Intra-specific differences in metabolic rates shape carbon stable isotope trophic discrimination factors of muscle tissue in the common teleost Eurasian perch (Perca fluviatilis)

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Abstract

1. Stable isotopes represent a unique approach to provide insights into the ecology of organisms. δ13C and δ15N have specifically be used to obtain information on the trophic ecology and food web interactions. Trophic discrimination factors (TDF, Δ13C and Δ15N) describe the isotopic fractionation occurring from diet to consumer tissue and these factors are critical for obtaining precise estimates within any application of δ13C and δ15N values. It is widely acknowledged that metabolism influences TDF, being responsible for different TDF between tissues of variable metabolic activity (e.g. liver vs. muscle tissue) or species body size (small vs. large). However, the connection between the variation of metabolism occurring within a single species during its ontogeny and TDF has rarely been considered. 2. Here, we conducted a 9-month feeding experiment to report Δ13C and Δ15N of muscle and liver tissue for several weight classes of Eurasian perch (Perca fluviatilis), a widespread teleost often studied using stable isotopes, but without established TDF for feeding on a natural diet. In addition, we assessed the relationship between the standard metabolic rate (SMR) and TDF by measuring their oxygen consumption of the individuals. 3. Our results showed a significant negative relationship of SMR with Δ13C, and a significant positive relationship of SMR with Δ15N of muscle tissue, but not with TDF of liver tissue. SMR varies inversely with size, which translated into a significantly different TDF of muscle tissue between size classes. 4. In summary, our results emphasize the role of metabolism in shaping specific TDF (i.e. Δ13C and Δ15N of muscle tissue), and especially highlight the substantial differences between individuals of different ontogenetic stages within a species. Our findings thus have direct implications for the use of stable isotope data and the applications of stable isotopes in food web studies.

Introduction:

Stable isotope analysis has become an established tool of ecologists for numerous applications, including research on ecosystem functioning (e.g. Mehner et al. 2016), animal migration (e.g. Hobson 1999), ecophysiological processes (e.g. Gannes et al. 1998), and parasitism (e.g. Lafferty et al. 2008). Furthermore, it provides a useful tool for elucidating trophic interactions in food-web research (Post 2002, Boecklen et al. 2011, Layman et al. 2012). For these purposes, the ratio of carbon (^{12}C/^{13}C, expressed as δ^{13}C values) and nitrogen (^{14}N/^{15}N, expressed as δ^{15}N values) stable isotopes have been widely used. While δ^{13}C values can be used to track the origin of the carbon source in organisms´ diet and the base of the food web, δ^{15}N values are especially useful for determining the organisms´ trophic level (DeNiro and Epstein 1978, 1981, Peterson and Fry 1987). Combining these two approaches can provide information on resource and habitat use, thus allowing inference of the ecological niche of individuals, species or communities (Bearhop et al. 2004, Newsome et al. 2007, Martínez del Rio et al. 2009).

For many applications of stable isotope analyses in ecology, estimates of the isotopic spacing between a consumer and its food (trophic discrimination) are needed. Trophic discrimination factors (TDF, Δ^{13}C and

Δ^{15}N) represent the difference in values of δ^{13}C (or δ^{15}N) between the consumer and its diet. Most studies rely on average values for this parameter found in the literature, but the use of inaccurate TDF has been described as a major source of uncertainties in the use of mixing models to calculate the contributions of food items to the diet of a consumer (Phillips et al. 2014). Therefore, to allow precise interpretations of isotope data, appropriate TDF values obtained from relevant species-specific trophic interactions are necessary (Martínez del Rio et al. 2009, Wolf et al. 2009).

TDF may vary considerably within and between species (Post 2002), influenced e.g. by diet quality (Gaye-Siessegger et al. 2003), feeding rates (Barnes et al. 2007), and the metabolic processes which shape the rate of diet incorporation (MacAvoy et al. 2005, MacAvoy et al. 2006, Pecquerie et al. 2010). Metabolism describes the sum of all anabolic (synthesizing) and catabolic (degrading) processes of living organisms. In animals, metabolism is driven by the oxidation of organic molecules with the consumption of oxygen during cellular respiration. Part of this energy is used during anabolic processes to produce macromolecules (i.e. carbohydrates, proteins or lipids). This will lead to an increase in tissue mass, resulting in growth, or to replacement of tissue, which are both important underlying processes shaping isotopic equilibration, potentially influencing TDF. Therefore, metabolic rate has strong implications for the rate at which isotopes are incorporated (Carleton and Martínez del Rio 2010). It is generally acknowledged, that more metabolically active tissues (e.g. liver) have faster turnover, resulting in lower TDF due to the preferential incorporation of^{12}C in the excreted metabolites, compared to tissues with slower turnover (e.g. muscle) (McIntyre and Flecker 2006, Xia et al. 2013, Matley et al. 2016). However, this framework has rarely been applied to the overall metabolism of an organism.

Fundamental differences in metabolic rates exist across the animal kingdom with higher mass-specific metabolic rates in smaller species compared to larger ones (Kleiber 1947). In addition to phylogenic differences in metabolism, metabolic rates also vary over ontogeny in individuals of the same species (Wieser 1984, Chabot et al. 2016). To achieve high growth rates in younger individuals, these ontogenetic live stages are characterized by high metabolic rates (e.g. Hou et al. 2008, Yagi et al. 2010). In addition, a whole research field studies the consequences of metabolic differences between individuals irrespective of ontogenetic stages, and their influence on general behavior and performance (Metcalfe et al. 1995, Careau et al. 2008, Biro and Stamps 2010), including social dominance, aggressive behavior, and activity levels (Røskaft et al. 1986, Reidy et al. 2000).

One component of an organism's metabolism is standard metabolic rate (SMR) which is the minimum metabolic rate needed for subsistence (Hulbert and Else 2004, Chabot et al. 2016). Oxygen consumption, measured in a respirometer is typically used as a proxy for SMR. This baseline is ecologically relevant in how it translates to differences in "maintenance costs" and thereby fitness, between conspecifics (Burton et al. 2011). Previous studies have been able to correlate individual metabolic rates to the differences of TDF found between species, sexes and laboratory strains of endothermic animals with a high metabolism, such as birds and rodents (Ogden et al. 2004, MacAvoy et al. 2006, MacAvoy et al. 2012), but this concept has not been broadened to ectothermic organisms with a slower mass-specific metabolic rate, such as temperate fish.

In this study, we examined TDF for Eurasian perch (*Perca fluviatilis*), which is a ubiquitous fish in Europe and Asia (Froese and Pauly 2020). It is the dominant predatory species in many aquatic habitats including freshwater (Mehner et al. 2007) and brackish systems (Ådjers et al. 2006), playing a fundamental role in structuring food webs (e.g. Svanbäck et al. 2015, Bartels et al. 2016, Marklund et al. 2019). Nonetheless, species-specific stable isotope TDF for perch feeding natural diets have not been established. Like many vertebrate predators, perch grow several orders of magnitude in body size over their lifetime (e.g. Hjelm et al. 2000), making this species an excellent model for studying the relationship between metabolism and TDF over ontogeny.

The motivation for this study was two-fold. First, we wanted to experimentally derive Δ^{13}C and Δ^{15}N for different weight classes of Eurasian perch, to allow more accurate estimates of trophic positions, ecological niches and other potential food-web inferences for this common teleost. Second, we aimed at identifying the role of metabolic rate on TDF. We predicted that the higher mass-specific standard metabolic rate of juvenile fish will result in lower TDF than those seen in adult perch.

Material and Methods:

Fish collection, husbandry and experimental design

Between August 21st and 28th 2018, we sampled perch from the lake Erken (59°50'09.6"N, 18°37'52.3"E) in Central Sweden by angling from littoral and pelagic habitats. We caught additional young-of-the year juveniles by beach seining. Using cooled and aerated boxes, we brought the fish to the aquarium facility at Uppsala University.

Fish were anesthetized using 60 mg L^{-1} benzocaine and weighted to the nearest 0.01g. We grouped the fish according to their habitat of origin and the body weight at the beginning of the experiment into weight classes: <20 g (juveniles of approximately 4 g), 20-30 g, 30-40 g, and 40-50 g. Perch of the weight class 20-30 g were caught in both littoral and pelagic habitats. Due to potential differences, we analyzed this weight class habitat-specifically. 3-9 perch individuals were placed together in 30l –aquaria (50 x 25 x 25cm) with the bottom covered by a 3cm thick layer of sand. In order to maintain tanks with approximately equal sized shoals, tanks with fish from the same size class and same habitat were combined after periodic sampling. Room temperature of the facility was set at 15 C°. Aquaria were equipped with a flow-through system of fresh tap water. As the tap water became colder during the winter months (November to March), we noticed a drop in the tank water temperatures from 18 C° to 14 °C. We fed the fish daily with commercially available chironomids, with an amount corresponding to approximately 15% of the individual wet weight^{-day}. Unfortunately, we noticed strong variation in isotopic signatures of chironomids and were forced to switch suppliers to obtain more stable signatures in perch diet (final food: δ^{13}C: -30.6 14.0 started the feeding experiment to estimate TDF in perch on October 10th 2018 (day 0). We ended the trials between July 12th (day 275) and 25th 2019 (day 288), i.e. the experiment ran slightly longer than 9 months. During this time, perch had approximately doubled their original body mass on average (132.7 % weight gain ± 89.4, Table 1). This is an approximate value as we did not track the weight increase individually, but set initial weights as the average values of the respective weight class. We assumed perch to be in isotopic equilibrium with the chironomid diet based on the equations for isotopic half-life of vertebrate muscle tissue and liver reported in Vander Zanden et al. (2015) and the predictive turnover equations presented by Thomas and Crowther (2015). For both predictions, we assumed isotopic equilibrium in 4-5 times the half-live (Thomas and Crowther 2015). Before the fish were sacrificed at the end of the experiment with an overdose of benzocaine, we conducted metabolic trials to obtain individual SMR (see section below). We weighed the sacrificed fish to the nearest 0.01g and measured total length to the nearest mm. We dissected a sample of the dorsal muscle tissue and the entire liver for stable isotope analyses and dried the tissue in a drying oven at 60°C for 48 hours.

Over the course of the experiment, we sacrificed a subset of 5 individuals every 6-10 weeks to observe the development of the TEFs over time. In total, we analyzed the Δ^{13}C and Δ^{15}N in muscle and liver for 48 and 47 individuals respectively. In addition to the fish sacrificed over the course of the experiment, 28 individuals were maintained for the entire 9-month period and SMR measurements were taken for 26 of them.

The study was approved by the Uppsala Animal Ethic Committee with permit number: C59/15.

Stable isotope analyses and calculation of trophic discrimination factors

We ground oven-dried tissues samples to a fine powder using a mortar and pestle, and transferred approximately 1 mg of the powder to tin capsules. Elemental and stable isotope analyses of carbon and nitrogen were conducted at the University of California, Davis Stable Isotope Facility, California, USA, using a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). We express our results using the δ notation, thus, referring the ratios of samples to the international standards (Vienna Pee Dee Belemnite and Air for carbon and nitrogen, respectively). Measurement error was 0.20.30.01; average ± SD), no lipid normalization was performed (Kiljunen et al.

2006). However, C/N in liver tissue (4.4 ± 0.6 average ± SD) exceeded the recommended ratio of 3.4 and we therefore used a mathematical lipid normalization on the $\delta^{13}C$ values to account for the bias of carbon fractionation occurring during lipid synthesis (Kiljunen et al. 2006, Sweeting et al. 2006, Skinner et al. 2016). As recommended by Skinner et al. (2016), we used the percent lipid model introduced by Post et al. (2007), together with the normalization model described by Kiljunen et al. (2006).

We calculated TDF (i.e. $\Delta^{13}C$ and $\Delta^{15}N$) for perch as

$$X_{\text{perch}} = \delta\Xi_{\text{perch}} - \delta\Xi_{\text{Chironomid}},$$

using average values for $\delta\Xi_{\text{Chironomid}}$, where Xstands for the heavy isotope of C (^{13}C) and N (^{15}N).

Measurements of standard metabolic rate (SMR)

SMR was measured using intermittent flow respirometry system (Loligo Systems, Viborg, Denmark). A detailed description of the respirometry set-up is described in Andersson et al. (2020). Briefly, our set-up was comprised of four acrylic respirometry chambers, submerged in two aquaria (two chambers per tank) which contained water maintained at 18.1 ± 0.07 °C (average ± SD) and air-stones to maintain oxygen at air-saturation levels. Oxygen concentration in each chamber was measured using fiber-optic optodes connected to flow-through oxygen cells in conjunction with a Wiltrox4 oxygen meter (Loligo Systems). Chambers were size matched to each fish. Measurement loops for the trials consisted of a 180 sec flush phase, a 30 sec wait phase and a 210 sec measurement phase. Measurement loops for calculating background oxygen consumption rate (MO_2 (m gO_2 h^{-1})) consisted of a 180 sec flush phase, a 30 sec wait phase and a 900 sec measurement phase. The entire system was drained and flushed with bleach between trials to prevent the build-up of microbes over the course of the experiment. At the beginning of each trial, the system was refilled with aerated tap water that had been maintained at 18 ± 0.5°C for a minimum of 24h.

Fish were fasted for approximately 24 hours before the start of the respirometry trial. Individuals were removed from their husbandry tank using a mesh net and transferred to the respirometer in a 9-liter bucket filled with aerated 18 ± 0.5°C tap water. Fish were placed in the respirometer in the afternoon and remained in the respirometer for a minimum of 19 hours and 43 minutes. Following the trial, fish were killed with an overdose of benzocaine and frozen for stable isotope analysis. Measurements for background respiration were taken directly before and after each trial. AutoResp software (Loligo Systems, version 2.2.0), was used to calculate MO_2 (mgO_2h^{-1}) from the linear decrease in dissolved oxygen over each measure phase. Chamber specific background respiration was calculated by fitting a linear regression between all of the starting and ending background values with $R^2 > 0.1$ grouped by chamber. The linear regressions between all values, as opposed to just values specific to each trial, were used so that measures with $R^2 < 0.1$ could be excluded, while still allowing for chamber specific background, which accounts for slight differences in calibration between each chamber. Each trial was adjusted for background respiration by subtracting fitted values estimating background respiration from measures of $MO_2$for each fish at each timepoint. Background corrected measures were divided by fish weight to calculate mass-specific MO_2(mgO_2 kg^{-1} h^{-1}) and any estimate of MO_2 with an $R^2 < 0.95$ was removed prior to calculating SMR. SMR was calculated as the mean of the lowest 10% of MO_2measures (Baktoft et al. 2016, Andersson et al. 2020). R-code for calculations of all metabolic measures is available on the data repository Zenodo (http://doi.org/10.5281/zenodo.4433723).

Statistical analyses

To analyze the variation in TDF between weight classes, we conducted four different ANCOVAs with subsequent Bonferroni posthoc comparisons using $\Delta^{13}C$ and $\Delta^{15}N$, for the two tissue types muscle and liver. We used final weight as a covariate with the attempt to adjust for the variation explained solely by growth (Fry and Arnold 1982). Additionally, we conducted four linear regressions with the $\Delta^{13}C$ and $\Delta^{15}N$, for muscle and liver tissue respectively as dependent variables and SMR as the predictor in order to analyze the relationship between TDF and SMR. We compared SMR between the different weight classes using an ANCOVA with subsequent Bonferroni posthoc comparisons, including final weight as a covariate. The assumptions of normal distribution and homogeneities of variances were met for all parametric analyses. We used IBM SPSS (version 25) for statistical analyses.

Results:

Over the course of the experiment, δ^{13}C of muscle tissue gradually decreased from an average of -25.9 measured after 8 weeks of feeding on a chironomid diet to -26.9 (Figure S1a), while the δ^{15}N of muscle tissue gradually increased from 14.2 during the same time interval. Compared to muscle tissue, lipid-normalized stable isotope values in liver tissue were more depleted in ^{13}C, leading to more negative values of δ^{13}C (Figure S1c). δ^{15}N of liver decreased from 16.5 generally more enriched in ^{15}N compared to muscle tissue (Figure S1d).

Δ^{13}C of perch muscle ranged from 2.6 for Δ^{15}N from 0.5 to 3.0 Δ^{13}C of perch liver tissue ranged from 0.0 and for Δ^{15}N from 0.2 to 2.0 d). For muscle tissue, Δ^{13}C differed significantly between weight classes (ANCOVA: $F_{5,28}$: 4.584, P= 0.008), with significantly lower TDF in juvenile individuals (i.e., weight class <20 g at the start of the experiment) compared to all other weight classes. In addition, muscle Δ^{13}C of 20-30 g individuals caught in the pelagic zone was significantly lower than in individuals of 40-50 g (Figure 1a). Δ^{15}N in muscle tissue differed significantly between weight classes (ANCOVA: $F_{5,28}$: 18.365, P< 0.001), with significantly higher Δ^{15}N observed in <20 g individuals compared to all other weight classes (Figure 1b). In this ANCOVA, final weight had a significant effect on the Δ^{15}N of muscle tissue (ANCOVA: $F_{4,28}$: 7.403, P= 0.012), while this covariate was not significant for the Δ^{13}C of muscle tissue. No significant differences were found in Δ^{13}C and Δ^{15}N of liver tissue between weight classes using final weight as a covariate (Figure 1c,d).

SMR between the individuals varied substantially among perch groups and ranged from 55.6 to 106.5 mg O_2 kg $^{-1}$hr^{-1} (Table 1). SMR differed significantly between perch of the different weight classes (ANOVA: $F_{4,26}$: 4.685, P= 0.008) and final weight as a covariate was not significant. SMRs were highest in <20 g individuals, but Bonferroni-adjusted pairwise comparisons showed that only differences between high SMR in 20-30 g individuals caught in the pelagic zone and the low SMR in individuals of 40-50 g were significant (Figure 2).

For muscle tissue, linear regression showed that SMR had a significant negative effect on the Δ^{13}C (t=-4.424, P< 0.001, Figure 3a) and a significant positive effect on Δ^{15}N (t= 2.657, P= 0.014, Figure 3b), respectively. In contrast, this relationship was not significant for Δ^{13}C or Δ^{15}N in liver tissue (Figure 3 c, d).

Discussion:

Metabolic rate can be viewed as the most fundamental biological rate, explaining the pace at which organisms take up, transform, and expend energy (Brown et al. 2004). Thus, we also predict that the individual metabolic rate would profoundly shape TDF. To the best of our knowledge, this framework has only been applied to the study of TDF in small mammals (i.e. mice and rats: MacAvoy et al. 2006, MacAvoy et al. 2012), or birds (Ogden et al. 2004). However, in temperate ectothermic teleosts, where mass-specific metabolic rate is comparably slower, this process was assumed to be negligible. This view needs a revision, as our results show a clear and gradual relationship between TDF of muscle tissue (i.e. Δ^{13}C and Δ^{15}N) and metabolic rate on the individual level. Thus, our results highlight that individual metabolic rate could be one of the factors explaining variable TDF within a single species. However, we could not detect any association between metabolic rate and TDF in liver tissue. Differences in metabolic rates and therefore TDF were especially pronounced between small and large perch. Thus, in accordance with Herzka (2005), our results highlight the need for establishing different TDF for specific ontogenetic stages to allow more precise interpretation of isotopic data.

Trueman et al. (2005) reported differences in individual growth rates of Atlantic salmon (*Salmo salar*) to be associated with variable TDF. The authors suggested that this pattern could be explained by intraspecific differences in metabolism, but direct measurements of metabolic rates were not included. In many studies of TDF in ecothermic species, isotopic change has been attributed to growth rather than metabolism (Hesslein et al. 1993, Bosley et al. 2002), but see (Herzka et al. 2001, Tarboush et al. 2006, Sun et al. 2012). Unfortunately, we are not able to separate the isotopic change into contributions of growth and metabolic rate *sensu* Fry and Arnold (1982) as we did not track the precise individual weight increase. We approached this issue by including the final weight as a covariate in our models. Here, the strongest differences in TDF could be detected comparing juvenile to adult perch. Along with the highest SMR, juveniles also had the strongest approximate weight increase (Table 1). When measuring individual metabolic rate immediately before assessing their TDF we found fine scale differences that correlated strongly with Δ^{13}C and Δ^{15}N of muscles indicating that changes in metabolic rates could translate into variations in TDF (Kleiber 1932, 1947, Boecklen et al. 2011).

Results of posthoc comparisons of SMR between perch with an initial weight of 20-30 g caught in different habitats (pelagic and littoral) were not significant, but when analyzed separately, a significant difference appears with pelagic having a higher SMR (t-test: $t_{7.633}$ = -2.406; P= 0.044), similar to previous results (Andersson et al. unpublished data). Generally, it is known that such intra-specific differences in metabolic rates within adult individuals exist in many species, including fish (Biro and Stamps 2010). However, less is known about differences between individuals living in different habitats. In many Swedish lakes including Erken, which is the origin of the perch used in this study, littoral and pelagic perch of the intermediate class size differ in their individual specialization for respective food items, which is even translated into adaptations of their morphology. While pelagic perch predominately ingest pelagic zooplankton and have a more streamlined body form, littoral perch include benthic macroinvertebrates in their diet to a higher degree and are characterized by a deeper body (Svanbäck and Persson 2004, Marklund et al. 2019). Potentially, habitat-specific differences in activity levels could be related to the differences found in SMR (Myles-Gonzalez et al. 2015, Watz et al. 2015). Pelagic perch need to be endurance swimmers in order to catch the smaller fast-moving prey, while littoral perch forage on larger prey items of lower mobility (Svanbäck and Eklöv 2004). Future research is needed to resolve the underlying causes for the differences found in SMR between littoral and pelagic perch. Interestingly, average Δ^{13}C in muscle tissue of $20 - 30$ g pelagic perch was lower compared to littoral perch of the same weight class (Table 1), but this difference was not significant. Thus, our data suggest a trend that the inverse relationship (i.e. elevated SMR leads to lower Δ^{13}C), holds true not only between juveniles and adults, but also between the habitat-specific individuals of the same weight class.

While the effect of SMR was strong for Δ^{13}C and Δ^{15}N in in muscle tissue, we did not observe any relationship between SMR and TDF of liver tissue, indicating that individual metabolic rate has a stronger effect of tissue types with slower isotopic turnover. Generally, liver had lower TDF (Δ^{13}C: 1.1 0.5) compared to TDF of muscle tissues (Δ^{13}C: 3.7 0.5; Δ^{15}N: 1.3 change in the isotope values between consumer and prey. Our results are in line with the findings of other studies on tissue-specific difference in TDF in fish (Buchheister and Latour 2010, Matley et al. 2016), and the observed pattern might be due to different biochemical composition of the tissue types, e.g. the abundances of specific amino acids (Pinnegar and Polunin 1999).

Our values of Δ^{15}N in muscle tissue are relatively low compared to the highly cited average value of 3.4 However, variation in Δ^{15}N between taxa is large, which is primarily attributed to different nitrogen assimilation and excretion modes (Gaye-Siessegger et al. 2003, Vanderklift and Ponsard 2003). Thus, fractionation is typically low when the C:N ratio of the diet is also low, as it was for our chironomid diet (4.7± 0.2) (Vanderklift and Ponsard 2003). For example, McCutchan et al. (2003) reported Δ^{15}N of 1.4 ± 0.21 in consumers raised on a invertebrate diet, whereas consumers raised on a high-protein diet showed a Δ^{15}N of 3.3 ± 0.26. Protein is the principal source of energy for perch, which are ammoniotelic organisms, and thus characterized by a relatively high N use efficiency that is linked to lower Δ^{15}N (Trueman et al. 2005). Furthermore, a previous study of perch stoichiometry reported that C:N of perch varied with size, indicating that stoichiometric demands vary over ontogeny (Vrede et al. 2011). This could potentially affect assimilation and excretion rates and could have contributed to the variation of TDF between size classes as we have observed in this experiment, but further experiments are needed to include this factor.

In contrast, our derived values of Δ^{13}C for muscle tissue were rather high compared to the typically assumed value of 0.3 (Post 2002) or 0.4 have reported similarly high values (e.g.Pinnegar and Polunin 1999, Barnes et al. 2007, Busst and Britton 2016). Vollaire et al. (2007) reported Δ^{13}C of 4.02 ± 0.13 feeding on artificial diet. A potential reason for variation in Δ^{13}C could be a process termed "isotopic routing", where resource constituents, such as proteins, lipids and carbohydrates are allocated to different tissue types (Schwarcz 1991). Altogether, we thus agree with Wolf et al. (2009) and acknowledge that further studies are urgently needed to understand variation of Δ^{13}C in fish.

Variation in Δ^{15}N, specifically in liver tissue was higher compared to that of Δ^{13}C. We assume that this variability can be attributed to the fact that the δ^{15}N of the diet was rather high (14.0 Commercially raised chironomids are maintained in large flumes and cannibalism might occur which would result in higher δ^{15}N of individual organisms. Another aspect that could potentially influence the variation in Δ^{15}N, but also in Δ^{13}C is the individual food intake, which was shown to influence TDF (Bosley et al. 2002, Barnes et al. 2007). In our study, perch shoals were fed at high feeding rates (approximately 15% of the individual wet weight$^{-\text{day}}$), and left-over food was removed. However, this does not imply that all fish individuals fed until satiation. Strong hierarchies exist in perch shoals (Magnhagen 2012) and were observed in some of our tanks. Dominance behavior of single individuals might have prevented subordinates the access to food. This artefact of the experimental design adds to the previously mentioned confounding aspects and highlight again the complexity and difficulty involved in experimentally assessing general and widely applicable TDF.

In conclusion, our data emphasize the role of metabolic rate in shaping specific TDF (i.e. Δ^{13}C and Δ^{15}N of muscle tissue). Especially, our results highlight the substantial differences between individuals of different ontogenetic stages within a species.

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Author Contributions: KS did the conceptualization and wrote the first draft. KS, MLA, FC, and PE collected data; KS and MLA analyzed the data. All authors helped writing and gave final approval for publication.

Data availability : Data and R scripts are available from the Zenodo Digital Repository (http://doi.org/10.5281/zenodo.4433723).

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Figures:

Figure 1: Δ^{13}C and Δ^{15}N of muscle and liver tissue of different weight classes in perch. Weight classes with the same letter are not significantly different (Bonferroni pairwise comparisons of ANCOVA with final weight as a covariate). Boxplots depict median, 25th and 75th percentile, and whiskers extend to maximum and minimum values, except for outliers (>1.5 times box height, represented by dots).

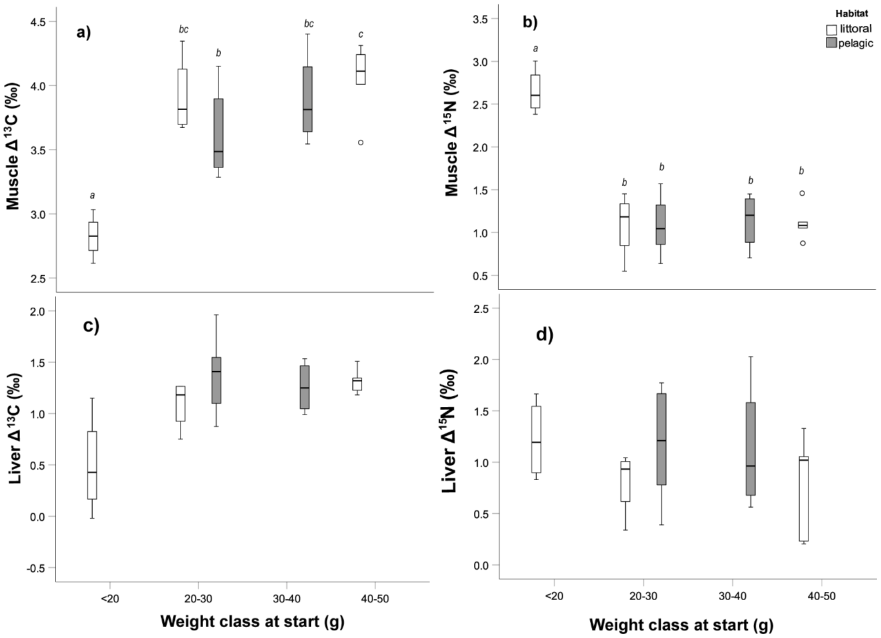

Figure 2: Standard metabolic rate (SMR) of different weight classes in perch. Weight classes with the same letter are not significantly different (Bonferroni pairwise comparisons of ANCOVA with final weight as a covariate). Boxplots depict median, 25th and 75th percentile, and whiskers extend to maximum and minimum values.

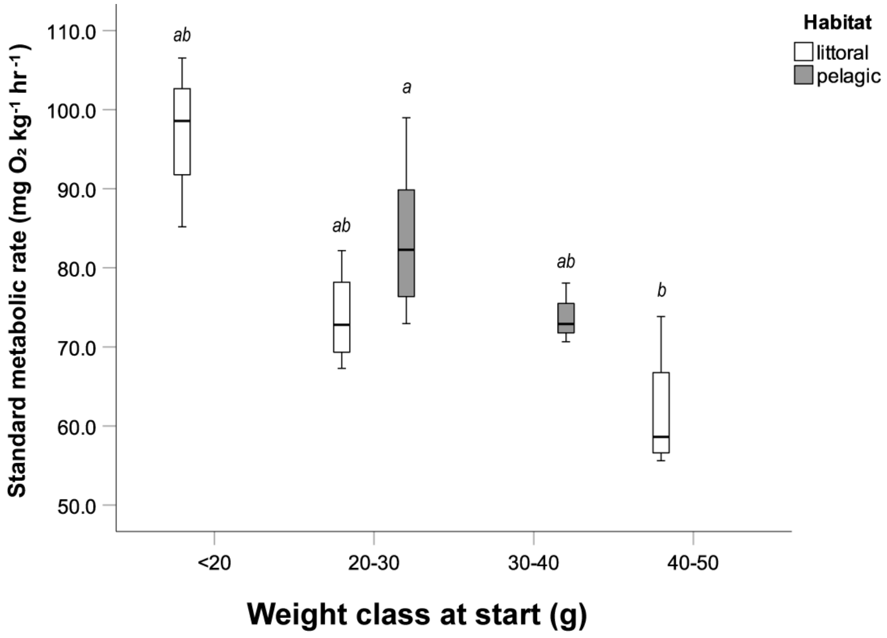

Figure 3 : Relationship between standard metabolic rate (SMR) and Δ^{13}C, and Δ^{15}N of muscle and liver tissue in perch individuals, respectively. Colors depict different weight classes of perch at the beginning of the experiment. Regression lines (including regression coefficient R^2) in a) indicate significant linear regressions between Δ^{13}C and Δ^{15}N and SMR in muscle tissue.

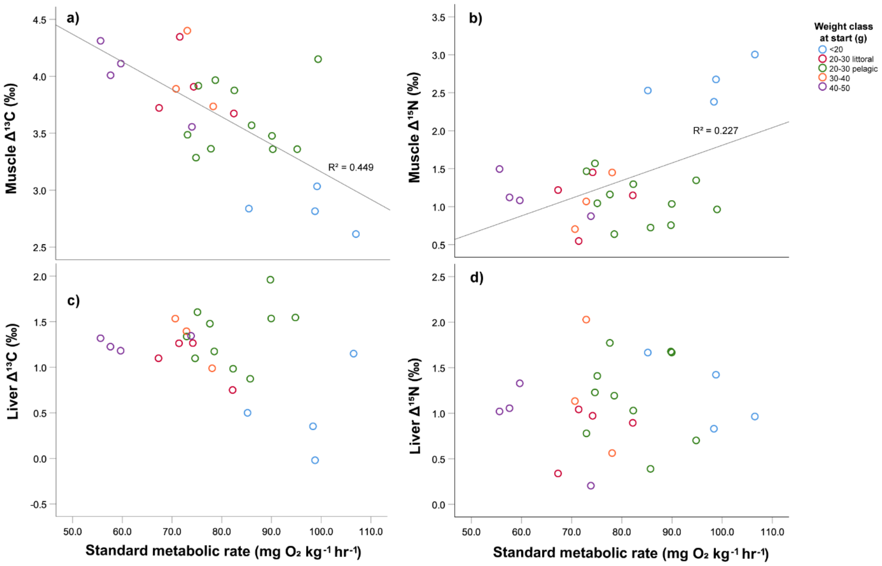

Table 1: Overview of averages (± standard deviations) of muscle and liver tissue TDF (Δ^{13}C and Δ^{15}N) for the different weight classes of perch, and standard metabolic rate (SMR), including sample sizes, weight at end of the experiment, and percent approximate change of weight (initial weight was not measured, but it was assumed to be the average of the respective weight classes).

Weight class at start (g)	Sample size	Habitat	Muscle Δ^{13}C (	Muscle Δ^{15}N (	Liver Δ^{13}C (	Liver Δ^{15}N (	SMR (mg O_2 kg $^{-1}$ hr^{-1})	Weight at end (g)	Pe ap ma cha we
<20 (juvenile)	4	littoral	2.8 ± 0.2	2.6 ± 0.3	0.5 ± 0.5	1.2 ± 0.4	97.2 ± 8.9	16.6 ± 1.0	31 ±
20-30	4	littoral	3.9 ± 0.3	1.1 ± 0.4	1.1 ± 0.2	0.8 ± 0.3	73.8 ± 6.3	56.2 ± 7.3	12 ±
20-30	11	pelagic	3.6 ± 0.3	1.1 ± 0.3	1.4 ± 0.3	1.2 ± 0.5	83.7 ± 8.8	57.7 ± 11.0	13 ±
30-40	4	pelagic	3.9 ± 0.4	1.1 ± 0.3	1.3 ± 0.3	1.1 ± 0.6	73. 8 ± 3.8	40.4 ± 23.1	73. 45.
40-50	5	littoral	4.0 ± 0.3	1.1 ±0.2	1.3 ± 0.1	0.8 ± 0.5	61.7 ± 8.3.	54.4 ± 21.2	56. 35.