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Seasonal Variations in the Use of Profundal Habitat among Freshwater Fishes in Lake Norsjø, Southern Norway, and Subsequent Effects on Fish Mercury Concentrations

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Abstract: This study is based on monthly sampling of fish from grates mounted at an industrial water intake, located at a depth of 50 m in Lake Norsjø (Southern Norway) during the year 2014, to investigate seasonal variations in the use of the profundal habitat and subsequent variations in total Hg-concentrations in profundal fish. Data on various fish present in a cold and dark hypolimnion of a large, deep, dimictic lake within the upper temperate zone of the Northern Hemisphere are rare. While predominant species such as A. charr (*Salvelinus alpinus*) and E. smelt (*Osmerus eperlanus*) were continuously present in this habitat, whitefish (*Coregonus lavaretus*) occupied this habitat primarily during wintertime, while other common species like brown trout (*Salmo trutta*), perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) were almost absent. Besides stomach analyses (diet) and biometry, stable isotope analyses ($\delta^{15}N$ and $\delta^{13}C$) and total mercury (Tot-Hg) analyses were carried out on the caught fish. The $\delta^{13}C$ signature and stomach analyses revealed a combined profundal-pelagic diet for all three species, A. charr with the most profundal-based diet. Length was the strongest predictor for Hg in whitefish and A. charr, while age was the strongest explanatory variable for Hg in E. smelt. A. charr was the only species exhibiting seasonal variation in Hg, highest during winter and spring.

Keywords: profundal habitat; Hg; Tot-Hg; stable isotopes; biomagnification; *Salvelinus alpinus*; *Coregonus lavaretus*; *Osmerus eperlanus*

1. Introduction

Methylated Hg is an environmental pollutant of concern in aquatic environments [1–4], as it is accumulated in biota, and concentrations rise in accordance with trophic position [5–10]. Fish and fish-eating wildlife often have toxic concentrations of total Hg (Tot-Hg) as a result [7]. In addition to trophic position, Hg-concentrations in fish are well documented to increase with increasing age [8,11] and length [8,11–14]. Contrarily, increasing weight at the same length or age results in lower Hg-concentrations, either by somatic growth dilution (SGD) [11,15–21], or by further concentrating Hg during starvation [22]. The combination of these two effects results in seasonal variations in Hg-concentrations in fish [23–30], however, some studies suggest that this is not the case in all populations [31–33].

Stable isotope ratio analyses of carbon ($\delta^{13}C = C^{13}/C^{12}$) and nitrogen ($\delta^{15}N = N^{15}/N^{14}$) are a highly valuable tool to trace the energy flow ($\delta^{13}C$) and trophic position ($\delta^{15}N$) in food webs [34–36], as the different isotopes have different abilities to form chemical bonds [37]. This means that molecules containing the heavier isotope are more stable, while molecules containing the lighter isotope are more

readily metabolized. Therefore, $\delta^{15}N$ increases at an average of 3.4‰ per trophic level [36,38], and $\delta^{13}C$ can be used to trace dietary carbon sources [36], as this ratio averagely varies with habitat [39]. Habitat and depth also influence Hg, usually meaning an increase of Hg-concentrations in biota with depth [8,11,40]. Comparisons between littoral and pelagic fish at similar trophic position indicate that pelagic fish exhibit higher Hg-concentrations [41–43], while Chumchal and Hambright [44] document no detectable difference.

There is extensive research available on Hg-concentrations and different explanatory variables, as well as seasonal variations, however, most of the literature is limited to the littoral and pelagic zone in lakes, as seasonal data is hardly accessible in the profundal zone. This study is based on fish sampled from an industrial water intake at Fjærekilen in Lake Norsjø (Southern Norway), which provides the unique opportunity to readily sample profundal fish throughout the year. Seasonal patterns in the use of the profundal habitat, as well as seasonal variations in Hg-concentrations in fish, were identified, and the main predictors of Hg-concentrations were investigated. For A. charr (*Salvelinus alpinus*) and whitefish (*Coregonus lavaretus*), length was found to be the most important predictor of Hg-concentrations, while age was most important for E. smelt (*Osmerus eperlanus*). Age, length, weight and $\delta^{13}C$ improved Hg-estimates for some of these three species. Seasonal variations in Hg-concentrations were confirmed for A. charr, with higher Hg-concentrations in spring and winter than in summer and autumn. This is likely to be a consequence of variations in the nutritional status of the fish.

2. Materials and Methods

2.1. Sampled Fish

In total, 471 fish were sampled in the water intake at a depth of ≈ 50 m in Fjærekilen, a bay south in Lake Norsjø. The most abundant species A. charr (n = 191) and E. smelt (n = 158) were present in the catch during all seasons, while whitefish (n = 117) were mainly caught between December and March (Table 1). Perch (*Perca fluviatilis*) (n = 4) and Northern pike (*Esox lucius*) (n = 1) were only sporadically present, and accordingly insufficient data was available for further analysis of these two species. Complete datasets were retrieved from 252 fish in total, 77 for A. charr, 99 for E. smelt and 76 for whitefish. These fish were used for Hg-modelling.

Table 1. Catch of each species each month, and analysed fish per month.

Month	A. Charr	E. Smelt *n* Caught	Whitefish	A. Charr	E. Smelt *n* Analysed	Whitefish
14 January	2	3	18	2	1	15
14 February	9	20	20	5	15	15
14 March	6	21	42	5	15	15
14 April	0	0	0	0	0	0
14 May	27	9	4	13	8	4
14 June	29	0	1	0	0	0
14 July	12	1	0	8	1	0
14 August	10	1	0	0	0	0
14 September	43	23	0	13	14	0
14 October	20	20	2	15	15	2
14 November	0	0	0	0	0	0
14 December	32	40	10	15	15	10
15 January	1	20	20	1	15	15
Total	191	158	117	77	99	76

2.2. Site Description

Lake Norsjø (59.29′ N, 9.36′ E) is a large (55.24 km^2), deep (middle depth = 87 m, maximal depth = 171 m) and oligotrophic lake [45,46] located in Telemark county in southeast Norway.

This study has been performed in Fjærekilen, which is a bay at the southern end of Lake Norsjø extending parallel to the discharge (Figure 1). The discharge to Hjellevannet in Skien is located in an adjacent bay to the north of the study site.

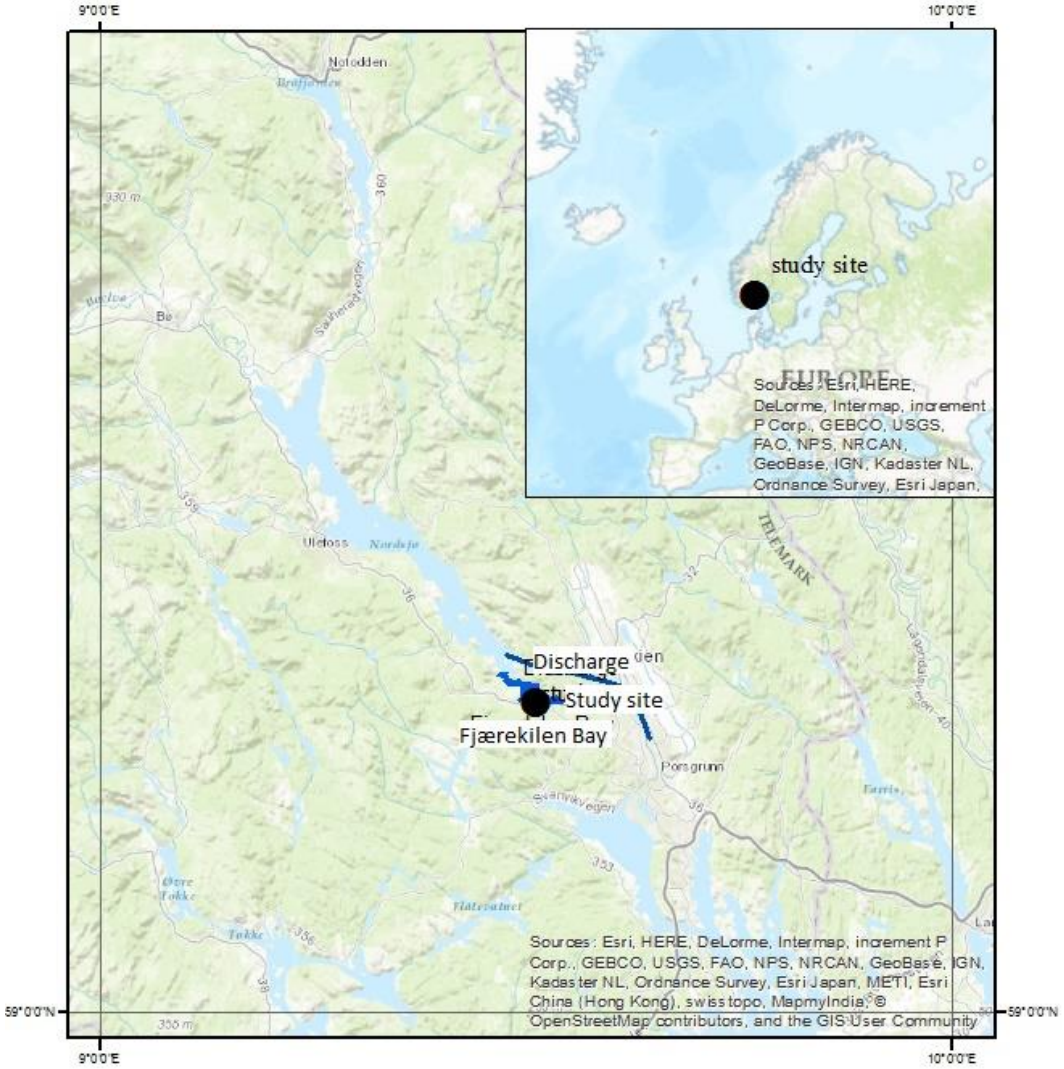

Figure 1. Map of the study area.

2.3. Sampling

The fish used in this study were acquired at an industrial water intake in Fjærekilen, which is located at a depth ≈ 50 m, 60–80 m off the shore, meaning that all fish were sampled at the same location within the profundal habitat. The fish were caught continuously at a grate (mesh size: 10 mm), which is mounted in an artificial pool inside the water intake tunnel, preventing fish being artificially transferred to the brackish fjord Frierfjorden. The grates cover the entire breadth of the water intake tunnel, and collect all fish passing through. Fish was sampled weekly between February 2014 and January 2015. The fish were frozen when collected, and the accumulated catch was stored in plastic bags every week. Additionally, fresh fish were acquired once every month during the sampling period from the grates. All fish were frozen in plastic bags sorted by sampling date and stored in a freezer ($\leq$20 °C) at the University College of Southeast Norway until analysed.

2.4. General Analysis

The collected fish were sorted, and randomly selected subsamples of approximately 20 individuals of each species were analysed each month. Total length of each fish was determined to the closest millimetre in a measuring cone, and weight was determined to the closest gram on a scale. The otoliths were removed, and subsequently burned over a propane torch before being sectioned transversally for later age determination under a stereomicroscope at a magnification of 48 × [47] (p. 80).

2.5. Benthic Invertebrates and Stomach Content Analysis

Benthic invertebrates were caught using two traps consisting of four bundles of hemp rope each, which were placed in the sediment and emptied once a month during the study period [48]. The traps were placed on both sides of the water intake. Additional benthic invertebrates were sampled each month using an Ekman bottom grab at the sites of the traps.

Stomach samples were taken from approximately five fish of each species each month covering the entire length range. However, as a considerable number of stomachs were empty, or diet items were digested beyond recognition, approximately two stomach samples per month could be used for further analysis for each species. The stomachs were preserved in 70% ethanol in glass bottles prior to analysis. Stomach content was identified under a stereomicroscope at a magnification of 48× to the closest taxa using a taxonomic key [49], and each item's occurrence was estimated visually in volume percent.

2.6. Preparation of Muscle Fillet Samples for SI and Hg Analysis

Approximately 2 g of muscle fillet were removed from the dorsal side of each fish under the dorsal fin. The samples were weighted on a scale at a precision to 0.1 g, before freeze-dried in a Heto Lyolab 3000 freeze-drier (Heto-Holten A/S, Allerød, Danmark) for at least 14 h at a temperature $\leq$30 °C. The drying process was aided by an infrared lamp. Dried samples were weighted on a scale with a precision to 0.0001 g. The dried samples were ground and homogenised using an agate pestle and a mortar. This procedure was also applied to the benthic animals, which were processed completely. Due to the animals' low mass, the accumulated catch of each taxonomic group for the respective month was analysed as a pooled sample.

2.7. Stable Isotope Analysis

Up to 15 fish of each species each month were selected for stable isotope analysis, covering the largest possible variety in age, length and weight. In addition, the pooled benthic invertebrate samples were analysed. Between 1.0 and 1.4 mg of the selected, freeze-dried samples were weighted on a scale, and stored in tin capsules of the types Elemental Microanalysis D1006 (6 × 4 mm) and Elemental Microanalysis D1008 (8 × 5 mm). The capsules were sent to the Norwegian Institute for Energy Technology (IFE) for stable isotope analysis. Results were delivered in the delta (δ) notation, which is measured in per mil (‰) deviation from a standard material, and calculated according to the following formula:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000, \quad (1)$$

where R represents the ratio of the heavier isotope ^{13}C or ^{15}N to the lighter ^{12}C or ^{14}N [8,40]. As standard material, Pee Dee belemnite limestone was used to calculate $\delta^{13}C$ [50], and atmospheric nitrogen for $\delta^{15}N$.

2.8. Hg Analysis

Freeze-dried dorsal muscle fillet samples were also used for determination of Tot-Hg-content in fish. Approximately 20 mg were used for each sample, weighted in on a Sartorius AX124 scale (precision: 0.0001 g). Total Hg was analysed by a Lumex Hg-analyser type Pyro-915 (Lumex Instruments, St. Petersburg, FL, USA) at the University College of Southeast Norway, and two replicates were

analysed for each sample. Measurements were repeated if both replicates deviated by more than 10%. The calibration of the equipment was confirmed using a standard sample of tuna (European Reference Material, ERM-CE 464), which was used as control after each 20th fish. Tot-Hg-content was estimated to be the average of the two replicate samples, and concentrations were transformed to resemble wet weight (ww.) using an individual conversion factor based on the weight loss of the fillet sample of each fish. The transformation was applied, because most nations are using wet weight concentrations of Tot-Hg in fish in their monitoring programs and consumption advice guidelines. Due to insufficient mass of the freeze-dried and ground samples, benthic invertebrates could not be analysed for Tot-Hg.

2.9. Data Analysis

Age, length, weight and Tot-Hg-concentrations were logarithmically transformed to match normal distributions using natural logarithms. Descriptive statistics were calculated for age, length, weight, $\delta^{13}C$, $\delta^{15}N$ and Tot-Hg-concentrations for each species and for the stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ of the pooled benthic invertebrate samples. Prior to model building, the logarithmically transformed age, length and weight and $\delta^{13}C$ and $\delta^{15}N$ were centered by subtracting the mean from each transformed observation in order to calculate an interpolated intercept, which represents the average specimen, based on a geometric average. In order to compare Tot-Hg-concentrations between species, all models were used to predict Tot-Hg for a set of explanatory variables, which were chosen in accordance with the maximum and minimum values in the dataset to avoid unnecessary extrapolation. The values for the explanatory variables used are 5.5 yr., 121.5 mm, 10.5 g, −29‰ and 10.14‰ for age, length, weight, $\delta^{13}C$ and $\delta^{15}N$, respectively. Months were grouped in seasons, classifying January, February and March as winter, April, May and June as spring, July, August and September as summer and October, November and December as autumn. Using the centered and transformed age, length and weight, and the centered stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ and the factor season as explanatory variables, and the transformed Tot-Hg-concentrations as response variable, the best fitting explanatory variable was determined by creating linear models for each species and each explanatory variable in R [51]. The models using only one explanatory variable at the time were compared using Akaike information criterion (AIC), where the model with the lowest AIC was chosen for further investigation. Subsequently, multiple linear regression models were created, adding one of the other potential explanatory variables at a time. These models were compared to the original model one by one, using the log likelihood ratio statistic estimated from maximum likelihood (ML) estimates for each model. Additional explanatory variables and two-way interaction terms of two already included variables were added to the model if the more complicated model resulted in a better fit, and the log likelihood ratio statistic was significant to a significance level of $\alpha = 0.05$. Once all significant explanatory variables and two-way interaction terms were added, the resulting model was refit using generalized least squares without specified variance covariates and restricted maximum likelihood estimation (REML), using the gls function from the nlme-package in R [52]. The standardised residuals of the REML-fit were plotted, and the plots were investigated for divergence from a normal distribution, heterogeneity, heteroscedasticity, and correlation to any of the potential explanatory variables. In case of divergence from the assumptions of multiple linear regressions, variance-covariates and insignificant fixed terms were added to the model according to the protocol described in [53] (pp. 90–92). All partial regressions were visualised as partial regression plots. For A. charr, the model was additionally visualised using the plot3d-function from the rgl-package [54], and the plot was extracted using the rglwidget-package [55]. For model interpretation, a significance level of $\alpha = 0.05$ was used, and results with a p-value between 0.05 and 0.10 were classified near significant.

The arithmetic mean volume percentage of each diet item was calculated for each population, including all fish with at least one identified stomach content item. A. charr individuals were grouped by total length, above and below 140 mm, as fish was only found in the diet for A. charr $\geq$140 mm.

The average diet overlap was estimated using Schoener's similarity index [56], calculated by the following formula:

$$D = 100 - 0.5\,\Sigma(|\,p_i - q_i\,|), \quad (2)$$

where p is the average volume percentage of one type of prey in the first group of fish, and q is the average volume percentage of the same item in the other group of fish. Diets are considered to overlap significantly if D exceeds 60% [57].

3. Results

3.1. Descriptive Statistics

A. charr (n = 77) varied in age from 3 to 19 years, with an average of 9 ± 4 years (Table 2). The individuals' lengths varied from 74 to 283 mm, with an average of 145 ± 51 mm, while average weight was 38 ± 45 g ranging from 3 to 178 g. A. charr exhibited average $\delta^{13}C$ and $\delta^{15}N$ signatures of −29.64‰ ± 1.51‰ and 11.69‰ ± 1.22‰, respectively, with individual variations in $\delta^{13}C$ ranging from −34.74‰ to −27.79‰, and from 6.89‰ to 13.51‰ for $\delta^{15}N$. The $\delta^{15}N$ range of 6.62‰ indicates an individual variation in trophic position by almost two trophic levels (Λ = 1.95) within the group of A. charr analysed, assuming a $\delta^{15}N$ enrichment by 3.4‰ per trophic level (Λ), as estimated by Minagawa and Wada and Post [36,38]. Tot-Hg-concentrations (ww.) varied between 0.07 ppm and 1.13 ppm with an average of 0.24 ± 0.21 ppm.

Table 2. Descriptive statistics for A. charr, E. smelt and whitefish including the variables age, length, weight, $\delta^{13}C$, $\delta^{15}N$ and Tot-Hg (ww.).

Variable	Species	*n*	Median	Mean ± SD	Min	Max	Min–Max
Age (year)	A. charr	77	9	9 ± 4	3	19	16
	E. smelt	99	2	2 ± 1	1	8	7
	Whitefish	76	4	5 ± 3	1	16	15
Length (mm)	A. charr	77	129	145 ± 51	74	283	209
	E. smelt	99	98	99 ± 6	87	113	26
	Whitefish	76	253	247 ± 36	130	310	180
Weight (g)	A. charr	77	20	38 ± 45	3	178	175
	E. smelt	99	4	4 ± 1	2	8	6
	Whitefish	76	131.5	131 ± 49	13	265	252
$\delta^{13}C$ (‰)	A. charr	77	−29.15	−29.64 ± 1.51	−34.74	−27.79	6.95
	E. smelt	99	−29.08	−29.14 ± 0.55	−32.38	−27.60	4.78
	Whitefish	76	−29.14	−29.12 ± 0.49	−30.21	−27.61	2.60
$\delta^{15}N$ (‰)	A. charr	77	12.01	11.69 ± 1.22	6.89	13.51	6.62
	E. smelt	99	10.19	10.41 ± 0.97	7.64	13.60	5.97
	Whitefish	76	8.35	8.60 ± 1.25	6.39	12.63	6.24
Tot-Hg (ppm ww.)	A. charr	77	0.14	0.24 ± 0.21	0.07	1.13	1.06
	E. smelt	99	0.20	0.22 ± 0.08	0.09	0.54	0.44
	Whitefish	76	0.18	0.20 ± 0.09	0.05	0.49	0.45

ww.: Wet weight; SD: Standard deviation.

E. smelt (n = 99) varied in age from 1 to 8 years, while the average age was 2 ± 1 years (Table 1). The length of E. smelt varied from 87 to 113 mm, with an average of 99 ± 6 mm. Average weight was 4 ± 1 g, ranging from 2 to 8 g. The average $\delta^{13}C$ and $\delta^{15}N$ signatures in E. smelt were −29.14‰ ± 0.55‰ and 10.41‰ ± 0.97‰, respectively, with individual variations in $\delta^{13}C$ from −32.38‰ to −27.60‰ and from 7.64‰ to 13.60‰ for $\delta^{15}N$. The range in $\delta^{15}N$ by 5.97‰ indicates an individual variation in trophic level (Λ) by 1.76 Λ within the group of E. smelt analysed. Tot-Hg-concentrations (ww.) in E. smelt averaged at 0.22 ± 0.08 ppm, and ranged from 0.09 to 0.54 ppm.

Whitefish (n = 76) varied in age from 1 to 16 years, with an average of 5 ± 3 years (Table 1). Whitefish length varied from 130 to 310 mm, with an average of 247 ± 36 mm. The average weight was 131 ± 49 g, ranging from 13 to 265 g. Whitefish had average δ^{13}C and δ^{15}N signatures of −29.12‰ ± 0.49‰ and 8.60‰ ± 1.25‰, respectively. While individual δ^{13}C signatures ranged from −30.21‰ to −27.61‰, the δ^{15}N signatures varied between 6.39‰ and 12.63‰. The range in δ^{15}N by 6.24‰ indicates an individual variation in trophic level (Λ) by 1.84 Λ for the whitefish analysed. Tot-Hg-concentrations (ww) ranged from 0.05 ppm to 0.49 ppm, and averaged at 0.20 ± 0.09 ppm.

Monthly pooled benthic invertebrate samples were obtained for caddisflies (*Trichoptera*), *Chironomidae* and *Asellus aquaticus* (Table 3). *Trichoptera* had an average δ^{13}C-signature of −27.98‰ ± 0.43‰ ranging from −28.66‰ to 27.17‰, while their δ^{15}N-signature varied between 3.28‰ and 7.96‰ and averaged at 5.46‰ ± 1.36‰. *Chironomidae* exhibited δ^{13}C-signatures between −33.61‰ and −26.27‰ with an average of −30.00‰ ± 1.20‰ and δ^{15}N-signatures between 8.21‰ and 10.69‰ with an average of 9.32‰ ± 0.42‰. The δ^{13}C-signatures of *Asellus aquaticus* varied between −32.21‰ and −25.25‰ with an average of −28.92‰ ± 0.78‰, while their δ^{15}N-signatures averaged at 6.13‰ ± 0.31‰, ranging from 4.37‰ to 7.32‰.

Table 3. Descriptive statistics for the stable isotope ratios δ^{13}C and δ^{15}N of the pooled benthic invertebrate samples.

Variable	Group	*n*	Median	Mean ± SD	Min	Max	Min−Max
δ^{13}C (‰)	*Trichoptera*	3	−28.10	−27.98 ± 0.43	−28.66	−27.17	1.49
	Chironomidae	5	−29.40	−30.00 ± 1.20	−33.61	−26.72	6.89
	Asellus Aquaticus	8	−28.63	−28.92 ± 0.78	−32.21	−25.25	6.96
δ^{15}N (‰)	*Trichoptera*	3	5.13	5.46 ± 1.36	3.28	7.96	4.68
	Chironomidae	5	9.09	9.32 ± 0.42	8.21	10.69	2.48
	Asellus Aquaticus	8	6.22	6.13 ± 0.31	4.37	7.32	2.86

3.2. Use of the Profundal Habitat

A. charr were present in the profundal habitat the whole year, with the highest occurrence in September and December (Table 1). E. smelt was also present all year, except for June, and most were caught in December. Whitefish were primarily caught during winter between December and March.

3.3. Stomach Content and Diet

3.3.1. Benthic Invertebrates

*Chironomidae sp. w*ere found in the stomachs of all species, and contributed to the diet with 44, 25 and 26 vol % for A. charr (n = 41), E. smelt (n = 31) and whitefish (n = 22), respectively. In E. smelt, *Chironomidae sp.* were only found between August and December (Figure 2a). *Pisidium sp.* were found in A. charr (2 vol %) and whitefish (16 vol %), but not in E. smelt. Ostracods were found in A. charr restricted to the period between August and February (1 vol %) (Figure 2b), and they were continuously present in E. smelt (9 vol %) and whitefish (4 vol %) (Figure 2a,c). *Phryganea grandis* were only found in A. charr, exclusively from March to June (5 vol %). Caddisflies (*Trichoptera*) were only found in E. smelt (2 vol %), while *Asellus aquaticus* was only found in whitefish, contributing to 2 vol %.

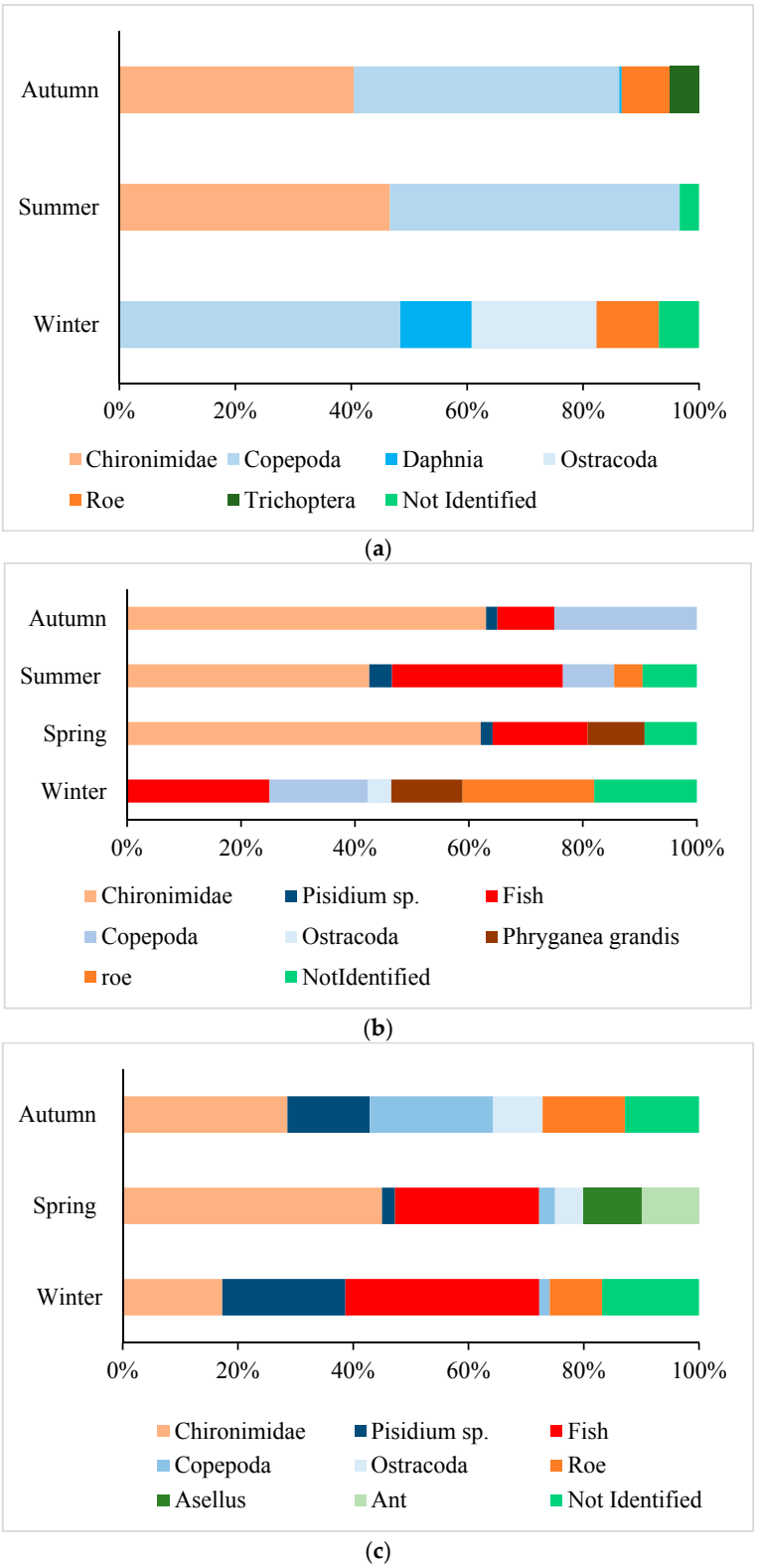

Figure 2. Seasonal variation in stomach content of (**a**) E. smelt (Winter n = 13; Summer n = 6; Autumn n = 12); (**b**) A. charr (Winter n = 8; Spring n = 12; Summer n = 10; Autumn n = 11); (**c**) Whitefish (Winter n = 11; Spring n = 4; Autumn n = 7).

3.3.2. Pelagic Invertebrates

Copepods were found in all investigated fish species, and constituted 12, 48 and 8 vol % in A. charr, E. smelt and whitefish, respectively. In A. charr, copepods were a seasonal item, only found from August to February. Cladocerans, i.e., *Daphnia* sp. were only found in E. smelt (5 vol %).

3.3.3. Fish and Other Items

Fish occurred in the stomach samples of A. charr (20 vol %) and whitefish (21 vol %). Regarding whitefish, fish were only found between January and May. Fish roe were seasonally present in all three fish species, primarily in September and February in A. charr (6 vol %), and in December and January in E. smelt (8 vol %) and whitefish (9 vol %). In whitefish, an ant (*Formicidae* spp.) was found (2 vol %), while unidentified remains constituted 11, 4 and 13 vol % in A. charr, E. smelt and whitefish, respectively.

The largest A. charr, individuals >140 mm (n = 20), consumed less *Chironomidae* sp., *Pisidium* sp. and copepods (34, 0 and 2 vol % compared to 54, 4 and 21 vol %), but more roe (9 vol % compared to 3 vol %) than smaller individuals, <140 mm (n = 21) (Figure 3). Additionally, the largest individuals consumed fish (≈40 vol %). Approximately 10 vol % of the stomach content of both groups remained unidentified. Schoener's similarity index [57,58], indicated no significant overlap in the diets of A. charr above and below 140 mm of length (D = 51%).

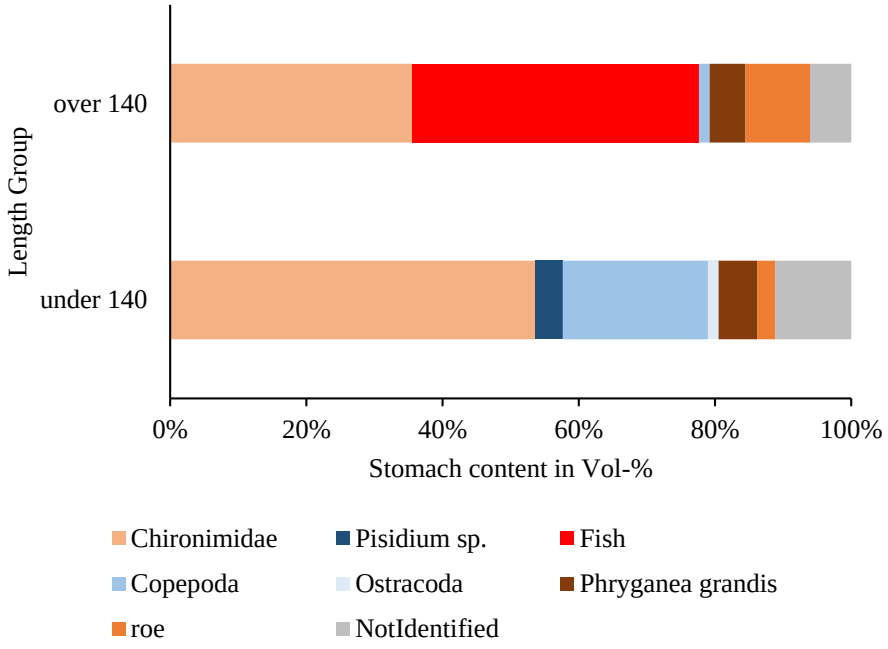

Figure 3. The diet of A. charr individuals larger than 140 mm (n = 20) compared to the diet of A. charr individuals smaller than 140 mm (n = 21).

3.4. Hg-Models

3.4.1. Model Intercepts and Residual Standard Error

Model intercepts were significant for all species, with A. charr exhibiting the lowest intercept for autumn data of −1.825 {degrees of freedom (df) = 29, standard error (SE) = 0.070, t = −26.23, $p < 0.001$}, followed by a general intercept for whitefish of −1.703 (df = 72, SE = 0.031, t = −55.10, $p < 0.001$), and the highest general intercept for E. smelt of −1.553 (df = 94, SE = 0.027, t = −58.56, $p < 0.001$). This corresponds to estimated Tot-Hg-concentrations (ww.) of 0.16 ppm for A. charr in autumn with a length of 137 mm and a δ^{15}N of 11.69‰. Average E. smelt, which were 2 years of age, 99 mm in length,

4 g in weight, and had a δ^{13}C-signature of −29.14‰, exhibited an estimated Tot-Hg-concentration of 0.21 ppm. The average whitefish, measuring 244 mm, weighing 118 g, and being 4 years old, had an estimated Tot-Hg-concentration of 0.18 ppm. Residual standard errors for the Hg-models are estimated to 0.499, 0.264 and 0.269 for A. charr, E. smelt and whitefish, respectively. The predicted Tot-Hg-concentrations for the dataset for comparison exhibit values of 0.09 and 0.22 ppm ww. for whitefish and E. smelt, respectively. A. charr varies in predicted Tot-Hg (ppm ww.) between 0.12 and 0.13 in summer and autumn, respectively, and 0.17 and 0.18 in winter and spring, respectively.

3.4.2. Length

The partial linear regression between the centered and transformed length and logarithmically transformed Tot-Hg was significant and positive for all species (Figure 4), meaning that Tot-Hg-concentrations increase with increasing length. The slopes were estimated to 1.592 (df = 71, SE = 0.131, t = 12.20, $p < 0.001$), 1.927 (df = 94, SE = 0.622, t = 3.10, p = 0.003) and 4.944 (df = 72, SE = 0.851, t = 5.81, $p < 0.001$) for A. charr, E. smelt and whitefish, respectively.

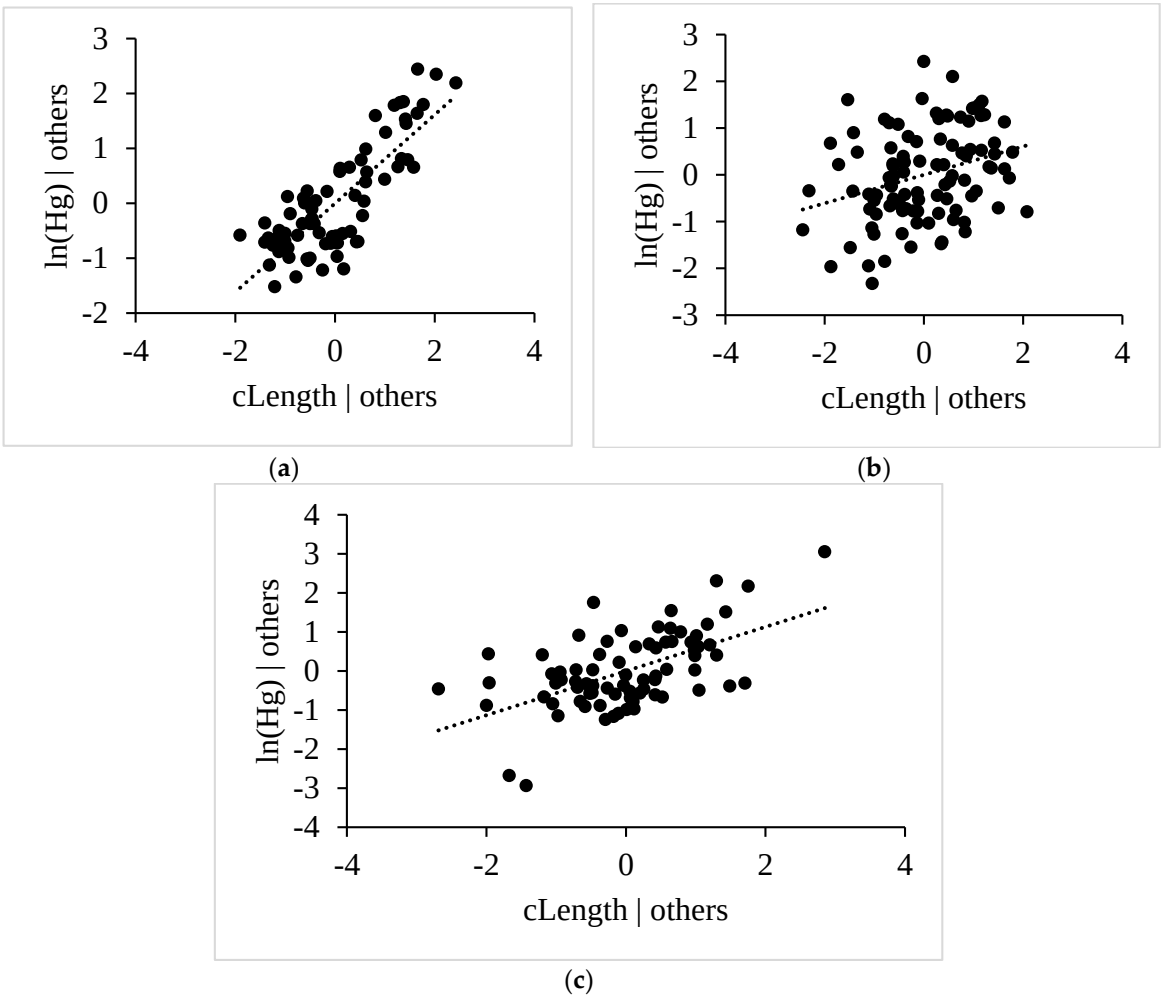

Figure 4. Partial linear regressions using the centered, transformed length as explanatory variable, and logarithmically transformed Tot-Hg as response variable, both corrected for all other variables included in the models for; (**a**) A. charr; (**b**) E. smelt; (**c**) whitefish.

3.4.3. Age

The partial linear regressions between the centered and transformed age and logarithmically transformed Tot-Hg were positive and significant for E. smelt and whitefish, with slopes of 0.186

(df = 94, SE = 0.060, t = 3.09, p = 0.003) and 0.238 (df = 72, SE = 0.058, t = 4.09, p < 0.001), respectively (Figure 5). For A. charr, however, including a partial regression with age as an explanatory variable did not improve the model.

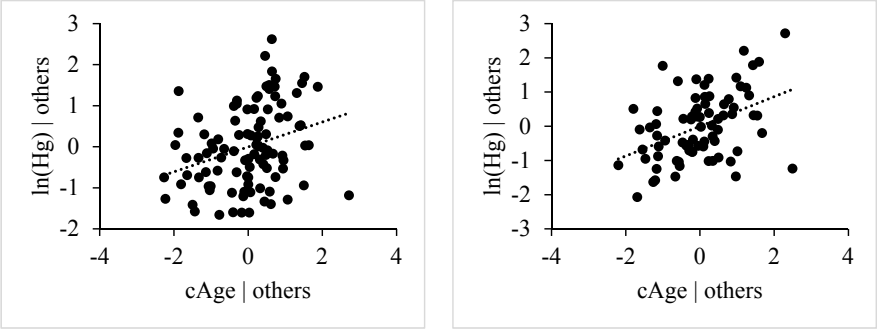

Figure 5. Partial linear regressions between the centered, transformed age and logarithmically transformed Tot-Hg-concentration; (**a**) added variable plot for E. smelt; (**b**) added variable plot for whitefish.

3.4.4. Weight

Despite the general tendency of fish with higher weight having higher Tot-Hg-concentrations, partial linear regressions between centered and transformed weight and logarithmically transformed Tot-Hg were negatively significant with slopes of −0.532 (df = 94, SE = 0.116, t = −4.57, p < 0.001) and −1.081 (df = 72, SE = 0.255, t = −4.24, p < 0.001) for E. smelt and whitefish, respectively (Figure 6). This effect is caused by the high correlation between length and weight of 0.645 and 0.962 for E. smelt and whitefish, respectively. However, as the partial regression using weight as explanatory variable is significant for E. smelt and whitefish, weight provides additional information for the estimation of Tot-Hg-concentrations in muscle fillet tissue of these species. Adding a partial linear regression with weight as an explanatory variable did not improve the Hg-model for A. charr.

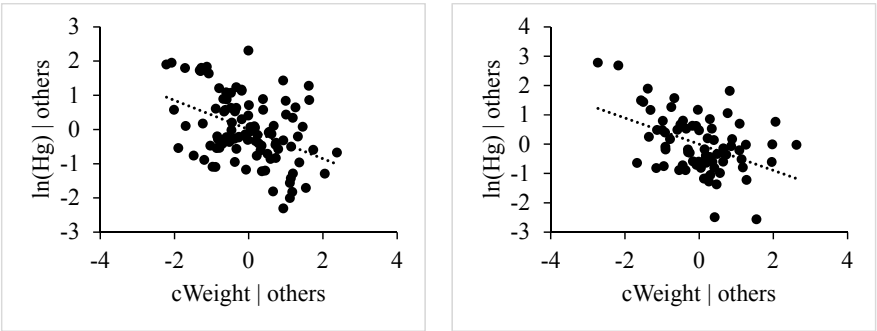

Figure 6. Partial linear regressions using centered, transformed weight as an explanatory variable and logarithmically transformed Tot-Hg-concentration as a response variable. Both variables are corrected for all other explanatory variables in their respective models; (**a**) for E. smelt; (**b**) for whitefish.

3.4.5. Stable Isotope Ratio $\delta^{13}C$

The centered stable isotope ratio of carbon, $\delta^{13}C$, was significantly, negatively correlated to the logarithmically transformed Tot-Hg-concentration in E. smelt (Figure 7) with a slope of −0.157 (df = 94, SE = 0.049, t = −3.18, p = 0.002). A partial linear regression between $\delta^{13}C$ and Tot-Hg, however, neither improved the model for A. charr or whitefish.

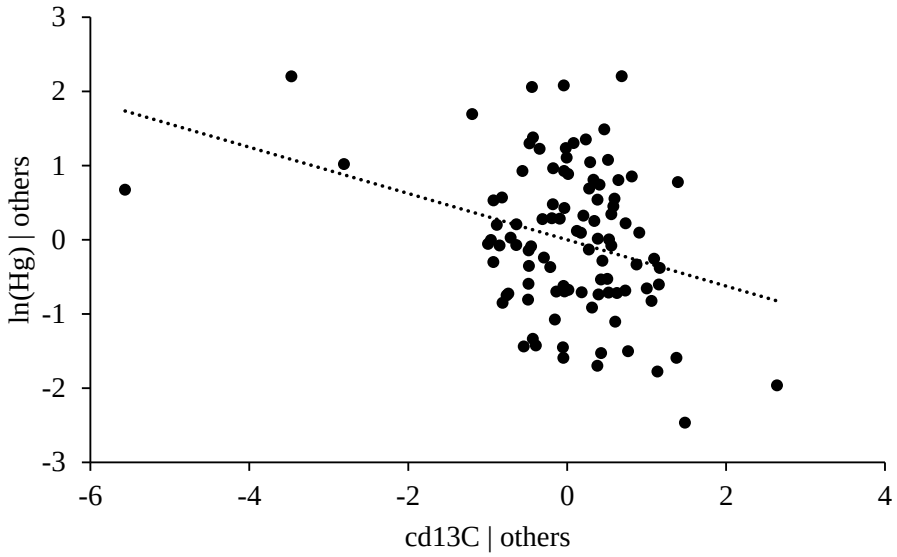

Figure 7. Partial linear regression between δ^{13}C and Tot-Hg for E. smelt. All variables are corrected for the other explanatory variables included in the model.

3.4.6. Stable Isotope Ratio δ^{15}N

The stable isotope ratio of nitrogen, δ^{15}N, as an explanatory variable did not improve the models for whitefish and E. smelt, and was thus omitted. However, a non-significant partial linear regression with the slope of 0.016 (df = 71, SE = 0.030, t = 0.55, p = 0.586) was included in the model for A. charr (Figure 8) due to heteroscedastic residuals in relation to δ^{15}N. In addition, δ^{15}N was incorporated in the A. charr model as a variance-covariate, estimating the variance at the centered c$\delta^{15}N_i$ by the following formula:

$$var\left(\varepsilon_i\right) = \sigma^2 \times e^{2*0.1543*cd^{15}N_i} \quad (3)$$

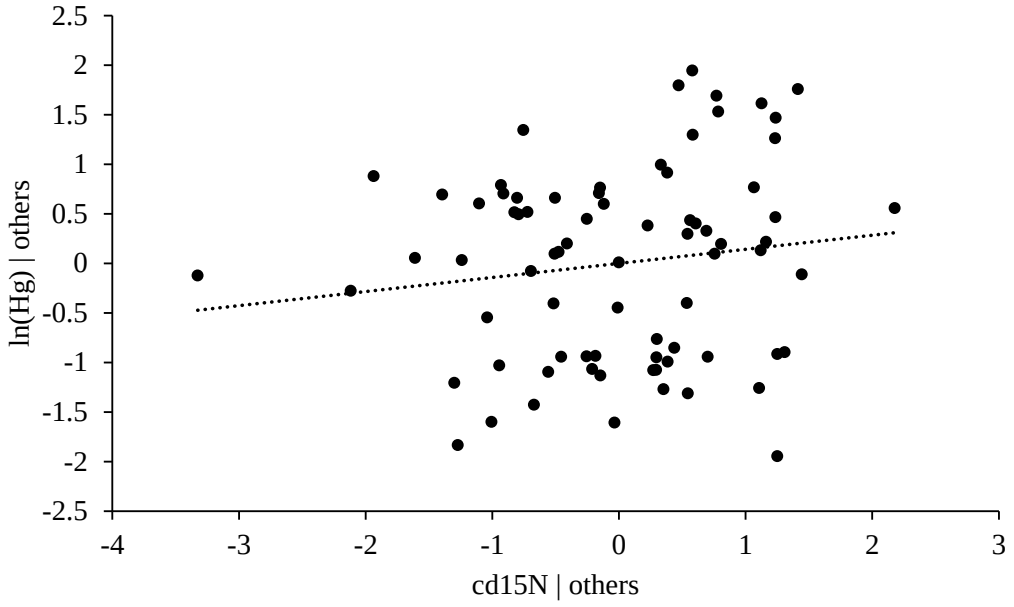

Figure 8. Partial linear regression between centered δ^{15}N and logarithmically transformed Tot-Hg in A. charr. This regression was not significant, however, it was included due to heterogeneous residuals of the multiple linear regression model for Tot-Hg in A. charr.

3.4.7. Season

Including the factor season improved the model for A. charr significantly, resulting in different intercepts per season. The lowest intercept (−1.849) was estimated for summer, which was not significantly different from the autumn intercept of −1.825 (df = 20, SE = 0.109, $t = -0.22$, $p = 0.826$). The winter intercept (−1.528) was near significantly higher (df = 12, SE = 0.167, $t = 1.78$, $p = 0.079$) than the autumn intercept, and the highest intercept in spring (−1.470) was significantly higher (df = 12, SE = 0.100, $t = 3.55$, $p < 0.001$) than the autumn intercept. This indicates that the average A. charr with a length of 137 mm and a δ^{15}N of 11.69‰ exhibits average Tot-Hg-concentrations (ww.) of 0.23, 0.16 and 0.22 ppm in spring, summer, and autumn and winter, respectively. Interaction terms involving season and length or δ^{15}N were not significant, thus only the intercept of the partial regressions depends on season (Figure 9). However, as variances, thus standard deviations, differed with season, it was also used as a variance-covariate (varIdent structure) in the model for A. charr, with the largest standard deviation in winter (4.37). The second largest standard deviation (3.85) occurred in summer, followed by a standard deviation of 3.32 in autumn, and the smallest standard deviation (1.84) in spring. The factor season was not significant for E. smelt and whitefish.

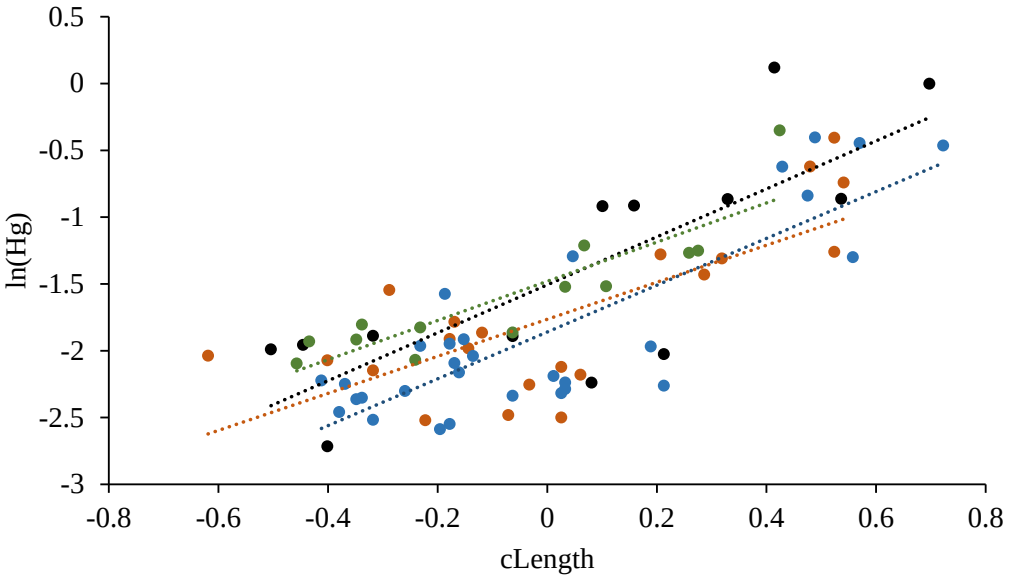

Figure 9. Linear regressions for A. charr, using centered, transformed length as an explanatory variable and logarithmically transformed Tot-Hg-concentration as a response. Seasons are coloured as green (spring), orange (summer), blue (autumn) and black (winter).

4. Discussion

4.1. Age, Size and Weight Distributions, Stable Isotope Ratios and Tot-Hg

The average δ^{13}C ratios of all three species caught in the profundal zone are similar, i.e., between −30‰ and −29‰ (Table 1). According to Vander Zanden and Rasmussen [39], profundal diet has the most depleted carbon signature, on average −30.5‰, followed by the pelagic, on average −28.4‰. The pooled benthic invertebrate samples from the area around the water intake exhibit similar average δ^{13}C-signatures as found in the fish species (Table 3), i.e., between ca. −28‰ and −30‰. The average δ^{15}N of *Trichoptera* (5.46‰), the only primary consumer sampled in this study, additionally resembles the profundal average δ^{15}N of 5.2‰ estimated by Vander Zanden and Rasmussen [39]. Consequently, all three species investigated in this study feed on a mixture of pelagic and profundal diet, also confirmed by the stomach analyses. A. charr appeared to consume most profundal prey, as it was previously found to be the weaker competitor against whitefish [58,59], thus forced to occupy the less

energetically favourable profundal niche [60–62]. This was also reflected in the highest $\delta^{15}N$ ratios measured for A. charr (Table 1), as profundal primary consumers produce higher baseline $\delta^{15}N$ than pelagic zooplankton [39]. The largest range in $\delta^{15}N$, exhibited by A. charr, however, was a result of the combination of benthivorous small individuals and rather piscivorous individuals. E. smelt primarily feeds on zooplankton, mainly pelagic copepods (primary consumers). However, some omnivorous, benthic organisms, such as *Chironomidae* sp., were found in the diet, and may cause the $\delta^{13}C$ signatures to resemble more profundal levels, as well as increased span in trophic position. However, as E. smelt primarily feeds on small, short lived organisms, temporal variations in dietary stable isotope ratios are to be expected [35,63–65], and $\delta^{13}C$ ratios in zooplankton may reach values resembling profundal organisms [66]. Whitefish exhibit the lowest values of $\delta^{15}N$, however, the signatures are fairly similar to those of E. smelt. A combination of profundal and pelagic prey was found in the stomach samples of whitefish, and the range in trophic position by 1.84 Λ is most likely caused by different feeding habitats and some piscivory.

The distributions of Tot-Hg for the sampled fish species appeared to be influenced by habitat [8,11,40], trophic position [5–8,10] and age distributions [8,11]. Due to the similar, profundal habitat, the average Tot-Hg-concentrations (ww.) for A. charr (0.24 $\pm$ 0.21 ppm), E. smelt (0.22 $\pm$ 0.08 ppm) and whitefish (0.20 $\pm$ 0.09 ppm) did not differ substantially. However, A. charr exhibited the largest range, highest values and lowest median Tot-Hg-concentrations (Table 1), which was likely caused by a catch of mainly small and young fish from a species with the highest potential to accumulate Tot-Hg due to high maximum age [8,11,67], a profundal diet consumed all year [8,11,40,60], and the highest average $\delta^{15}N$ [5–8,10]. For the standard dataset of explanatory variables, A. charr exhibited intermediate predicted Tot-Hg-concentrations (0.12–0.18 ppm ww.), likely due to their more profundal diet compared to whitefish, and their larger size compared to E. smelt. The Tot-Hg-concentrations, measured in E. smelt and whitefish, were similar. Whitefish spawn in the profundal zone [60], but they have access to pelagic, perhaps even littoral prey, as they do not occupy the profundal zone all year [60]. Consequently, the Tot-Hg-concentration in the diet of whitefish is decreased when they do not consume profundal prey [8,11,40]. Contrarily, E. smelt was not shown to ingest any littoral prey, which may be one reason for the higher average Tot-Hg-concentrations measured in E. smelt. Additionally, E. smelt matures at an age of 2–4 years [68], which often leads to stagnating growth [69]. Consequently, Tot-Hg will not further be diluted by increasing tissue mass in mature E. smelt [15,16,20,21]. The early stagnation in growth and the pelagic to profundal diet of E. smelt likely leads to E. smelt having the highest concentrations of Tot-Hg (0.22 ppm ww.) corrected for a standard set of explanatory variables. As whitefish only occupy the profundal zone for spawning during winter [60], and they exhibit higher growth rates than E. smelt, their predicted Tot-Hg-concentration (0.09 ppm ww.) for the standard dataset was the lowest in this study.

4.2. Use of the Profundal Habitat

All fish species sampled occurred in the profundal zone in similar patterns as reported by Borgstrøm and Saltveit [60]. A. charr was caught all year, with the highest presence in autumn, as they are likely forced to occupy the profundal niche by competition with whitefish [58,59,61,62]. E. smelt was also caught in the profundal zone all year, but fewer individuals were caught in summer. E. smelt is an important prey species for larger fish, primarily brown trout (*Salmo trutta*), and E. smelt is reported to undergo diurnal vertical migrations feeding in the epilimneon at night and staying close to the bottom at daytime [70,71]. However, as predator avoidance should be most pronounced in the growth season, when there were few E. smelt caught in the profundal zone, it is more plausible, that E. smelt feed on benthic invertebrates in the profundal zone, when zooplankton is scarce. The use of the profundal habitat of E. smelt may be size-dependent, as no E. smelt with a length exceeding 113 mm were caught in this study. Cannibalistic individuals of E. smelt with lengths up to 135 mm are observed in many Norwegian populations of E. smelt [72] (pp. 68–69), including the population in Lake Norsjø [73]. Whitefish was the only species caught, which was completely absent during

summer, and the largest numbers were caught in January through March. Analogously, Borgstrøm and Saltveit [60] reported most whitefish were caught (200–300 per week) in January and February, with decreasing numbers in spring, and no whitefish caught in summer. This seasonal occurrence is caused by the different behaviour of three distinct whitefish morphs in Lake Norsjø, littoral whitefish, stream whitefish, and winter whitefish, the latter spawning at 15–70 m depth in January and February [74]. Borgstrøm [75], who sampled whitefish with gill nets, only caught whitefish at 25–50 m depth during spawning. Conclusively, all whitefish caught in this study belong to the winter whitefish population, which utilises the profundal habitat for spawning and subsequent feeding on roe during winter. Therefore, most of the whitefish caught are spawning, adult individuals, however, also few immature individuals were caught.

4.3. Ontogenetic Diet Shift in A. Charr

An ontogenetic diet shift can be observed in the stomach samples of A. charr at a length of 140 mm. The diet shifts form predominantly *Chironomidae* sp., some pelagic prey such as copepods, and other items like *Phryganea grandis* and roe to a diet mainly based on fish, *Chironomidae* sp., *Phryganea grandis* and roe. Subsequent to the diet shift, Tot-Hg-concentrations and length continued to increase, while the increase in $\delta^{15}N$, thus trophic position, stagnated. The ontogenetic diet shift in A. charr, which have invertebrate consumption and cannibalism as different stages in the same life history strategy, has been proposed by e.g. Finstad et al. [76]. Another explanation for the differences in the two groups is a dimorphism with invertebrate eating dwarfs and cannibalistic giants [77], which could persist permanently [78]. Parker and Johnson [79], for example, have observed phenological differences between A. charr morphs such as different numbers of gill rakers. However, molecular techniques have only revealed slight genetic differences at first [80–83], and different phenotypes were rather thought to be a result of genetic and environmental components in combination [84,85]. More recently, evidence for larger genetic differences in A. charr was found, especially if different populations inhabit different niches [86–91]. Further investigations in Lake Norsjø are necessary in order to determine, whether A. charr undergoes an ontogenetic diet shift, or if there are two different life history strategies. For this purpose, differences in gill raker counts could be examined.

4.4. Factors Determining Tot-Hg-Concentrations (Model Results)

4.4.1. Length, Age and Weight

Length exhibited significant, positive partial regressions to Tot-Hg in all fish species, and length is frequently used as proxy for Hg-concentrations [8,11–14]. Age was significantly, positively correlated to Tot-Hg in E. smelt and whitefish, as older fish have accumulated more Tot-Hg throughout their longer lives [8,11]. The partial linear regressions between weight and Tot-Hg were significant and negative for E. smelt and whitefish, and it is important to note that they are corrected for effects also explained by age or length. Hg is diluted by organic matter, either through algal bloom dilution (ABD) [17,18], or through SGD in fish [15,16,20,21], two effects that cannot be separated without laboratory procedures [92]. SGD occurs at higher rates in fish with high growth rates, but also in fish gaining weight, thus it is likely the cause of lower Tot-Hg-concentrations at higher weight corrected for length. The opposite effect has also been reported in starving fish, which exhibit relatively high concentrations of Tot-Hg [22] and low weight corrected for length.

4.4.2. Habitat Effect and $\delta^{13}C$

The only species investigated with a significant partial regression between $\delta^{13}C$ and Tot-Hg is E. smelt, which exhibits higher Tot-Hg-concentrations with more depleted $\delta^{13}C$. Consequently, E. smelt vary in diet and possibly habitat [8,11,40], and Tot-Hg-concentrations are influenced by that variance. There are several studies reporting that littoral fish accumulate less Hg than pelagic fish at the same

trophic level [41–43], and as Hg is influenced by depth [11], a profundal diet likely leads to higher Tot-Hg-concentrations than a pelagic diet.

4.4.3. Biomagnification and $\delta^{15}N$

Hg is reported to bioaccumulate and biomagnify, and predators may have concentrations million times higher than the surrounding water [9], which can reach toxic levels in fish and fish eating wildlife [7]. This effect is usually linked to an increase in Hg by trophic position measured in $\delta^{15}N$ [5–8,10], however, no partial correlation between $\delta^{15}N$ and Tot-Hg has been significant in this study. Conclusively, $\delta^{15}N$ did not contribute additional information crucial to estimating Tot-Hg-concentration in fish, it may, however, function as a proxy for Tot-Hg-concentrations, as it may be correlated to length or age, which it is for E. smelt and whitefish. These two species also appeared to feed on homogenous diets throughout all length classes, resulting in a reduced effect of $\delta^{15}N$ on Tot-Hg-concentrations. The $\delta^{15}N$ signatures were only included in the model for A. charr, and showed an insignificant positive trend, indicating a slight increase in Tot-Hg-concentrations with increasing $\delta^{15}N$. A. charr appeared to increase in $\delta^{15}N$ up to the ontogenetic diet shift to piscivory, then only length and Tot-Hg continued to increase (Figure S1). Tot-Hg-concentrations increase substantially, once A. charr being piscivorous, however, $\delta^{15}N$ did not seem to increase further at that point. Thus, the residual variance of Tot-Hg-concentrations increased with increasing $\delta^{15}N$, as high values of $\delta^{15}N$ covered the increase in Tot-Hg subsequent to reaching the maximum trophic position.

4.4.4. Seasonal Variation

Seasonal variations in Tot-Hg-concentrations were significant for A. charr, which exhibit significantly higher concentrations in spring and near significantly higher concentrations in winter than in autumn. This seasonal pattern is likely caused by ABD and SGD [11,15–21], as dilution lowers Tot-Hg-concentration during the growth season (summer). A. charr may then be starving during winter, which leads to near significantly higher Tot-Hg-concentrations [22], and significantly higher Tot-Hg-concentrations in spring before the onset of the growth season. Similar seasonal variations related to growth rates and condition have been reported in littoral and pelagic habitat and streams [23–30]. The different residual variances per season are likely caused by different sample sizes, however, the highest variance in winter may also be supported by different reactions to starvation. The individual resource demand is dependent on size, and large animals need more food in order to sustain themselves [93–96], meaning that their habitat must provide a higher resource density to avoid starvation [96,97]. Byström et al. [98] found that small A. charr could even be able to sustain close to optimal growth rates in ice-covered lakes during winter, which indicates that small A. charr should not be subject to starvation in Lake Norsjø, while larger individuals probably are. However, even small A. charr may be subject to starvation or reduced growth during winter in Lake Norsjø as A. charr only compete with whitefish for profundal resources from late autumn to spring, when whitefish occurs in the profundal zone [60].

5. Conclusions

Tot-Hg-concentrations in fish increased with length and age in the profundal zone, while a less depleted $\delta^{13}C$ signature, and lower weight, corrected for length, resulted in higher Tot-Hg-concentrations. A slight Increase in Tot-Hg with increasing $\delta^{15}N$ or trophic position was found in A. charr. Both the use of the profundal habitat and Tot-Hg-concentrations may vary seasonally. Winter whitefish in Lake Norsjø were only found in the profundal habitat during their spawning period in winter. Tot-Hg-concentrations varied with season for A. charr, and were highest in spring and lowest in summer, likely as an effect of nutritional status.

Supplementary Materials: The following are available online at file:///E:/1-manuscripts/environments/environments-152406/environments-152406-suppl.-original/SupplementS1.html, Figure S1: RGL Model for A. charr; The following are available online at www.mdpi.com/2076-3298/3/4/29/s1, Supplement S2: R-code and model construction.

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Author Contributions: Espen Lydersen conceived and designed the experiments; Tom Robin Olk, Tobias Karlsson and Asle Økelsrud performed the experiments; Tom Robin Olk analysed the data; Tom Robin Olk, Tobias Karlsson, Asle Økelsrud and Espen Lydersen wrote the paper.

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Abbreviations

The following abbreviations are used in this manuscript:

ABD	Algal bloom dilution
AIC	Akaike information criterion
Df	Degrees of freedom
Gls	Generalised least squares
Hg	Mercury
ML	Maximum likelihood
ppm	Parts per million
REML	Restricted maximum likelihood
SD	Standard deviation
SE	Standard error
SGD	Somatic growth dilution
Tot-Hg	Total Mercury
ww.	Wet weight

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