

Life history traits of the fish community in Lake Annecy: evidence from the stable isotope composition of otoliths

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ABSTRACT

Key-words:
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Stable isotope analysis (SIA) of fish otoliths shows great potential for exploring the ecology of fish, but this method has not been applied to the study of lacustrine fish communities. Both sequential and whole-otolith SIAs were performed on six fish species of Lake Annecy and their results compared to muscle SIA. The first purpose of these investigations was to test the use of $\delta^{18}\text{O}_{\text{oto}}$ values for reconstructing fish thermal history and delimiting spatial distribution in a stratified lake. Comparison of species-specific fractionation equations and the general equation developed for freshwater fishes showed that the general one was the best suited for thermal estimation of Lake Annecy fishes and suggested that inter-specific differences or specific “vital effects” are not the only reason for apparent difference in fractionation. Thermal estimations based on SIA were consistent with descriptions of thermal habitats in the literature, except in the case of roach (*Rutilus rutilus*). Based on the current results, roach appears to live in a colder habitat than do perch (*Perca fluviatilis*). The high water transparency and thermal stratification of Lake Annecy could explain this distribution. Moreover, perch juveniles were found to live in two different thermal niches. This finding highlights the great plasticity of the species. Second, the potential use of $\delta^{13}\text{C}_{\text{oto}}$ values to reconstruct variation in diet and metabolism was assessed. The proportion of metabolic carbon (M) contributing to otolith carbon varies markedly among species. Comparison with $\delta^{13}\text{C}_{\text{muscle}}$ values shows no direct relationship between $\delta^{13}\text{C}_{\text{oto}}$ and diet at either the intra-individual or the inter-specific level. A strong linear relationship between either M or the isotopic offset between otolith and muscle ($\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$) and $\delta^{18}\text{O}_{\text{oto}}$ values was found; this relationship reveals the dependence of M on the ambient temperature at which the species occurs. This relationship might be general and could be used to explore variation in fish metabolism in the future.

RÉSUMÉ

Traits d'histoire de vie de la communauté de poissons dans le lac d'Annecy : l'apport de la composition en isotopes stables des otolithes

Mots-clés :
isotopes stables,

L'analyse des isotopes stables (SIA) des otolithes de poissons montre un grand potentiel pour explorer l'écologie des poissons, mais cette méthode n'a encore jamais été appliquée à l'étude des communautés de poissons lacustres. Les SIA séquentielles et globales d'otolithe ont été réalisées sur six espèces de poissons du lac d'Annecy et leurs résultats comparés aux SIA du muscle. Le premier objectif

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de ces investigations était de tester l'utilisation de valeurs $\delta^{18}\text{O}_{\text{oto}}$ pour reconstruire l'histoire thermique des poissons et préciser leur distribution spatiale dans ce lac stratifié. La comparaison des équations de fractionnement spécifiquement développées pour une espèce et l'équation générale pour les poissons d'eau douce a montré que l'équation générale était la plus adéquate pour l'estimation de l'histoire thermique des poissons du lac d'Annecy. Il est également suggéré que des différences inter-spécifiques ou « effets vitaux » spécifiques n'expliquent pas complètement la différence apparente de fractionnement isotopique. Les estimations thermiques basées sur la SIA étaient compatibles avec les descriptions des habitats thermiques dans la littérature, sauf dans le cas du gardon (*Rutilus rutilus*). Selon les résultats de l'étude, le gardon semble vivre dans un habitat plus froid que celui de la perche (*Perca fluviatilis*). La grande transparence de l'eau et la stratification thermique du lac d'Annecy pourraient expliquer cette répartition. En outre, les jeunes perches occupaient deux niches thermiques différentes ce qui met en évidence la grande plasticité de l'espèce. Deuxièmement, l'utilisation potentielle des valeurs $\delta^{13}\text{C}_{\text{oto}}$ pour reconstruire les variations de l'alimentation et du métabolisme a été évaluée. La proportion de carbone métabolique (M) contribuant au carbone de l'otolithe varie considérablement entre les espèces. La comparaison avec les valeurs $\delta^{13}\text{C}_{\text{muscle}}$ montre qu'il n'y a pas de relation directe entre $\delta^{13}\text{C}_{\text{oto}}$ et le régime alimentaire, ni au niveau intra-individuel, ni au niveau inter-spécifique. Une forte relation linéaire entre soit M , soit la différence isotopique en carbone entre l'otolithe et le muscle ($\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$) et les valeurs $\delta^{18}\text{O}_{\text{oto}}$ a été trouvée; cette relation montre que la valeur de M est fortement dépendante de la température ambiante à laquelle vit l'espèce. Cette relation pourrait être générale et utilisée pour explorer la variation du métabolisme des poissons dans l'avenir.

INTRODUCTION

Stable isotope analysis (SIA) has enjoyed great success in ecological studies for the past three decades (Michener and Lajtha, 2007). SIA is widely used for studying the ecological characteristics of fishes, including geographical distribution and origin, movements and migrations, trophic interactions, population dynamics and biomagnifications of contaminants (Deutsch and Berth, 2006; Dufour and Gerdeaux, 2001; Hoeninghaus and Zeug, 2008; Jardine *et al.*, 2013). Various fish tissues can be analysed, but soft tissue, generally muscle, has typically been preferred. It is easy to collect and prepare material and it provides the means to analyse several isotopic tracers ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{34}\text{S}$).

However, isotope turn-over in fish muscle is low, and somatic growth is usually discontinuous. Muscle SIA primarily reflects the food consumed during the season of rapid growth (Perga and Gerdeaux, 2005) and the reconstruction of ontogenetic shifts in diet or in habitat (Bergman and Greenberg, 1994; Werner and Gilliam, 1984) requires repeated fish sampling and analysis over time. SIA of otoliths has recently become of increasing interest in fish ecology studies. Otoliths are paired calcified (CaCO_3) structures, usually consisting of aragonite (Degens *et al.*, 1969). Their incremental growth and permanent nature theoretically provides a continuous time series of a fish's environment during the entire life span of the individual (Campana, 1999). Developments in micrometric sampling techniques associated with classical mass spectrometry or *in situ* SIA have enabled the retrospective reconstruction of the complexity of life chronology (Aubert *et al.*, 2012; Hanson *et al.*, 2010; Wurster *et al.*, 1999).

Oxygen and carbon isotopes in otoliths ($\delta^{18}\text{O}_{\text{oto}}$ and $\delta^{13}\text{C}_{\text{oto}}$) provide records of the ambient water conditions and the metabolic parameters of the fish. The temperature-dependent fractionation between otolith aragonite and ambient water has been used to reconstruct the thermal histories of fish based on information on the $\delta^{18}\text{O}$ value of water ($\delta^{18}\text{O}_{\text{water}}$) and the use of a valid temperature fractionation equation (Patterson *et al.*, 1993; Hoie *et al.*, 2004). Records

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of $\delta^{18}\text{O}_{\text{oto}}$, usually in combination with $\delta^{13}\text{C}_{\text{oto}}$, have been used to delineate fish habitat, define stock structure and track fish movements, migrations or depth positioning (Carpenter *et al.*, 2003; Dufour *et al.*, 2007; Huxham *et al.*, 2007; Thorrold *et al.*, 2001) when the various habitats present distinctive physicochemical compositions (temperature, salinity, $\delta^{18}\text{O}_{\text{water}}$ values) or are thermally stratified. Carbon isotopes are deposited under circumstances where they are not at equilibrium with the dissolved inorganic carbon (DIC, $\delta^{13}\text{C}_{\text{DIC}}$) present in the water. Ambient DIC carbon mixes with metabolic carbon ($\delta^{13}\text{C}_{\text{meta}}$) derived from food (Kalish, 1991; Schwarcz *et al.*, 1998; Tohse and Mugiya, 2002). Variations in $\delta^{13}\text{C}_{\text{oto}}$ have been argued to reflect a host of parameters: standard and active metabolism, diet, trophic position, DIC and depth (Schwarcz *et al.*, 1998). Furthermore, the $\delta^{13}\text{C}_{\text{oto}}$ value of otoliths is correlated with the specific respiration rate of individual fish estimated with $\delta^{18}\text{O}_{\text{oto}}$ values (Dufour *et al.*, 2007; Solomon *et al.*, 2006; Wurster and Patterson, 2003). These values have been used to track ontogenetic changes in diet, trophic level over life or temperature habitat of different morphs of a species (Jamieson *et al.*, 2004; Schwarcz, *et al.*, 1998; Kahilainen *et al.*, 2014). However, applications have been restricted due to uncertainties regarding the nature of the relationship with controlling parameters (Dufour *et al.*, 2007).

Previous otolith SIAs have primarily provided records resolving environmental and behavioural dynamics. Most otolith SIAs have quantified individual variability or change over time in a population or have compared different populations or stocks of a single species (Jamieson *et al.*, 2004; Sako *et al.*, 2007; Weidman and Miller, 2000; Wurster *et al.*, 2005). To our knowledge, the present study is the only research to compare $\delta^{18}\text{O}_{\text{oto}}$ values among several species, and no study has been performed with the specific purpose of comparing the records of life history traits of various species of a fish community. This paper presents SIAs of the principal species of the fish community in Lake Annecy, a deep stratified lake located in the French Alpine region (Haute-Savoie). The first aim of the study was to explore the potential use of $\delta^{18}\text{O}_{\text{oto}}$ values to reconstruct fish thermal histories and delimit spatial distributions within the fish community: are the thermal habitats estimated from $\delta^{18}\text{O}_{\text{oto}}$ consistent with the known vertical positions of the different species? The second aim of the study was to explore the potential use of $\delta^{13}\text{C}_{\text{oto}}$ values to reconstruct both intra-individual and inter-specific variations in diet and metabolism: does the fraction of metabolic carbon in the otolith differ among the species? Is there a relationship between this carbon fraction and the temperatures at which the fish live?

MATERIALS AND METHODS

> STUDY SITE

Lake Annecy, France, is at 446 m a.s.l. and is situated 50 km south of Lake Geneva. Lake Annecy is of glacial origin, with steep banks and a relatively flat bottom, a surface area of 2800 hectares, and a maximum depth of 65 m. The length and maximum width are 15 and 4 km, respectively. The physical, chemical and biological characteristics of the lake have been under monthly or bi-monthly monitoring for more than a decade (Dufour and Gerdeaux, 2007; Gerdeaux and Perga, 2006). Lake Annecy is monomictic and oligo-mesotrophic, with a total phosphorus concentration that has never exceeded $10 \mu\text{g}\cdot\text{L}^{-1}$. The Lake Annecy mean winter water temperature is between 4.5 and 6 °C. In summer, the thermocline is situated at approximately 15 m depth, and the hypolimnion temperature remains at ~5 °C. Epilimnion temperatures vary according to depth and exhibit regular seasonal variations. Maximum temperatures, around 22 °C, are encountered in August (Dufour and Gerdeaux, 2007; Dufour *et al.*, 2007; Gerdeaux and Dufour, 2012).

> FISH SPECIES SELECTION AND COLLECTION

We sampled the principal species of the fish community of Lake Annecy. The Lake Annecy fish community is primarily composed of salmonids, which represent more than 80% of the

yield of the fishery in Lake Annecy. Whitefish (*Coregonus lavaretus*, Linné, 1758) and Arctic char (*Salvelinus alpinus*, Linné, 1758) are the main species whereas brown trout (*Salmo trutta*, Linné, 1758) are less well represented. Northern pike (*Esox lucius*, Linné, 1758) and perch (*Perca fluviatilis*, Linné, 1758) represent 10% of the yield. Burbot (*Lota lota*, Linné, 1758) and certain cyprinids, along with roach (*Rutilus rutilus*, Linné, 1758) and tench (*Tinca tinca*, Linné, 1758) represent the remaining 10% of the total catch (Gerdeaux *et al.*, 2001).

For this study, six species were chosen that present distinctive trophic habits and habitat selection (Appendix 1). In the lake Annecy, Arctic char and whitefish are pelagic cold-water species, whereas roach, perch, Northern pike and tench are inshore warm-water eurythermic species. Northern pike is a top predator feeding primarily on fish. Whitefish is mainly zooplanktivorous and feed opportunistically on chironomids when these invertebrates rise to the surface to moult into adults. Arctic char feeds on benthic invertebrates and sometimes on fish. Roach and tench are omnivorous, as are juvenile perch. Adult perch are often piscivorous. Specimens were captured using 30 × 6 m experimental gill nets anchored at the bottom and set overnight at various depths. Adult and juvenile whitefish, arctic char, perch and roach were caught on August 31, 2005 and in the first week of September 2005. One adult perch and Northern pike were obtained from a local professional fisherman on the west shore on February 20, 2006. Adult tench were sampled on the west shore on July 12, 2006 (Appendix 1). For each individual, approximately 5 g of white muscle tissue and the two sagittae, the largest of the three otolith pairs, were sampled. Data from otolith SIAs obtained on whitefish collected in 2004 (Dufour and Gerdeaux, 2007; Dufour *et al.*, 2007) and Northern pike collected in 2008 (Gerdeaux and Dufour, 2012) were added to the present dataset.

> MUSCLE PREPARATION AND ANALYSIS

Muscle samples were kept frozen until they were prepared in the laboratory. They were freeze-dried overnight and reduced to a fine powder with a ball mill. Approximately 1 mg was weighed with a high-precision microbalance and placed in tin cups for $\delta^{13}\text{C}$ analysis.

Measurements were performed with a VG-Sira 10 or VG-Optima mass spectrometer coupled to a Carlo-Erba NA 1500 CHN element analyser at the Scottish Crop Research Institute (Scotland). Accuracy and precision were determined to be better than $\pm 0.15\%$ (SD). The amount of carbon and nitrogen (weight:weight) in each sample was measured by the thermal conductivity detector of the CHN analyser; the analytical precision was 0.2%.

Lipids can have a confounding influence on $\delta^{13}\text{C}_{\text{muscle}}$ values. The C:N atomic ratio calculated from elemental measurements obtained by CHN analysis was used to assess lipid content. Because lipids do not contain nitrogen there is a positive relationship between the C:N atomic ratio and lipid content (Kline *et al.*, 1998). The C:N values (< 4) of fish muscle tissues indicated a low lipid content in Lake Annecy (Janjua and Gerdeaux, 2011). For this reason, no treatment or C:N normalization of $\delta^{13}\text{C}_{\text{muscle}}$ values were used to eliminate lipids or correct $\delta^{13}\text{C}_{\text{muscle}}$ values.

> OTOLITH PREPARATION AND ANALYSIS

The sagittae were cleaned in 70% ethanol, and air dried. Because of their small size, whole otoliths of juveniles for all species as well as for adult tench were used. One otolith per individual was ground with a pestle and mortar into a fine powder. An aliquot (100 μg) from this homogenised material was used for stable isotope analysis. For each adult fish (except for tench), one otolith was embedded into epoxy resin (Fluka-BioChemica[®]) following the procedure of Secor *et al.* (1991). The resin blocks were subsequently polished by hand until growth banding and the core were clearly visible on the sagittal plane. The blocks were then placed on glass slides. Slides were attached to a stage under the fixed high-precision dental drill of a computer-controlled Micromill microsampling apparatus (New Wave[®]; Dettman and Lohmann, 1995) that permitted a resolution of a few μm . The innermost part of the core

and visible growth marks on sagittal sections were digitized in real time as a series of three-dimensional coordinates that were interpolated to create main sampling paths (Wurster *et al.*, 1999). Arrays of intermediate sampling paths were calculated between main sampling paths. The resulting powder samples ('microsamples') drilled from each sampling path at a depth of 25 to 50 μm were collected manually using a scalpel tip. The critical mass of aragonite for a single isotope analysis was 80–100 μg . When the amount of carbonate microsample obtained from one sampling path did not attain this critical mass, carbonate microsamples generated from two consecutive sampling paths were combined. The same sampling procedure was applied to the five species. However, the otolith shape and size differ among species. These morphological differences resulted in differences in the ease of sampling and in differences in microsample number per individual. Moreover, the relatively high mass of carbonate necessary for an analysis and the combination of consecutive sampling paths resulted in a relatively low spatial and temporal resolution. Thus it was estimated that the temporal resolution varies from 2 to 6 months depending on the species.

Stable isotope analyses were performed with a Continuous Flow Isotope Ratio Mass spectrometry (CF-IRMS) Finnigan MAT Delta plus XL equipped with GasBench II at the Stable Isotope Geochemistry Laboratory of the University of Lausanne (Switzerland). Carrara marble II (180–250 μm /1.98 Hu) is used as internal standard for calibration every 10 samples with quantities ranging from 94 μg to 221 mg. Analytical precision was 0.10‰ and 0.13‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values respectively (Spötl and Vennemann, 2003). $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values were reported relative to the VPDB standard and plotted against the distance from the core to the ventral edge on the sagittal section (width in μm) to generate intra-individual isotopic profiles.

> THERMAL HISTORY RECONSTRUCTION

Several studies have quantified the temperature dependence of otolith oxygen isotopes through the development of specific temperature-dependent fractionation equations based on fish growing in natural and laboratory environments (*e.g.*, Hoie *et al.*, 2004; Kalish, 1991; Patterson *et al.*, 1993; Thorrold *et al.*, 1997). Temperature reconstructions using these equations are relatively precise (<1 °C; Hoie *et al.*, 2004). Slopes are either statistically indistinguishable or similar to that reported by Kim *et al.* (2007) for inorganic aragonite but intercepts differ. Regarding freshwater fishes, three fractionation equations (Eq. (1)), (Patterson *et al.*, 1993) (Eq. (2)), (Storm-Suke *et al.*, 2007) (Eq. (3)), (Godiksen *et al.*, 2010) are available:

$$1000 \ln \alpha = 18.56 (1000T^{-1}) - 33.49 \text{ developed for multiple genera in natural systems (1)}$$

$$1000 \ln \alpha = 20.69 (1000T^{-1}) - 41.69 \text{ developed for two } \textit{Salvelinus} \text{ species (2)}$$

$$1000 \ln \alpha = 89.90 (1000T^{-1})^2 - 617.19 (1000T^{-1}) + 1089.24 \text{ developed for Arctic char (3)}$$

where α is the fractionation factor between water and otolith aragonite, and T is temperature in Kelvin. The relationship between α and $\delta^{18}\text{O}_{\text{oto}}$ is as follows:

$$\alpha = (\delta^{18}\text{O}_{\text{oto}} + 10^3) / (\delta^{18}\text{O}_{\text{water}} + 10^3). \quad (4)$$

An equation for marine fish, *Micropogonias undulates*, was developed by Thorrold *et al.* (1997):

$$1000 \ln \alpha = 18.56 (1000T^{-1}) - 32.54. \quad (5)$$

> ESTIMATION OF THE SOURCES OF CARBON IN OTOLITHS

Carbon isotopes from otoliths are deposited under conditions of non-equilibrium with the ambient water because a substantial proportion of carbon is derived from metabolic sources (~10–60% of total C deposited; Kalish, 1991; Schwarcz *et al.*, 1998; Solomon *et al.*, 2006). The two sources have very different isotopic values and $\delta^{13}\text{C}_{\text{oto}}$ falls in between

(Campana, 1999). The proportion M of this metabolic carbon source incorporated into otolith aragonite can be expressed by a simple mass-balance equation between the two sources:

$$\delta^{13}\text{C}_{\text{oto}} = M \delta^{13}\text{C}_{\text{met}} + (1 - M) \delta^{13}\text{C}_{\text{DIC}} + \varepsilon_{\text{total}} \quad (6)$$

where $\varepsilon_{\text{total}}$ is an enrichment factor between endolymphatic bicarbonates and aragonite and other fractionation steps.

A more detailed model including enrichment factors between food, blood, endolymph, and otolith is presented by Solomon *et al.* (2006). Assuming that fish $\delta^{13}\text{C}_{\text{muscle}}$ represents an integrated estimate of $\delta^{13}\text{C}_{\text{met}}$ and a $\varepsilon_{\text{total}}$ value equal to 0 (DeNiro and Epstein, 1978; Vander Zanden and Rasmussen, 2001), M can also be expressed as:

$$M = (\delta^{13}\text{C}_{\text{oto}} - \delta^{13}\text{C}_{\text{DIC}}) / (\delta^{13}\text{C}_{\text{muscle}} - \delta^{13}\text{C}_{\text{DIC}}). \quad (7)$$

> $\delta^{18}\text{O}_{\text{WATER}}$ AND $\delta^{13}\text{C}_{\text{DIC}}$ IN LAKE ANNECY

Variations in $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ have been previously recorded in the various fish habitats of Lake Annecy (Dufour *et al.*, 2007). It is difficult to select $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ mean values that represent the average values over the life of a fish. Variability is higher in the littoral than in the pelagic zone, even more than in the hypolimnion (Dufour and Gerdeaux, 2007). Lake Annecy waters exhibit regular seasonal and vertical variations in $\delta^{18}\text{O}_{\text{water}}$ (Danis *et al.*, 2004; Gerdeaux and Dufour, 2012). Because of the stability in $\delta^{18}\text{O}_{\text{water}}$ values among years and because the residence time of Lake Annecy water is more than four years (Danis *et al.*, 2004), 2001 monthly averaged $\delta^{18}\text{O}_{\text{water}}$ values calculated by Danis *et al.* (2004) for epilimnion and hypolimnion can be considered representative for all years considered in the present study. The epilimnion and hypolimnion monthly mean $\delta^{18}\text{O}_{\text{water}}$ values varied from -9.35% to -8.60% VSMOW and from -9.35% to -9.10% VSMOW, respectively. The $\delta^{18}\text{O}_{\text{water}}$ mean values chosen for this study depended on *a priori* knowledge of species habitat preferences obtained from the literature and on author knowledge of the fish community of Lake Annecy. According to previously described values, a range of variation in $\delta^{18}\text{O}_{\text{water}}$ between -8.95% and -9.35% was used for all fish except for Arctic char. The uncertainty of the results would be greater for roach and perch in particular, as these species live in the coastal and/or in the pelagic zone. For Arctic char, which always remain in deep waters, a range of $\delta^{18}\text{O}_{\text{water}}$ between -9.35% and -9.10% was used.

The maximum variation in $\delta^{13}\text{C}_{\text{DIC}}$ values encountered by fish (tench, roach, pike, perch) in the littoral zone was from -6.5% in summer to -9.0% in winter. The fish living around the thermocline (whitefish) encountered $\delta^{13}\text{C}_{\text{DIC}}$ values between -8.0% at 15 m depth to -6.8% at 10 m during the growing season. Fish living deeper in the hypolimnion (Arctic char) encountered almost no variation in the values, which ranged narrowly in the neighbourhood of -9.5% .

> STATISTICAL ANALYSES

Average isotopic values were compared with parametric tests (ANOVA, t-test). Non-parametric tests (Kruskal-Wallis (K.W.), Mann-Whitney (M.W.)) were used if the distributions were not normally distributed or if variances were unequal. All analyses were performed with SPLUS[®] software.

RESULTS

> INTRA-OTOLITH $\delta^{18}\text{O}$ AND $\delta^{13}\text{C}$ PROFILES

Low-resolution isotopic profiles were generated to reveal intra-otolith isotopic variations for five of the six studied species (Figure 1). At the species level, no general clear pattern was

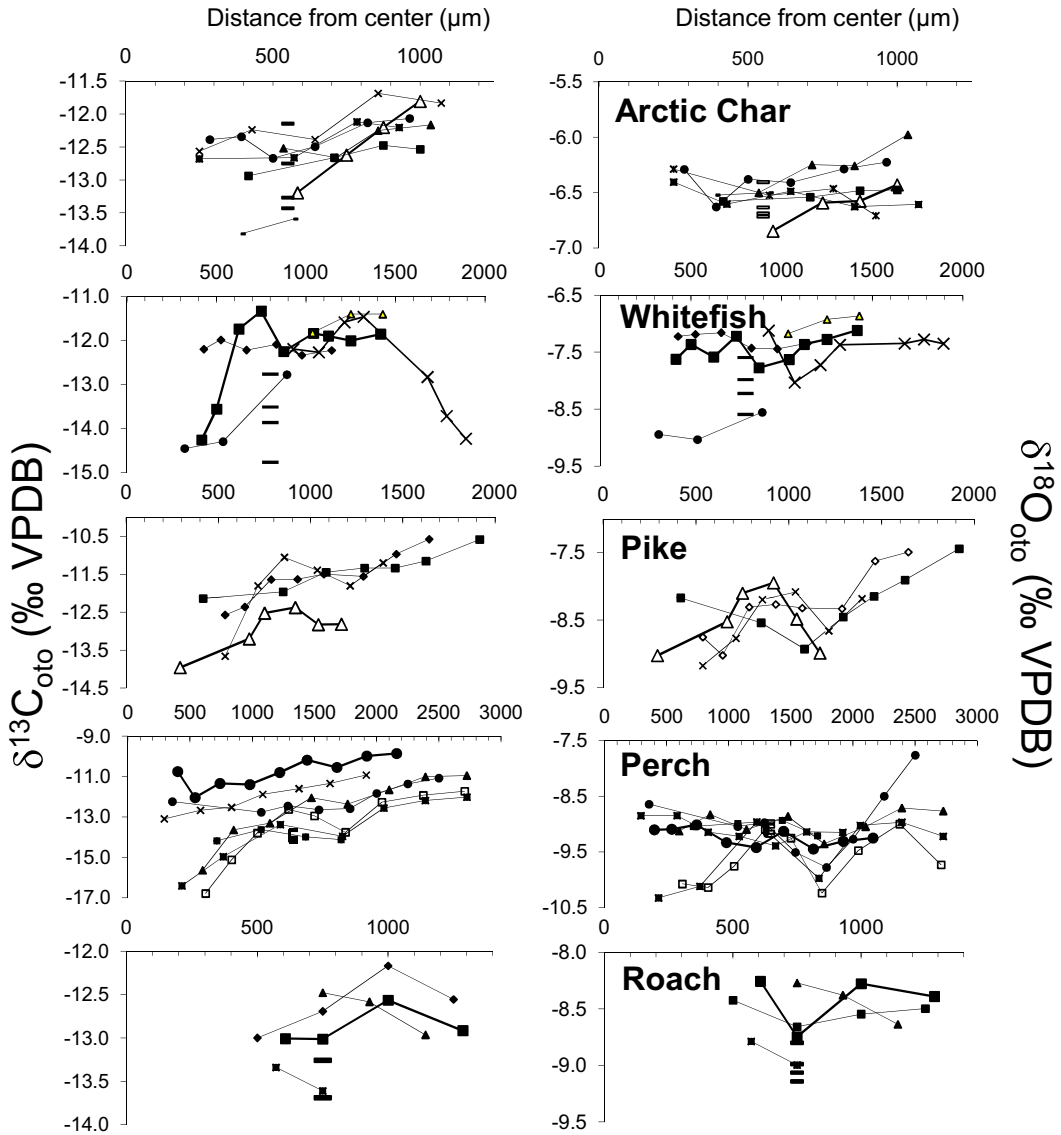


Figure 1
 Intra-otolith variation in $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{oto}}$ ‰, VPDB, left column) and $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{oto}}$ ‰, VPDB, right column) for Arctic char (*Salvelinus alpinus*), whitefish (*Coregonus lavaretus*), pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from Lake Annecy fish community. Each line represents one individual isotopic profile obtained by micromilling the otolith from the center (nucleus) to the edge.

observed for roach and whitefish, while pike, Arctic char and perch profiles usually exhibited similar patterns in seasonal and ontogenetic variations. However, perch profiles exhibited two patterns of $\delta^{18}\text{O}_{\text{oto}}$ and $\delta^{13}\text{C}_{\text{oto}}$ values at the beginning of life. There was a higher intra-individual variation in $\delta^{13}\text{C}_{\text{oto}}$ values than in $\delta^{18}\text{O}_{\text{oto}}$ values for the pike and perch species.

> OTOLITH $\delta^{18}\text{O}$ AND THERMAL RECONSTRUCTION

$\delta^{18}\text{O}_{\text{oto}}$ values ranged for adults and juveniles from -9.4 to -7.3‰ for pike, -9.3 to -6.9‰ for whitefish, -9.1 to -8.3‰ for roach, -7.0 to -6.0‰ for Arctic char, -10.3 to -7.8‰ for perch and -8.9 to 8.7‰ for tench (Figures 1 and 2). There were significant differences in adult $\delta^{18}\text{O}_{\text{oto}}$ values among species (ANOVA, $F = 102.67$, $p < 0.001$). The lifetime mean $\delta^{18}\text{O}_{\text{oto}}$ values for individual adults ranged from $-9.3\text{‰} \pm 0.5$ for perch and $-6.2\text{‰} \pm 0.2$ for Arctic char. The mean values did not differ between pike and whitefish ($|t| = 0.90$, $df = 9$, $p > 0.05$)

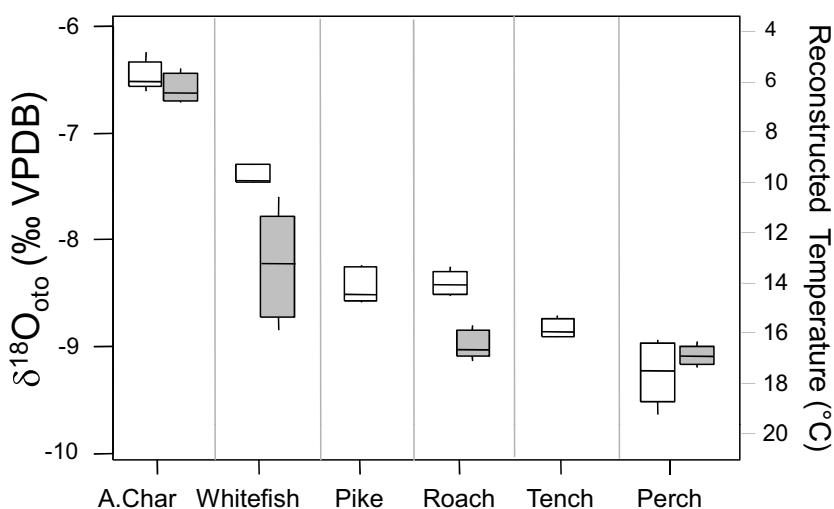


Figure 2

Total variation in otolith $\delta^{18}\text{O}_{\text{oto}}$ values ($\delta^{18}\text{O}_{\text{oto}}$ ‰, VPDB) of adults (white) and juveniles (shaded) of six fishes of Lake Annecy. Temperatures ($^{\circ}\text{C}$) are calculated using the Patterson et al. (1993) fractionation equation and water $\delta^{18}\text{O}$ values of -9.35‰ measured for Lake Annecy.

and between pike and roach ($|t| = 0.88$, $df = 7$, $p > 0.05$). Perch mean value was significantly lower to that of all other species ($p < 0.05$) (Figure 2).

Juvenile perch presented the lowest mean $\delta^{18}\text{O}_{\text{oto}}$ values, Arctic char the highest mean $\delta^{18}\text{O}_{\text{oto}}$ values (Figure 2). There was no significant difference in mean $\delta^{18}\text{O}_{\text{oto}}$ values between adults and juveniles for Arctic char ($|t| = 0.228$, $df = 10$, $p = 0.824$), perch ($|t| = 0.835$, $df = 10$, $p = 0.424$) and roach ($t = 1.733$, $df = 8$, $p = 0.121$) (Figures 1 and 2). The $\delta^{18}\text{O}_{\text{oto}}$ values differed significantly between juvenile and adult whitefish (M.W. $U = 16$, $n = 5$, $p = 0.016$).

Temperature ranges reconstructed using Patterson's equation (Eq. (1)) and taking all adult microsample values and juveniles values were 9°C to 18.6°C for pike, 6.5°C to 17.5°C for whitefish, 10.4°C to 24°C for perch, 12.6°C to 18.4°C for roach, 2.8°C to 8.9°C for Arctic char (Figure 2). While temperatures were reconstructed using a range of $\delta^{18}\text{O}_{\text{water}}$ values to take into account spatial and seasonal variability, Figure 2 presents results obtained with a single value ($\delta^{18}\text{O}_{\text{water}} = -9.35\text{‰}$) for more clarity. A positive offset of $\sim 2^{\circ}\text{C}$ is obtained when temperatures are reconstructed with $\delta^{18}\text{O}_{\text{water}}$ value of -8.95‰ .

> $\delta^{13}\text{C}$ VALUES OF OTOLITH AND MUSCLE

For adult specimens, $\delta^{13}\text{C}_{\text{oto}}$ values ranged from -14.0 to -10.6‰ for pike, -14.5 to -11.3‰ for whitefish, -15.0 to -12.2‰ for roach, -13.9 to -11.5‰ for Arctic char, -16.8 to -9.9‰ for perch and -14.6 to -12.9‰ for tench (Figures 1 and 3). Mean $\delta^{13}\text{C}_{\text{oto}}$ values for adult fish ranged from -13.5‰ for tench to -12.0‰ for pike (Figure 3). The measured value for tench is the only one which is statistically different from the other five species (ANOVA, $f = 2.5$, $p = 0.04$).

$\delta^{13}\text{C}_{\text{muscle}}$ values for adult ranged from -35.2‰ to -27.2‰ for all species, with a maximum intra-species variation of 4.2‰ for perch (Figure 3). There are significant difference between species (ANOVA; $F = 31.314$, $p < 0.001$). Arctic char presented the lowest $\delta^{13}\text{C}_{\text{muscle}}$ values, and tench presented the highest $\delta^{13}\text{C}_{\text{muscle}}$ values; pike, roach and perch values are close to that of tench. The whitefish $\delta^{13}\text{C}_{\text{muscle}}$ values were closer to those of Arctic char values than to those of any other species (Figure 3). Pike, roach, perch and tench $\delta^{13}\text{C}_{\text{muscle}}$ values did not differ significantly (t test, $p > 0.2$). Arctic char and whitefish values differed from each other and from that of the four other species (t test, $p < 0.001$).

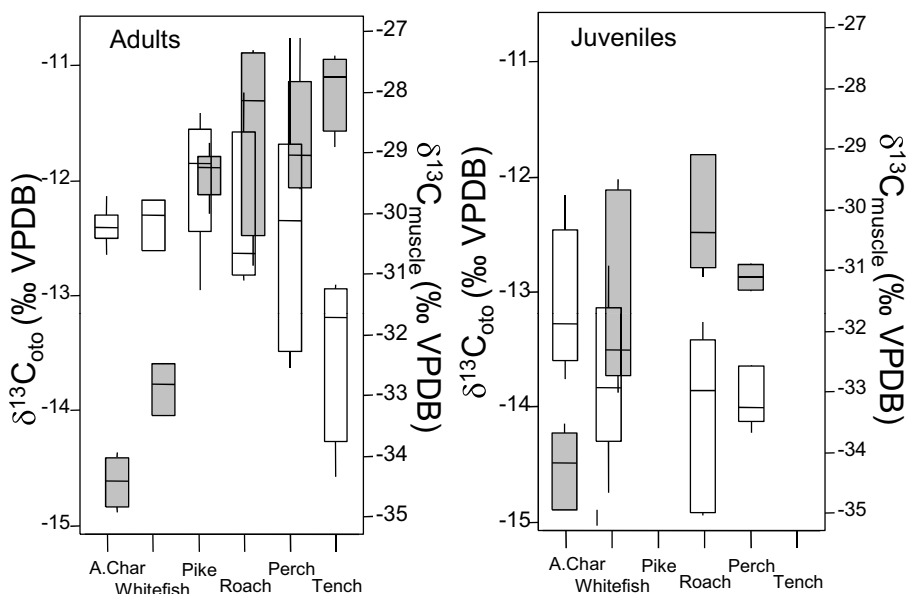


Figure 3

Total variation in otolith $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{oto}}$ ‰, VPDB) and muscle $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{muscle}}$ ‰, VPDB) of adults and juveniles of six fish species of Lake Anney community. The $\delta^{13}\text{C}_{\text{muscle}}$ values are in shaded area.

Juveniles presented $\delta^{13}\text{C}_{\text{muscle}}$ values similar to those of adults for Arctic char ($t = 0.78$, $df = 6$, $p = 0.47$), whitefish ($t = 1.91$, $df = 4$, $p = 0.13$) and roach ($t = -0.96$, $df = 5$, $p = 0.38$), and lower $\delta^{13}\text{C}_{\text{muscle}}$ values than adults for juvenile perch ($t = -8.96$, $df = 1$, $p < 0.001$) (Figure 3).

> METABOLIC CARBON IN OTOLITH AND THERMAL HABITAT

The difference between $\delta^{13}\text{C}_{\text{oto}}$ and $\delta^{13}\text{C}_{\text{muscle}}$ values, $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$, varied between 14‰ and 23‰ among species (Figures 4 and 5). $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ was greatest for Arctic char living in the cold waters of the lake and was lowest for fish such as perch, tench and roach, which usually stay in the warm upper layers of the lake. There was a strong linear correlation between $\delta^{18}\text{O}_{\text{oto}}$ and $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ for all individuals included in this study ($R^2 = 0.81$, $p < 0.001$) (Figure 4).

The estimated proportion of M (metabolic carbon sources) incorporated into otolith aragonite, calculated from equation (7), varied between 0.1 and 0.35 among species (Figure 5). There was a significant, negative linear relationship between M and $\delta^{18}\text{O}_{\text{oto}}$, based on pooling of all the studied specimens ($r^2 = 0.5$, $p < 0.001$) (Figure 5).

DISCUSSION

> RECONSTRUCTION OF FISH LIFE HISTORY PATTERNS FROM LOW-RESOLUTION MICROMILLING

The present study was designed to provide insight into the thermal and metabolic history of Lake Anney fishes at the level of the community, using otolith SIA. We used a micromilling technique (except for tench) from the core to the periphery to retrieve the intra-individual variation of adult fish at low resolution. Previous studies conducted on whitefish or pike have shown that it was possible to determine life history traits with a time step of the order

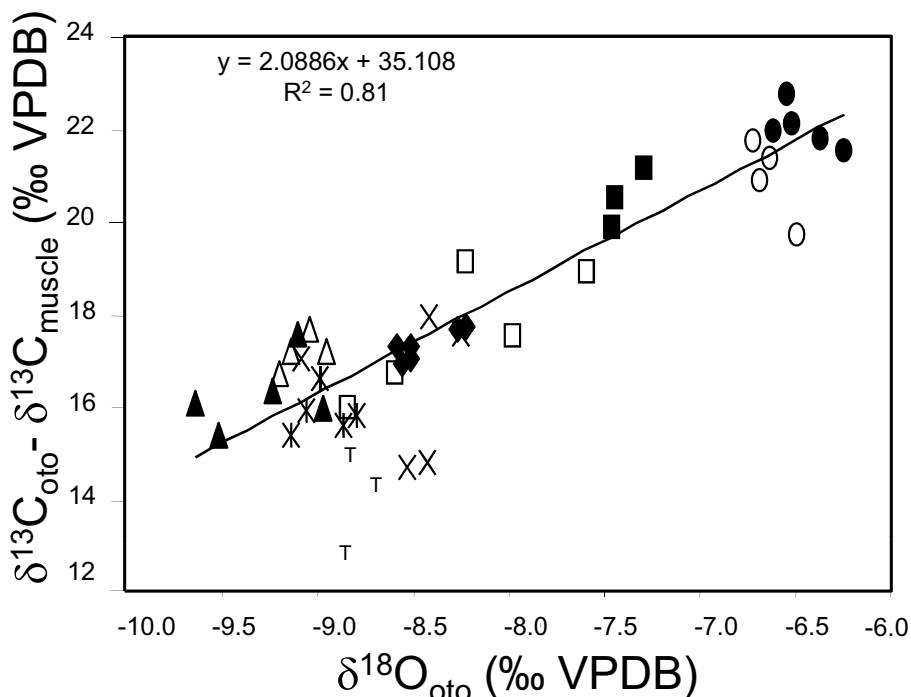


Figure 4
 Relation between the difference in $\delta^{13}\text{C}$ values between otolith and muscle ($\Delta\delta^{13}\text{C}_{\text{otolith-muscle}}$, ‰, VPDB) and otolith $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{otolith}}$ ‰, VPDB) of adults for six fish species (pike \blacklozenge , perch \blacktriangle , arctic char \bullet , whitefish \blacksquare , roach \times , T tench) and juveniles (perch \triangle , arctic char \circ , whitefish \square , roach $*$) of Lake Annecy community.

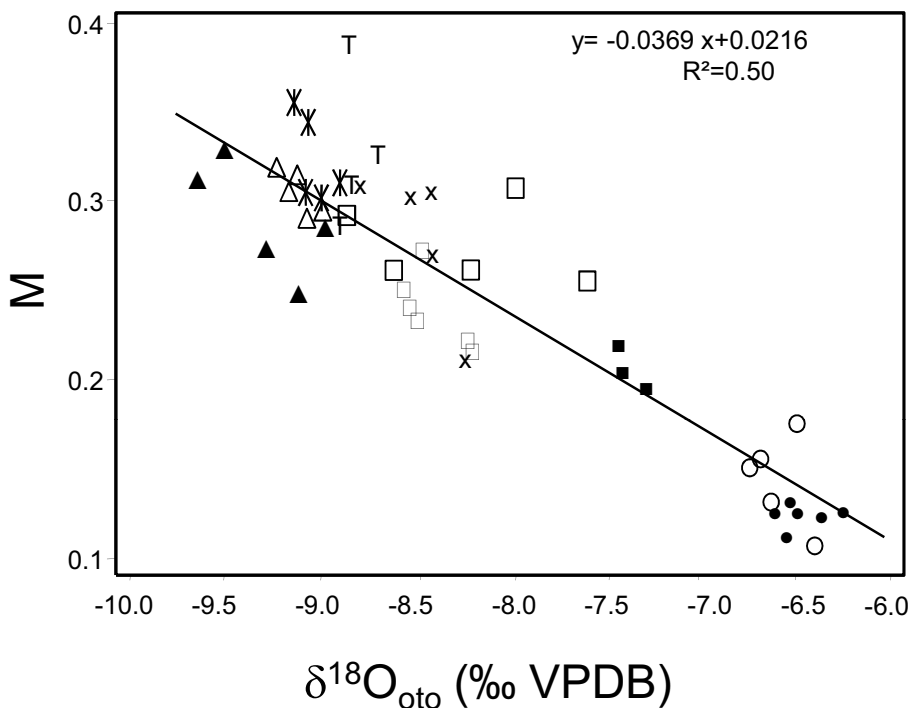


Figure 5
 Relation between the contribution of metabolic carbon (M) to otolith and otolith $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{otolith}}$ ‰, VPDB) values of six fish species (pike \blacklozenge , perch \blacktriangle , arctic char \bullet , whitefish \blacksquare , roach \times , T tench) of Lake Annecy community.

of months with a high-resolution micromilling procedure associated with classical mass spectrometry (Dufour *et al.*, 2007; Gerdeaux and Dufour, 2012). This approach is time-consuming and costly. Besides that, this study did not plan to describe individual fish histories. It aimed primarily to describe inter-specific variation in life history parameters recorded by otoliths and to test whether these findings were consistent with the current knowledge of the species' ecology. For this purpose, we determined $\delta^{18}\text{O}_{\text{oto}}$ and $\delta^{13}\text{C}_{\text{oto}}$ values for inter-specific comparisons by averaging the set of values over all the profiles (Figure 2). This procedure was preferred to the averaging obtained after grinding the entire otolith because, in this case, the part represented by the last year of life less overwhelmingly dominated the total otolith mass and, then, the mean otolith isotopic value. For tench, the whole otolith was ground, and we are aware that the average value does not have exactly the same meaning as it does for the other species.

The time average of the SIA profiles likely differs between species because of otolith shape and size. The studied species present markedly differing otolith shapes. The perch otolith is relatively flat, the growth marks are easily distinguishable by colour contrasts and their layout is relatively simple (only slightly wavy). In this case, the designed Micromill sampling paths easily match and recover synchronous aragonite deposits. With the chosen micromilling procedure, only pike and perch presented a range of intra-otolith $\delta^{18}\text{O}_{\text{oto}}$ variation greater than 1‰ (Figure 1). This is not the case for roach otoliths, for which growth marks are more difficult to identify and present a complicated (very wavy) layout. Accordingly, extraction of non-synchronous deposits might have led to a stronger averaging of the isotopic values. As a consequence, no clear intra-individual variations were noted, even at the inter-annual scale, for roach. Furthermore, the sub-seasonal scale of variation previously described for whitefish (Dufour and Gerdeaux, 2007; Dufour *et al.*, 2007) could not be retrieved here, whereas good quality profiles were obtained for perch (Figure 1). Interestingly, the data showed clear inter-individual differences for perch, suggesting two different patterns in life history traits. For all perch profiles, lower $\delta^{13}\text{C}_{\text{oto}}$ and $\delta^{18}\text{O}_{\text{oto}}$ values were found at the beginning of life, but a $\delta^{18}\text{O}_{\text{oto}}$ difference of ~1‰ at the beginning of life effectively discriminated two groups of individuals (Figure 1). Differences in $\delta^{13}\text{C}_{\text{oto}}$ were also observed for certain individuals. This finding suggests that part of the population occupies the coastal environment, whereas the other part remains in the pelagic environment during the first year of life. The ecological plasticity of perch has been well described in Swedish lakes (Svanback and Eklov, 2006; Svanback *et al.*, 2008) and is also demonstrated here for Lake Annecy for the first time.

> CONSTRAINTS ON THERMAL HISTORY RECONSTRUCTION

Variations in $\delta^{18}\text{O}_{\text{oto}}$ values obtained after otolith micromilling or grinding were used to reconstruct the ranges of temperature experienced by the six Lake Annecy species selected for study (Figure 2). Such reconstruction, based on fractionation equations, required that the value of $\delta^{18}\text{O}_{\text{water}}$ is known. Fractionation equations are highly sensitive to this parameter, the variability of which is often neglected. A 0.1‰ variation in the $\delta^{18}\text{O}_{\text{water}}$ value (which corresponds to the analytical precision) results in a change of approximately 0.4 °C in the temperature calculated using equation (1). Despite the monitoring of $\delta^{18}\text{O}_{\text{water}}$ (and of $\delta^{13}\text{C}_{\text{DIC}}$) for the different ecological zones, the choice of suitable $\delta^{18}\text{O}_{\text{water}}$ (and $\delta^{13}\text{C}_{\text{DIC}}$) mean values that represent the average value over the life of a fish is difficult. The choice of the appropriate value must be based on *a priori* knowledge of species habitat preferences, obtained from the literature and from the authors' knowledge of the fish community of Lake Annecy. Moreover, the variability is higher in the epilimnion than in the hypolimnion and in the epilimnion of the littoral than of the pelagic zone (Dufour and Gerdeaux, 2007). This will result in an increased variability in $\delta^{18}\text{O}_{\text{oto}}$ providing potentially a better way to discriminate fish habitat in the lake for roach and perch in particular because they live both in coastal and/or pelagic zones. However, the use of $\delta^{18}\text{O}_{\text{water}}$ values will also increase the uncertainty of the calculated temperatures that will be greater for littoral/pelagic species than for benthic species.

Another constraint on the results is the choice of the fractionation equation used for such temperature estimates. Several studies have suggested that oxygen isotope fractionation between otolith aragonite and ambient water was species-specific and recommended the use of species specific equations (Godiksen *et al.*, 2010). Several authors have noticed significant differences among thermal estimates (Thorrold *et al.*, 1997; Hoie *et al.*, 2004; Storm-Suke *et al.*, 2007). For Storm-Suke *et al.* (2007) and Rowell *et al.* (2005), this inconsistency precludes the broad use of any single fractionation equation for accurate thermal reconstruction of different species. It requires the use of a fractionation equation based on closely related species. Patterson's (1993) equation (Eq. (1)) was developed for a mixed assemblage of freshwater fish genera and has been used in previous studies on Lake Annecy. It was found to provide the best fit for both whitefish and pike (Gerdeaux and Dufour, 2012). Equation (1) was published prior to the equations that incorporated appropriate adjustments for the genus *Salvelinus* and for Arctic char (Godiksen *et al.*, 2010; Storm-Suke *et al.*, 2007). The availability of equations (2) and (3) provides an opportunity to test this assumption for the Lake Annecy Arctic char analysed in the present study. Arctic char usually live in the deep part of the lake hypolimnion, where the ambient temperature remains at approximately 5 °C (Klemetsen *et al.*, 2003). Thirty-six of 37 temperature values calculated for Arctic char using equation (1) varied between 4 °C to 8.9 °C. Apart one very low value (2.8 °C), reconstructed temperatures are close to the minimum recorded in the deep hypolimnion of Lake Annecy. Indeed, the water temperature in Lake Annecy is never less than 4 °C; moreover, below a depth of 30 m, the water temperature is always less than 7 °C. The temperature calculations using equations (2) and (3) yield values approximately 2 and 3.5 °C, respectively, below the minimum observed in Lake Annecy (<4 °C). Geffen (2012) also found that equations (2) and (3) underestimated the temperatures for plaice (*Pleuronectes platessa*) captured at the same time in the North Sea. Conversely, temperatures calculated based on equation (5) (Thorrold *et al.*, 1997) are ~4 °C higher than values calculated with equation (1); these values are unrealistically high. Equations (1) (Patterson *et al.*, 1993), developed for mixed assemblages of freshwater fishes, gives better calculations especially for Arctic char in Lake Annecy. These results suggest that interspecific differences or specific "vital effects" are not the only reason for apparent difference in fractionation. Additionally, and perhaps more importantly, the differences in temperature range and experimental design between the different calibration studies could explain the observed variations among studies in the fractionation factors. Otolith treatment method used in some studies (Storm-Suke *et al.* 2007; Godiksen *et al.*, 2010) includes the roasting of samples at high temperature (350 °C). It is well-known that aragonite is metastable and alters to calcite that has its own fractionation factor. It is possible that roasting has, at least partially, caused some recrystallization of aragonite into calcite. The use of the equations developed using this treatment may still be appropriate on any roasted otolith but might be inappropriate on untreated aragonite for which some correction (offset) should be applied. The large differences in temperatures derived between temperature equations developed specifically for Arctic char and the general equation of Patterson *et al.* (1993) observed in the present study might be due to this treatment difference. Therefore, we preferred to use a general equation, even if a specific equation was available for one of the species. Because the fractionation factor might vary in response to different causes, very precise temperature reconstruction, even for populations of the same species, might be difficult. Here, the goal is to estimate the thermal habitat, and the use of equation (1) appears suitable.

> WHAT $\delta^{18}\text{O}$ TELL ABOUT THE THERMAL DISTRIBUTION OF LAKE ANNECY FISH?

The range of calculated temperatures at which the fish appeared to live, based on SIA, is highly consistent with the range of life traits known from the literature. Reconstructed temperature ranges were calculated from all microsample $\delta^{18}\text{O}_{\text{oto}}$ values using equation (1) and two $\delta^{18}\text{O}_{\text{water}}$ value ranges: from -9.35‰ to -8.95‰ for all pelagic and/or coastal species and from -9.35 ‰ to -9.10‰ for Arctic char, which always remain in deep waters. Ranges

were 9 °C to 18.6 °C for pike, 6.5 °C to 17.5 °C for whitefish, 10.4 °C to 24 °C for perch, 12.6 °C to 18.4 °C for roach, and 4 °C to 8.9 °C (except one value at 2.8 °C) for Arctic char (Figure 2). The mean temperature for tench was 15.5 °C. Except for Arctic char, for which some calculated temperatures are lower than those observed in the lake in winter, the minimum calculated values were close to the values given in the literature at which the metabolism of somatic growth becomes active. For roach, this result is in accordance with the findings from feeding experiments in which the growth of roach is low or zero at temperatures below 12 °C (vanDijk *et al.*, 2002). The growth of perch starts above 10 °C (Karas, 1990). In a literature review Armstrong and Hawkins (2008) reported that the standard metabolic rate of pike is very low, below 8 °C. Thus, the lowest reconstructed values using equation (1) correspond quite well with the thermal thresholds cited in the literature. It is above these thresholds that the metabolism of growth starts for pike, perch and roach.

Regarding the maximum well-being temperature for each species, data from the literature are quite variable. In this context, our calculations are consistent with the various upper limits of the ranges of temperature. The values for pike are generally close to 20 °C. In Canadian lakes, the estimated preferred temperature of adult pike is ~23–24 °C (McCauley and Casselman, 1981). In Europe, optimum growth is considered to occur between 10 and 23 °C (Keith and Allardi, 2001) or, more, approximately around 20 °C (Bruslé and Quignard, 2001; Craig, 1996) and 25 °C (Bevelhimer *et al.*, 1985). In the literature, the data for roach are variable. Souchon and Tissot (2012) indicated that roach use a very wide temperature range, with a minimum ranging from 0 to 12 °C and a maximum ranging from 25 to 38 °C. Broughton and Jones (1978) noted that the growth of juvenile roach begins at temperatures above 14 °C. vanDijk *et al.* (2002) have shown experimentally that roach preferred temperatures above 25 °C during the day. Tench has a mean reconstructed temperature at 15.5 °C, quite low considering that the species is often found in warm waters of the littoral zone. Tench fry show maximum growth to 24 °C (Backiel, 1986). Perch provide more consistent results, with literature data generally citing temperature ranges between 18 °C and 27 °C (Hokanson, 1977).

The lower reconstructed temperatures for roach than for perch raise questions. In Lake Annecy, the transparency of water (Secchi disk ≤ 10 m) is, perhaps, the factor that changes the spatial segregation of fish and the temperatures at which they live. Due to competition for habitat and food in oligotrophic lakes with highly transparent water, roach might be displaced from the littoral zone by the presence of perch; the latter species is better able to forage in macrophyte patches (Svärdson, 1976; Winfield, 1986). The vertical positioning of the species could also be related more closely to avoiding predation by fish and birds than to finding a foraging optimum. In summer, very few fish stay in the littoral zone of the lake. The only fish observable are juvenile perch. Roach fry are never observed in the same areas. Currently, only a limited number of studies are available to support or contradict our observations based on $\delta^{18}\text{O}_{\text{oto}}$ values. However, *in situ* studies of the vertical distribution of different species in lake with fishing nets or by echo sounding often show vertical spatial segregation, in which roach are positioned in higher water layers than those occupied by perch (Persson, 1986; Horppila *et al.*, 2000). Kahl and Radke (2006) and Vasek *et al.* (2009) found that roach occurs during the day in the layer just above the thermocline (3 to 4.5 m) and occupies the upper epilimnion (0–3 m) at night, whereas perch occupy the lower epilimnion and upper metalimnion (1.5–6 m). In Lake Annecy, the epilimnion is much thicker, approximately 15 m, and the water transparency is always greater than 6 m (as measured by a Secchi disk). In Saldenbach Reservoir, which has a wide and deep pelagic zone, Kahl and Radke (2006) concluded that the spatial segregation of perch and roach is related to the temperature gradient. None of these studies provide any indication of the water transparency. Kahl and Radke's data were derived from a comparative analysis of various lakes of different sizes and depths, with simultaneous exclusion of confounding factors such as fish species composition, productivity, food or water transparency. Thus, more investigations regarding the perch-roach interaction in wide and deep lakes are necessary.

In this study, the use of otolith SIAs to study various fish species in Lake Annecy successfully located the positions of the thermal habitats of the species. The results for perch and roach

highlight an interesting question of niche segregation previously mentioned by Svanback *et al.* (2008); this question has not yet been fully investigated.

> WHAT $\delta^{13}\text{C}_{\text{OTO}}$ VALUES TELL ABOUT FISH DIET AND METABOLISM?

SIA of carbon (associated with nitrogen) is routinely used in fish ecology and food-web studies through the documentation of fish diets and trophic positions. Although these studies are largely based on muscle SIA, SIA of otoliths has recently gained interest. According to previous authors of SIA studies, standard and active metabolism, diet, trophic position, DIC and depth are factors influencing $\delta^{13}\text{C}_{\text{oto}}$ values at the intra or inter-specific levels (Schwarcz *et al.*, 1998; Sherwood and Rose, 2003). It is well known that $\delta^{13}\text{C}_{\text{oto}}$ values are not precipitated in equilibrium with $\delta^{13}\text{C}_{\text{DIC}}$ values (e.g. Weidman and Millner, 2000). Predicted $\delta^{13}\text{C}_{\text{aragonite}}$ values using an enrichment factor averaging 2.7‰ for inorganic aragonite (Romanek *et al.*, 1992) and $\delta^{13}\text{C}_{\text{DIC}}$ values measured for Lake Annecy waters should range from -6.3‰ to -3.8‰. All Lake Annecy fish have considerably lower $\delta^{13}\text{C}_{\text{oto}}$ values than those predicted as previously described for whitefish (Dufour *et al.*, 2007).

Simple two component mass-balance equations such as equation (7) are used in the literature to estimate the contribution of metabolic sources (e.g. Kalish, 1991). For Lake Annecy fish, the range of M estimates is rather large (~0.1 for Arctic char to ~0.35 for roach; Figure 5) but falls within the published estimates. Absolute M estimates should be used with caution, and comparisons with previous estimates are difficult: (1) previous works have been conducted either on adults or juveniles while difference in ontogenetic growth rate might influence the contribution of the proportion of metabolic carbon; (2) the values of the fractionation factor (ϵ) between diet and otoliths and between total blood bicarbonate and otoliths differ among studies. The effect of these factors on M estimates is substantial (Solomon *et al.*, 2006; Rowell, 2006; Dufour *et al.*, 2007). Clearly, experimental studies in which carbon source components are labelled and the fractionation factor is independently determined are needed. Such investigations are beyond the scope of this study, in which we assumed the same fractionation factor for all studied species.

As a proportion of otolith carbon derives from metabolism, it has been considered that $\delta^{13}\text{C}_{\text{oto}}$ could provide the ability to reconstruct diet information for marine (Jamieson *et al.*, 2004; Weidman and Miller, 2000) and freshwater fish (Sako *et al.*, 2007). In the current study, the potential relationship between $\delta^{13}\text{C}_{\text{oto}}$ with diet was explored indirectly by comparison with $\delta^{13}\text{C}_{\text{muscle}}$ values. Lake Annecy species show large variation (7‰) in mean specific $\delta^{13}\text{C}_{\text{muscle}}$ values (Figure 3). In lakes, planktonic-pelagic consumers present lower $\delta^{13}\text{C}$ values than benthic-littoral consumers as a result of different exposure levels to water turbulence of primary producers (periphyton and phytoplankton) at the bottom of the food web (France, 1995). The $\delta^{13}\text{C}_{\text{muscle}}$ values measured in the present study well reflect the consumption of pelagic food for pelagic species (Arctic char) or littoral food for inshore species (roach, perch, tench) or a mix of both for whitefish (Figure 3). Contrasting with the large variations in $\delta^{13}\text{C}_{\text{muscle}}$, Lake Annecy species displayed a limited (~3‰) range of mean $\delta^{13}\text{C}_{\text{oto}}$ values among species (Figure 3). The $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ is not a constant offset and varies between 14‰ and 23‰ among species (Figure 4). Our data, therefore, do not support a direct influence of diet on inter-specific variation in $\delta^{13}\text{C}_{\text{oto}}$. This lack of a simple relationship with diet, approximated by $\delta^{13}\text{C}_{\text{oto-muscle}}$, is also observed at the individual level. Certain of the studied fishes present ontogenetic variations in $\delta^{13}\text{C}_{\text{muscle}}$ values with adult individuals exhibiting lower (whitefish) or higher (perch) values than juvenile individuals (Figure 3). However, when it exists, the ontogenetic change in $\delta^{13}\text{C}_{\text{oto}}$ values is always towards higher values and thus is in opposite direction to that of muscle for whitefish (Figure 3). Previous studies have explored the role of diet using $\delta^{13}\text{C}_{\text{oto-muscle}}$, as in the present study, or, more straightforwardly, using comparisons with dietary $\delta^{13}\text{C}$. Such comparisons avoid the issue of varying trophic enrichment between diet and muscle (Elsdon *et al.*, 2010) and the difference in turn-over between the two tissues. The lack of direct control by dietary $\delta^{13}\text{C}$ values of inter-specific variations in $\delta^{13}\text{C}_{\text{oto}}$ values was observed at the seasonal scale for Lake Annecy whitefish based on a comparison with

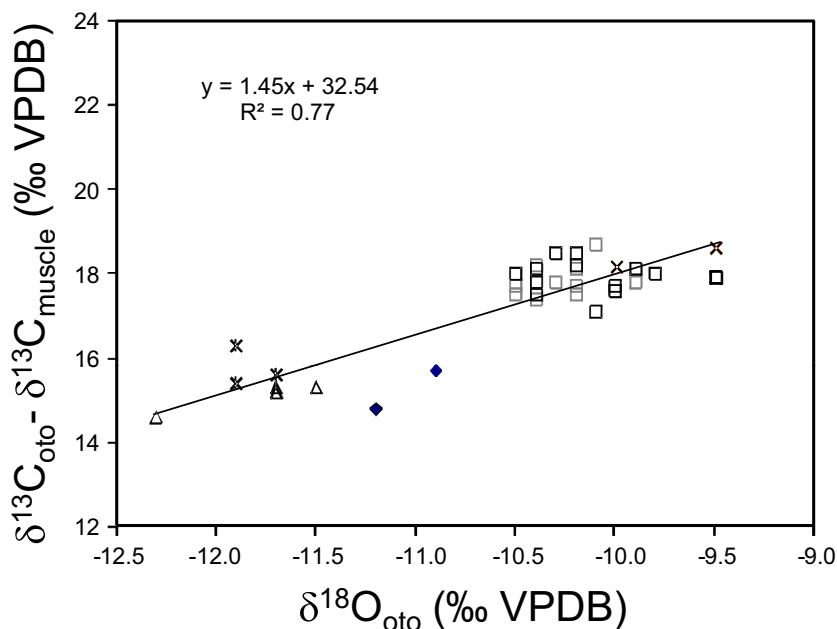


Figure 6

Relation between the $\delta^{13}\text{C}_{\text{oto}} - \delta^{13}\text{C}_{\text{muscle}}$ values and otolith $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{oto}}$ ‰, VPDB) values of six fish species (pike \blacklozenge , perch \blacktriangle , whitefish form “gangfelchen” \square , whitefish form “blaufelchen” \square , burbot \times , pikeperch $*$) of Lake Constance community.

the $\delta^{13}\text{C}$ values of zooplankton monitored over several years (Dufour *et al.*, 2007). In contrast, in an experimental study of *Fundulus heretoclitus* fed on five isotopically distinct diets, Elsdon *et al.* (2010) found a strong correlation of bulk $\delta^{13}\text{C}_{\text{oto}}$ with both diet and muscle $\delta^{13}\text{C}$, and bulk $\delta^{13}\text{C}_{\text{oto}}$ reflected the same total change in $\delta^{13}\text{C}$ observed among the diet treatments. Even more intriguing are the results obtained by different studies of cod (*Gadus morhua*) that have produced contradictory conclusions. The direct influence of the dietary signal has been experimentally demonstrated by feeding two groups of individuals on isotopically different diets (Radtke *et al.*, 1996). A comparison of the $\delta^{13}\text{C}_{\text{muscle}}$ values of two cod stocks from the western and eastern Baltic Sea revealed that they feed on different organisms with different $\delta^{13}\text{C}$ values but that this dietary difference was not reflected in differences between the $\delta^{13}\text{C}_{\text{oto}}$ values (Deutsch and Berth, 2006). At the intra-individual level, the hypothesis of the control of cod ontogenetic $\delta^{13}\text{C}_{\text{oto}}$ by dietary shift suggested for wild fish (Jamieson *et al.*, 2004; Weidman and Miller, 2000), without constraints on ambient conditions and the composition of the diet, has not been supported by the analysis of reared fish fed on isotopically constant diets with ontogenetic and seasonal variation in $\delta^{13}\text{C}_{\text{oto}}$ values (Høie *et al.*, 2004).

The apparent discrepancy among the different results might be explained by variation in M at the intra-specific (seasonal, ontogenetic, population) or inter-specific levels. M is thought to be influenced by metabolic rate (Sherwood and Rose, 2003; Solomon *et al.*, 2006) and because temperature influences the metabolic rate of poikilothermic fish (Clarke and Johnston, 1999), M should increase with temperature. In the current study, a positive and significant linear relationship between $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ and $\delta^{18}\text{O}_{\text{oto}}$ values (Figure 4) as well as a negative and significant linear relationship between M and $\delta^{18}\text{O}_{\text{oto}}$ (Figure 5) were observed when all the studied specimens of the various species of the Lake Annecy community were pooled for analysis. $\delta^{18}\text{O}_{\text{oto}}$ values were used as thermal history descriptors in the current study, and the relationships found served to indicate a strong relationship between the temperatures experienced by the fish and both the $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ value and the proportion (M) of the metabolic carbon source incorporated into otolith aragonite. The $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ value was the greatest and the proportion of M the lowest for Arctic char living in the cold waters of the lake. The $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ value was the lowest and M the greatest for perch, tench and roach, which

usually occur in warm layers near the surface of the lake. The increase in the proportion of metabolic source into otoliths as a function of temperature is described by an inter-species relationship.

The more energetic is the fish, the more otolith carbon might be derived from a metabolic source (Sherwood and Rose, 2003). The increasing variation in M from Arctic char to perch and roach is consistent with the variation in respiratory rate among the different species available in the literature. Values of respiratory rate range from 40 mg O₂ kg⁻¹·h⁻¹ at 5 °C and 50 mg O₂ kg⁻¹·h⁻¹ at 10 °C for Arctic char (Holeton, 1973), approximately 75 mg O₂ kg⁻¹·h⁻¹ at 10 °C for whitefish (Madenjian *et al.*, 2006), 150 mg O₂ kg⁻¹·h⁻¹ at 20 °C for pike (Armstrong and Hawkins, 2008), 200 mg O₂ kg⁻¹·h⁻¹ for juvenile perch (Enders *et al.*, 2006), and 120 mg O₂ kg⁻¹·h⁻¹ at 23 °C for roach of 119 g (Hoelker, 2003). Thus, the fish-specific $\delta^{13}\text{C}_{\text{oto}}$ values depended on the metabolic rate in relation to the living temperature of species, not on the species themselves. Archived muscle and otolith data for Lake Constance fish community (whitefish, pike, burbot, perch, pikeperch *Sander lucioperca*) (Dufour *et al.*, 1998; Dufour, 1999) (Appendix 2) exhibit a linear relationship between $\Delta\delta^{13}\text{C}_{\text{oto-muscle}}$ and $\delta^{18}\text{O}_{\text{oto}}$ values (presented in appendix) similar to that of Lake Annecy that further supports our finding (Figure 6). Results of the present paper provide a rewarding starting point from which to conduct further studies on this generic relationship between the temperature of life of fish and the proportion of metabolic carbon incorporated in the otolith. To date, the existing data in the literature on both freshwater and marine fishes are too limited to allow these initial results to be compared.

CONCLUSION

The results of this study support the assertion that otolith SIA provides significant insights into the life history traits of fish in a lake community. Despite seasonal and spatial variations in $\delta^{18}\text{O}_{\text{water}}$ (and $\delta^{13}\text{C}_{\text{DIC}}$) values, $\delta^{18}\text{O}_{\text{oto}}$ values provide a consistent thermal history and vertical positioning of fish thermal habitats. The position of roach relative to perch raises the questions of competition between species, niche segregation and trade-offs in foraging efficiency that might result in divergent thermal habitats among populations. Moreover, perch $\delta^{18}\text{O}_{\text{oto}}$ (and $\delta^{13}\text{C}_{\text{oto}}$) values suggest two different habitat patterns, littoral *versus* more pelagic, during early life, highlighting the ecological plasticity of this species. These initial results encourage further studies of the thermal niche life history of this species, particularly in juveniles.

A linear relationship between the estimated metabolic carbon contribution to the otolith and the living temperature of fish, which includes all species of the studied community, was found. has also been found in Lake Constance. The hypothesis of a generic relationship provides a promising area of research. If this hypothesis is verified, it will allow the use of $\delta^{13}\text{C}_{\text{oto}}$ as a proxy element of the average trophic position of different species in a lake community as a complement to new methods developed for studies of the organic matrix of fish otoliths (Gronkjaer *et al.*, 2013; McMahon *et al.*, 2011, 2013).

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Appendix 1. Sampling date, size, weight, $\delta^{13}\text{C}$ values of otolith and muscle (‰, VPDB) and otolith $\delta^{18}\text{O}$ (‰, VPDB) of the fish studied in Lake Annecy.

Species	Sampling date	Total length (mm)	Weight (g)	$\delta^{13}\text{C}$ muscle	$\delta^{13}\text{C}$ otolith	$\delta^{18}\text{O}$ otolith
arctic char	23/01/2006	134	24.2	-35.2	-13.4	-6.7
arctic char	23/01/2006	134	25.2	-33.9	-12.1	-6.4
arctic char	23/01/2006	135	27.3	-34.2	-12.7	-6.6
arctic char	23/01/2006	137	25.4	-34.2	-13.3	-6.7
arctic char	18/01/2006	140	26.5	-33.5	-13.8	-6.5
arctic char	01/09/2005	250	154	-33.9	-12.4	-6.2
arctic char	01/09/2005	254	140	-34.1	-12.3	-6.4
arctic char	01/09/2005	255	152	-34.3	-12.4	-6.5
arctic char	01/09/2005	275	202	-34.4	-12.5	-6.6
arctic char	01/09/2005	275	192	-34.8	-12.7	-6.5
arctic char	01/09/2005	300	282	-34.9	-12.1	-6.5
perch	17/01/2006	99	10.2	-30.9	-13.6	-9.0
perch	17/01/2006	100	10.4	-31.0	-14.2	-9.2
perch	17/01/2006	107	11.5	-31.3	-13.6	-9.0
perch	17/01/2006	109	12.7	-31.3	-14.0	-9.1
perch	17/01/2006	112	14.2	-30.9	-14.0	-9.1
perch	31/08/2005	245	206	-28.6	-12.6	-9.0
perch	31/08/2005	260	188	-29.0	-13.6	-9.5
perch	31/08/2005	265	276	-29.6	-12.0	-9.1
perch	31/08/2005	280	288	-29.5	-13.4	-9.6
perch	31/08/2005	305	374	-27.1	-10.8	-9.2
perch	20/02/2006	310	438	-29.0	-12.4	-9.3
pike	01/09/2005	470	668	-30.0	-12.9	-8.5
pike	20/02/2006	480	892	-29.1	-11.8	-8.5
pike	20/02/2006	500	954	-29.6	-12.3	-8.6
pike	20/02/2006	500	958	-28.8	-11.9	-8.6
pike	20/02/2006	540	1214	-29.2	-11.4	-8.2
pike	20/02/2006	550	1240	-29.3	-11.6	-8.3
roach	18/01/2006	95	8.1	-30.9	-14.9	-9.1
roach	18/01/2006	96	8.5	-29.1	-13.5	-8.9
roach	18/01/2006	100	8.9	-29.1	-13.3	-8.8
roach	18/01/2006	104	10.7	-30.3	-13.7	-9.0
roach	18/01/2006	107	11.8	-30.4	-15.0	-9.1
roach	18/01/2006	114	12.1	-31.1	-14.0	-9.1
roach	18/01/2006	156	34.7	-27.5	-12.7	-8.4
roach	18/01/2006	167	45.8	-30.9	-12.9	-8.4
roach	18/01/2006	167	52.7	-28.8	-11.2	-8.3
roach	18/01/2006	175	54	-27.3	-12.6	-8.5
tench	12/07/2006	460	1637	-27.8	-13.3	-8.7
tench	12/07/2006	475	1719	-27.6	-14.6	-8.9
tench	12/07/2006	475	2023	-28.0	-13.0	-8.8
tench	12/07/2006	485	2049	-29.1	-12.9	-8.9
whitefish	31/08/2005	102	7.7	-32.3	-14.8	-8.0
whitefish	31/08/2005	110	10.7	-29.5	-12.8	-8.6
whitefish	31/08/2005	111	10.4	-32.5	-13.5	-7.6
whitefish	31/08/2005	113	9.9	-33.0	-13.9	-8.2
whitefish	01/09/2005	115	10.1	-29.8	-13.8	-8.8
whitefish	31/08/2005	405	620	-32.8	-12.3	-7.4
whitefish	31/08/2005	415	690	-33.3	-12.2	-7.3
whitefish	31/08/2005	430	684	-32.5	-12.6	-7.5

Appendix 2. Size, weight, $\delta^{13}\text{C}$ values of otolith and muscle (‰, VPDB) and otolith $\delta^{18}\text{O}$ (‰, VPDB) of 5 fish species of Lake Constance community (Dufour et al. 1998; Dufour, 1999). Samples were collected in 1995–1996. Muscle and otolith preparation and isotopic analysis were performed as described in Dufour et al. (1998) and Dufour et al. (2000).

Species	Total length (mm)	Weight (g)	$\delta^{13}\text{C}$ muscle	$\delta^{13}\text{C}$ otolith	$\delta^{18}\text{O}$ otolith
whitefish blaufelchen	395	613	-29.6	-11.8	-10.3
whitefish blaufelchen	345	459	-29.6	-12.2	-10.4
whitefish blaufelchen	**	**	-29.8	-12.1	-10.5
whitefish blaufelchen	350	473	-29.9	-11.2	-10.1
whitefish blaufelchen	350	473	-30.1	-11.9	-10.4
whitefish blaufelchen	340	444	-29.4	-11.3	-10.2
whitefish blaufelchen	305	352	-29.4	-11.9	-10.5
whitefish blaufelchen	340	444	-29.5	-11.8	-10.2
whitefish blaufelchen	330	417	-29.5	-12.0	-10.2
whitefish blaufelchen	305	352	-29.3	-11.7	-10.0
whitefish blaufelchen	295	328	-29.7	-11.9	-9.9
whitefish gangfelchen	310	365	-29.7	-11.2	-10.2
whitefish gangfelchen	300	340	-29.6	-12.1	-10.4
whitefish gangfelchen	320	390	-29.9	-11.4	-10.3
whitefish gangfelchen	290	316	-29.9	-12.1	-10.4
whitefish gangfelchen	355	487	-29.5	-11.3	-10.2
whitefish gangfelchen	305	352	-30.0	-11.9	-10.4
whitefish gangfelchen	295	328	-29.9	-11.8	-9.9
whitefish gangfelchen	325	404	-29.0	-11.9	-10.1
whitefish gangfelchen	345	459	-29.4	-11.7	-10.0
whitefish gangfelchen	335	431	-29.4	-11.4	-9.8
whitefish gangfelchen	360	502	-29.3	-11.7	-10.0
whitefish gangfelchen	320	390	-29.5	-11.5	-10.5
whitefish gangfelchen	320	390	-29.3	-11.4	-9.5
pike	590	1300	-25.9	-11.1	-11.2
pike	670	1300	-27.7	-12.0	-10.9
pikeperch	**	**	-27.5	-11.2	-11.9
pikeperch	**	**	-27.3	-11.9	-11.9
pikeperch	520	1194	-26.9	-11.3	-11.7
burbot	325	238	-30.9	-12.3	-9.5
burbot	340	250	-31.6	-13.5	-10.0
perch	165	48	-29.2	-13.9	-11.5
perch	200	90	-28.7	-13.4	-11.7
perch	180	64	-28.5	-13.9	-12.3
perch	165	48	-28.9	-13.7	-11.7

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