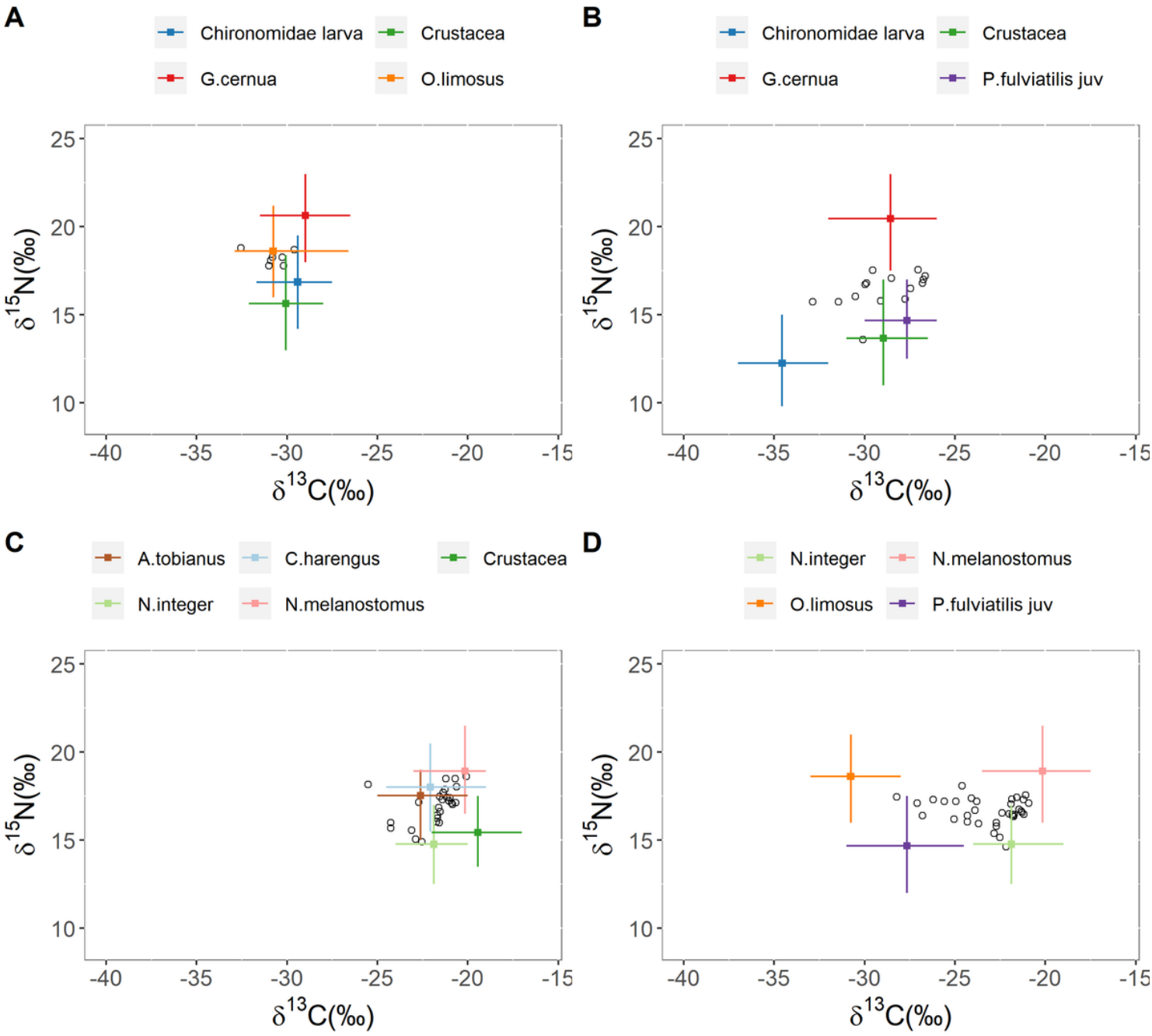

Figure 5

Results of SIAR Bayesian mixing model. A Station 1, B Station 2, C Station 3, D mixed group; black circles – perch specimens (consumers)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx
- Additionalfile2.pdf