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Isotopic half-life and enrichment factor in two species of European freshwater fish larvae: an experimental approach

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RATIONALE: Stable isotope ratios of carbon and nitrogen are valuable tools for field ecologists to use to analyse animal diets. However, the application of these tools requires knowledge of the tissue enrichment factor (TEF) and half-life (HL). We experimentally compared TEF and HL in two freshwater fish larvae. We hypothesised that chub had a better growth/tissue replacement ratio than roach, due to the use of a food closer to their natural diet.
METHODS: We determined the isotopic HL, the TEF and the contribution of growth or metabolic tissue replacement to dynamic isotopic incorporation. After yolk sac resorption, larvae were fed for 5 weeks with prey similar to their natural diet (*Artemia nauplii*) up to the isotopic equilibrium followed by Chironomid larvae. Stable isotope measurements were carried out using a continuous flow isotope ratio mass spectrometer coupled to an elemental analyser.
RESULTS: Changes in isotopic composition strongly followed the predictions of exponential growth and time-dependent models. The isotopic HL varied between 8.2 and 12.6 days and the TEF of nitrogen and carbon ranged from 1.7 to 3.1 ‰ and from –0.9 to 1.2 ‰, respectively. The incorporation of dietary ^{13}C was due more to the production of new tissue (between 56 and 79%) than to the metabolic process. Chub allocated more energy to growth than roach and the Chironomidae diet contributed more to the consumers' growth than the *Artemia* diet.
CONCLUSIONS: Metabolic rates seemed lower for chub than for roach, especially when they were fed with Chironomidae. A Chironomidae-based diet would be more profitable to chub, and the high associated growth rate could increase the development of the fish larvae. The HL and TEF were in the range of those reported in the literature. These results will be helpful for field-based studies, because they can help to increase the accuracy of models.

In the last few decades, stable isotope ratios of light biogenic elements have become valuable tools for studying trophic relationships and food web structure,[1,2] and also for estimating the energy content in young-of-the-year fish (or larvae).[3] The carbon stable isotope ratio (^{13}C/^{12}C, usually expressed as the δ^{13}C value) exhibits a weak increase per trophic level and is generally used to track the origin of the carbon source in die`tary reconstruction.[4] In contrast, the ^{15}N/^{14}N ratio (expressed as the δ^{15}N value) of consumers' tissues is often enriched in ^{15}N relative to their diets, thereby revealing both an animal's diet and its trophic level.[5] Combining the δ^{13}C and δ^{15}N values provides quantitative information on resource and habitat, which together define the ecological niche space[6] of species or communities. However, application of stable isotopes in dietary analyses requires knowledge of two major factors, the tissue enrichment factor (TEF, also called the discrimination factor) and the isotopic half-life (HL).[7] These two factors can vary significantly in relation to studied ecosystems and species as well as to individual life stage, contributing to error in quantitative mixing model outputs.[8]

The TEF (also denoted as Δ) corresponds to the difference between the stable isotope composition of a consumer and that of its diet, due to isotopic fractionation during metabolic processes.[9] Observed fractionation can fluctuate notably according to the type of tissue analysed, the presence of some classes of compounds[10,11] (e.g., lipids and lignin), and the diet or dietary protein content.[8] Many authors[4,7,12,13] have measured TEFs, and it is usually accepted that the difference between prey and consumer ranges from 1 to 4 ‰ for δ^{15}N values and from 0 to 1 ‰ for δ^{13}C values. Δ^{15}N differs for many groups of organisms and the variance of the estimated enrichment factor must be taken into account in trophic relationship studies.[7] Furthermore, the TEF value is required to build mixing models, which are used by stable isotope ecologists to estimate the contribution of multiple sources to the diet of a consumer.[14]

The isotopic HL is defined as the time that it takes for the isotopic composition of a given consumer tissue to reach an intermediate point corresponding to a mid-value between

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the original diet and the new one. The HL can change according to metabolic type (ectotherm versus endotherm), life stage of the consumer, and tissues or taxa analysed.[15,16] The HL is also different in growing organisms;[17] the more rapid the fish growth, the higher the HL. In the same way, other authors have evaluated the HL to be about 2 days in fish larvae and 1 year in adults.[17,18]

To better exploit and validate field data, ecologists have to determine the TEF and HL corresponding to the studied species, in order to estimate the timing of diet shift,[14] obtain information on the life history of the target species,[19] evaluate time and degree of diet specialisation,[20] and increase the accuracy and realism of mixing models. To meet the requirements of field ecologists, laboratory studies have examined the isotope HL and TEF, and developed models[14] adapted from an exponential growth model.[15] Hesslein *et al.*[21] observed that isotopic incorporation depends on the addition of tissue resulting from growth ('anabolism turnover') and tissue renewal ('catabolism turnover'). Energy from food was allocated in the metabolic process and growth, with a ratio which varied according to the development stage,[22] the type of food, and the environment (notably temperature, which controls biochemical reaction rate in poikilotherm species[23]). A study on the warm-water adult zebra fish (*Danio rerio*) demonstrated that metabolic tissue replacement accounts for 68 to 80% of the modification of the isotopic composition following a dietary shift.[24] However, the contribution of growth could account for more than 90% for the cold-water red drum larvae (*Sciaenops ocellatus*).[16]

Ten years after the call for more laboratory experiments by Gannes *et al.*,[25] a large number of measurements of incorporation rate have been made. However, the dynamics of isotopic incorporation have mostly been measured on one-compartment models,[20] without dissociating tissue growth and catabolic turnover. Furthermore, the number of experimental studies remains rather low compared with observational field studies.[18]

In this study we have selected two cyprinid larvae (young-of-the-year): chub (*Squalius cephalus*) and roach (*Rutilus rutilus*), both common freshwater species widely distributed in western Europe.[26,27] Throughout their larval stage, chub and roach mainly feed on zooplankton. However, later in life their diets diverge, with chub starting to feed on benthic invertebrates and roach becoming omnivorous.[28] Nevertheless, food acquisition and assimilation are important for both fish for growth and survival, particularly during early stages when individuals are highly vulnerable to competition, food shortage and other perturbations.[29,30]

The aim of this paper is to describe a controlled diet-shift experiment conducted on the larvae of *S. cephalus* and *R. rutilus* with two kinds of prey that are close to their natural diets. First, we measured the stable nitrogen and carbon isotope HL and TEF of the fish larvae using an exponential time-dependent model.[31] Secondly, using an exponential growth and time-dependent model,[21] we studied the contribution of metabolic tissue replacement or growth to the variations in isotopic composition of young-of-the-year larvae resulting from the diet shifts. We hypothesised that, during the second diet, chub would have a higher 'growth/tissue replacement' ratio than roach, due to the use of a food more in line with their natural diet.

EXPERIMENTAL

Experimental design

Our investigations were conducted in accordance with the guidelines for animal use and care and in compliance with Belgian and European regulations on animal welfare. Adult roach (*n* = 20) and European chub (*n* = 8) were sampled in the Meuse River at the fish pass of La Plante dam (Namur province, Belgium, 50°27′01.9″N; 4°51′40.6″E) during the spawning period (23–25 April 2015). Roach and European chub gametes were collected by stripping and ova were fertilised with the sperm of multiple males. The eggs were incubated at 12°C under a 12L:12D photoperiod, under static conditions with filtered and UV-sterilised water from the Meuse River. The water was replaced daily. Eggs and yolk sac larvae were collected for isotopic analysis. After yolk sac resorption, approximately 1000 larvae of each species were distributed between six tanks (110 L) at a constant temperature (15°C) and under a constant photoperiod (12L:12D). The water quality was kept stable by a filtration and cooling system, while detritus and uneaten food were siphoned daily. The initial $\delta^{13}C$ and $\delta^{15}N$ values of larvae before feeding were determined by sampling three larvae in each tank (t_0). Over 5 weeks (t_0 to t_5), the larvae were fed daily, in excess of five times a day, with freshly hatched *Artemia salina nauplii* ($\delta^{13}C = -22.7 \pm 0.1$ ‰ and $\delta^{15}N = 11.2 \pm 0.1$ ‰). From t_5 to t_{10} (5 weeks) a diet shift was performed, and the larvae were fed daily and slightly in excess with coarsely chopped frozen Chironomid larvae ($\delta^{13}C = -18.1 \pm 0.6$ ‰ and $\delta^{15}N = 8.6 \pm 0.8$ ‰). We selected these prey because they are close to the diet of cyprinid fish in their natural environment and are isotopically distinct from *Artemia nauplii*. During the dietary shift experiments, three larvae per tank (*n* = 18 per species and per sampling date) were randomly selected each week for isotopic analysis, after 24 h of food privation for gut clearance. Stable isotope measurements were performed on whole fish. One prey sample was collected daily (*n* = 77).

Fish larvae were anesthetised and killed using an excess of 2-phenoxyethanol (3 mL/10 L freshwater), measured (total length (TL) ± 1 mm), weighed with a microbalance (±0.1 mg), and rinsed with deionised water. Larvae (chub and roach) and diet samples (*Artemia nauplii* and Chironomidae) were dried at 60°C for at least 48 h and ground into a fine homogenous powder using a mortar and pestle. Due to their small mass, larvae were pooled at t_0 and t_1, and treated individually from t_2 to t_{10}.

Stable isotope ratio measurements were performed via continuous flow elemental analyser/isotope ratio mass spectrometry (CF-EA/IRMS) using a vario MICRO cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to an IsoPrime100 mass spectrometer (Isoprime, Cheadle, UK). Isotopic ratios were expressed using the international δ notation.[32] Sucrose (IAEA-C6, $\delta^{13}C = -10.8 \pm 0.5$ ‰, mean ± SD) and ammonium sulphate (IAEA-N2, $\delta^{15}N = 20.3 \pm 0.2$ ‰, mean ± SD) were used as certified reference materials. Both these reference materials were calibrated against the international isotopic references, i.e. Vienna Pee Dee Belemnite (VPBD) for carbon and atmospheric air for nitrogen. Standard deviations on multi-batch replicated measurements of lab standards (fish tissues), analysed interspersed among the samples (two lab standards for 15 samples), were 0.1 ‰ for both δ^{13}C and δ^{15}N values. Glycine (Merck, Darmstadt, Germany) was used as an elemental standard and the elemental contents were expressed as a percentage of dry mass.

Turnover times and fractionation factor estimations

First, to estimate change in isotopic ratio following the diet shifts, HLs were calculated with the time-dependent model adapted from Hobson and Clark:[31]

$$\delta X_{(t)} = \delta X_{(\infty)} - \left[\delta X_{(0)} - \delta X_{(\infty)}\right] e^{-\lambda t} \qquad (1)$$

where $\delta X_{(t)}$ is the isotopic ratio of C or N in the consumer tissue at time t, $\delta X_{(\infty)}$ is the asymptotic isotopic ratio (‰), $\delta X_{(0)}$ is the isotopic ratio before diet shift (‰), and λ is the turnover rate (time^{-1}).

Table 1. Mean total length (mm) and mean growth (g) of chub and roach larvae during the dietary shift experiment

	Time	Total length (mm)		Growth (g)	
	(weeks)	Mean	SE	Mean	SE
Chub	0	7.9	±0.3	0.4	±0.02
	5	14.9	±0.2	2.8	±0.6
	10	25.0	±0.6	14.4	±4.7
Roach	0	6.2	±0.4	0.1	±0.01
	5	14.8	±0.3	2.1	±0.1
	10	23.2	±0.8	9.6	±1.0

Secondly, based on the growth and time-dependent model adapted from Fry and Arnold,[15] we evaluated the contribution of growth (k) and metabolism (m) to the ^{13}C isotopic turnover following the diet shifts:

$$\delta^{13}C_{(t)} = \delta^{13}C_{(\infty)} - \left[\delta^{13}C_{(0)} - \delta^{13}C_{(\infty)}\ w_{(0)}/w_{(t)}\right] C^{t} \qquad (2)$$

where $w_{(0)}$ is the body mass (g) before the diet shift and $w_{(t)}$ is the body mass at time t. C represents the metabolic turnover to change in the isotope ratio δ^{13}C.

As young-of-the-year fish grow exponentially, we can assess the growth rate (k_g, expressed in day^{-1}) and the metabolic contribution (k_c) to isotopic turnover following the equation proposed by Hesslein *et al.*:[21]

$$k_g = \ln\left[w_{(t)}/w_{(0)}\right]/t \qquad (3)$$

where t is the time (days) between the two measurements.

$$k_c = \lambda - k_g \qquad (4)$$

Finally, according to Martínez del Rio and Anderson-Sprecher,[33] we used a nonlinear least-squares fitting procedure[34] and isotopic HL to estimate the parameters of Eqns. (1), (2) and (3) using the following equations:

$$HL = \ln(2)/\left(k_c + k_g\right) \qquad (5)$$

Tissue fractionation factors were calculated for the two diet shifts (*Artemia* sp. or Chironomidae) according to the following formula:

$$TEF = \delta X_{(\infty)\ consumer} - \delta X_{diet} \qquad (6)$$

where δX_{diet} is the mean diet isotopic composition.

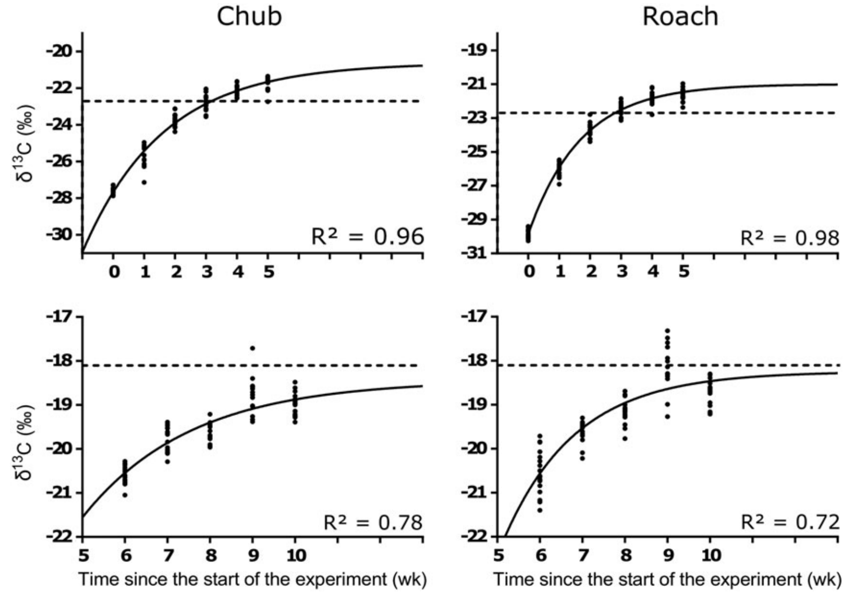

Figure 1. Carbon isotope ratios (mean ± SE) of chub (left) and roach (right) larvae, relative to laboratory experiment time (weeks), during the first *Artemia* diet (top) and the second Chironomidae diet (bottom). The curve (solid line) represents the best-of-fit nonlinear regressions using Hobson and Clark's[31] model (n = 114), and the dashed line represents the mean diet isotopic composition (n = 39).

RESULTS

The isotopic composition of both species displayed a strong temporal pattern during the dietary shift under controlled laboratory conditions. Chub and roach larvae grew by 2.4 g and 2.0 g, respectively, in the 5 weeks before the dietary shift, and by 11.6 g and 7.5 g in the 5 weeks after (Table 1).

The time-dependent model[31] performed adequately with our data, as the goodness-of-fits were between 0.78 and 0.98 (Figs. 1 and 2), except for the $\delta^{15}N$ value for the first feeding period (Fig. 2). The extrapolated equilibrium curves were close to the mean larval isotope values at t_5 or t_{10}, suggesting that larvae performed a complete turnover at the end of each diet experiment.

The carbon isotopic HLs were relatively brief, ranging between 8.2 and 12.7 days (Table 2), and showed a relative homogeneity between diet and species. The nitrogen isotopic HLs were also found in this range for the second diet (10.6 and 12.4 days for roach and chub, respectively). However, the nitrogen HLs calculated for the first diet were certainly overestimated, with values of 27.6 days for roach and 72.7 days for chub. Moreover, the model poorly described the temporal evolution of $\delta^{15}N$ values in larvae fed with *Artemia* sp. in both species (R^2 = 0.02 for roach and 0.08 for chub).

Ratios between the contribution of metabolism and growth (k_c/k_g) for $\delta^{13}C$ were smaller for the Chironomidae diet than for the *Artemia* diet (Fig. 3) and roach had a higher ratio than chub. The chub k_g accounted for 61% during the first diet and 79% during the second diet of the $\delta^{13}C$ incorporation, while the roach k_g were slightly lower (56 and 71%, respectively).

The isotopic composition of the *Artemia* diet proved to be relatively constant, because variability on this parameter was comparable to our instrument's analytical precision (SE = 0.1 ‰ for carbon and nitrogen). The variation in isotopic composition in Chironomidae was higher but still moderate (SE = 0.6 and 0.8 ‰, respectively, for carbon and nitrogen). These differences in variability between the two food sources did not affect the quality of the model predictions, with a high R^2 during each period of the experiment.

The TEFs were relatively homogenous between diet and species (Table 2). The $\Delta^{15}N$ were between 2.1 and 2.3 ‰ for the *Artemia* diet and between 1.9 and 2.4 ‰ for the Chironomidae diet. The $\Delta^{13}C$ varied widely, and were positive for the *Artemia* diet (2.1 ‰ for chub and 1.7 ‰ for roach) and negative for the Chironomidae diet (–0.3 ‰ for chub and –0.1 ‰ for roach).

DISCUSSION

Due to an exponential growth, ectotherm young-of-the-year chub and roach exhibited isotopic incorporation patterns that followed theoretical models.[9] As observed in ectotherm larvae, the measured isotopic HLs were small and isotopic incorporation was mainly due to growth.[16,35] Variation in fish isotopic composition during the experiment strongly matched with the exponential time-dependent model,[31] and goodness-of-fits were higher than 0.72 except for the nitrogen composition during the first feeding period (Table 2). The model poorly described this part of the experiment because the $\delta^{15}N$ values of both consumers remained unchanged from t_0 to t_5 (Fig. 2). This can probably

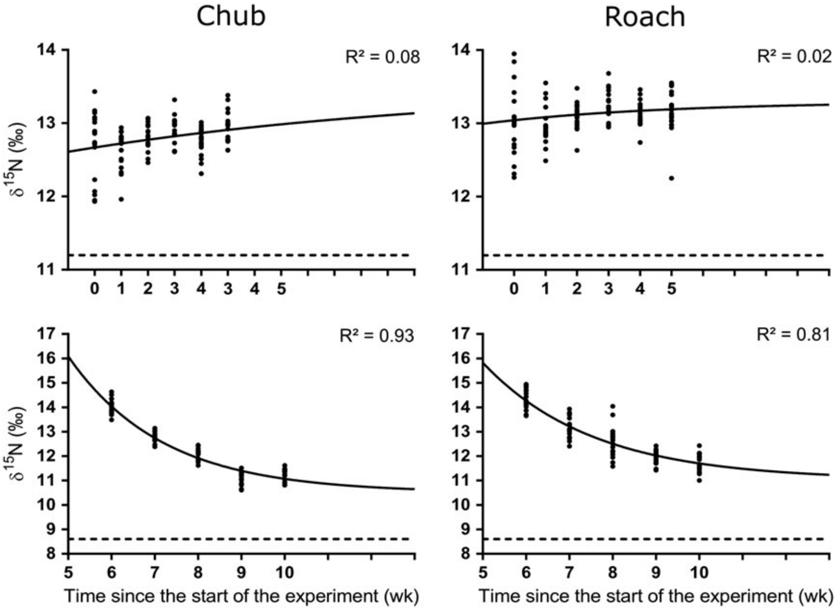

Figure 2. Nitrogen isotopic values (mean ± SE) of chub (left) and roach (right) larvae relative to lab experiment time (weeks), during the first *Artemia* diet (top) and the second Chironomidae diet (bottom). The curve (solid line) represents the best-of-fit nonlinear regressions using Hobson and Clark's[31] model (n = 114), and the dashed line represents the mean diet isotopic composition (n = 39).

Table 2. Half-life (HL), asymptotic isotopic ratio ($\delta X_{(\infty)}$) and tissue enrichment factor (TEF) estimations, calculated using nonlinear regressions of $\delta^{13}C$ and $\delta^{15}N$ values (time-dependent model adapted from Hobson and Clark[31]) following diet shift from *Artemia* sp. to Chironomidae

		First diet (Artemia sp.)					Second diet (chironomidae)				
				$\delta X_{(\infty)}$ (‰)					$\delta X_{(\infty)}$ (‰)		
Element	Species	R^2	HL (days)	Mean	SE	TEF (‰)	R^2	HL (days)	Mean	SE	TEF (‰)
$\delta^{13}C$	Chub	0.96	12.7	−20.6	± 0.03	2.1	0.78	12.4	−18.4	± 0.05	−0.3
	Roach	0.98	8.2	−21	± 0.02	1.7	0.72	8.2	−18.2	± 0.04	−0.1
$\delta^{15}N$	Chub	0.08	72.7	13.5	± 0.8	2.3	0.93	10.6	10.5	± 0.03	1.9
	Roach	0.02	27.6	13.3	± 0.14	2.1	0.80	12.4	11	± 0.06	2.4

be explained by the low variations between initial and final diets. Differences in isotopic value between prey and consumers at t_0 were roughly equivalent to the fractionation factor, making any attempt at calculating turnover times irrelevant.

Difference in TEFs between diets

Although TEFs are commonly used by scientists for quantifying the contribution of alimentary sources to the diet of consumers, they vary greatly according to the considered element and sample tissue, as well as the physiology of the studied species.[7] A meta-analysis by Le Vay and Gamboa-Delgado[36] reported a considerable variability in aquatic larvae and post-larvae $\Delta^{13}C$ and $\Delta^{15}N$ observed under laboratory conditions: from 0.4 to 4.1 ‰ for carbon and from 0.1 to 5.3 ‰ for nitrogen. The TEFs gathered in this meta-analysis come from various organisms, mainly crustacean larvae. Only one was measured in post-larval marine fish fed with natural or artificial food. These large ranges of observed TEFs nevertheless highlight the requirement of laboratory experiments to measure representative TEFs that can be efficiently applied to environmental or ontogenetic studies.

One review[37] proposed a global evaluation of adult fish isotopic TEFs around 1.7 ‰ for $\delta^{13}C$ values and 2.5 ‰ for $\delta^{15}N$ values; the estimations were mostly in line with our results for $\Delta^{15}N$ (between 1.9 and 2.4 ‰), as well as for $\Delta^{13}C$ during the first diet (between 1.7 and 2.1 ‰). However, for the period of the second diet, prey had a lower $\delta^{13}C$ value than consumers (–0.3 to –0.1 ‰). Less common than positive

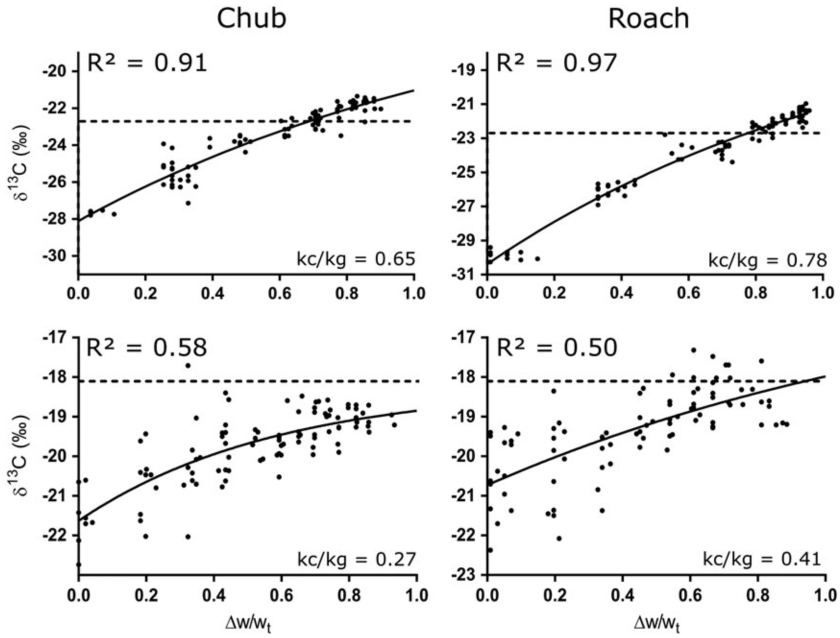

Figure 3. Carbon isotope ratios (mean ± SE) of chub (left) and roach (right) larvae relative to relative mass change ($\Delta w/w_t$), during the first *Artemia* diet (top) and the second Chironomidae diet (bottom). The curvature of the solid line (n = 114) decreases if the contribution of the growth (k_g) to isotopic incorporation increases. The dashed line represents the mean diet isotopic composition (n = 39).

values, negative $\Delta^{13}C$ were reported by McCutchan *et al.*,[9] with a minimum observed value of –2.7‰ (buckeye pupae fed with Plantago). As lipids are more ^{13}C-depleted than proteins, a negative TEF could be linked with the lipid content in the analysed tissue if consumer tissues were richer in lipid than their diet, although this was not the case in our experiment. Another explanation could be differences in the amino acid concentrations between the prey and the consumer, which influence the TEF;[20] however, this mechanism has been poorly studied.[38]

Differences in TEF between consumers were small, with a maximum close to 0.5 ‰, possibly due to the common diet, common requirements (i.e. larval growth phase) and their taxonomic proximity. TEFs observed between consumers were higher in the literature for the same type of diet; e.g., the $\Delta^{15}N$ of marine crustaceans fed with *Artemia* varied between 0.1 and 2.5 ‰.[36] For both consumers, we also noticed that the variation in $\Delta^{13}C$ was higher than in $\Delta^{15}N$ in this study (contrary to the literature[36]) and depended mainly on the type of diet (Table 2): $\Delta^{13}C$ differences were 1.8 to 2.4 ‰ greater for *Artemia* sp. than for the Chironomidae diet. This variation was smaller for $\Delta^{15}N$, between 0.2 and 0.4 ‰. In the literature it has been reported that $\Delta^{15}N$ and $\Delta^{13}C$ measured for the same taxa can vary with diet type from 0.8 to 6.6 ‰ and 0.4 to 7.0 ‰, respectively.[9,39]

Difference in isotopic incorporation rate

With the exception of the $\delta^{15}N$ HL during the first feeding period, isotopic incorporations fluctuated between 8 and 13 days according to species and diet. These results were close to those reported in similar studies.[16,17] For example, Bosley *et al.*[17] determined HLs in muscle tissue for three marine fish larvae species of between 1 to 17 days for carbon and 2 to 19 days for nitrogen.

In a literature review, Vander Zanden *et al.*[39] proposed an HL evaluation method for ectotherm vertebrates based on allometric relationships between organism weight and isotopic incorporation. With our data, the isotopic HL estimations were between 36 and 39 days for the first diet, and 51 and 57 days for the second diet (respectively, for roach and chub). These values are longer than those estimated in our study. The difference may be due to the temperature at which the larvae in our study were reared and hence to high growth rates, which is not taken into account by the model. Many authors[17,40] have highlighted a significant effect of temperature on isotopic HLs, notably in fast growth organisms such as larvae. Furthermore, growth rate influences isotopic HL, since a lower growth rate increases the isotopic HL while rapidly growing species have a short HL due to a high metabolic rate.[15] Although growth rate varies widely according to developmental stage, this parameter was not taken into account by the models of Vander Zanden *et al.*[39] In our study, young-of-the-year fish allocated a large amount of energy to growth (k_c) and tissue production rather than metabolic turnover (k_g) (Fig. 3). Similarly, Zuanon *et al.*[41] estimated a $\delta^{13}C$ HL for Nile tilapia (*Oreochromis niloticus*), a large freshwater fish, of around 52.5 to 66.5 days for larvae, and 120 days for juveniles. The turnover of adult whitefish could be longer than 1 year,[21] reflecting the reduction in growth and slowing of metabolism compared with younger individuals.

Contribution of growth to incorporation of dietary ^{13}C in consumer tissues

The contribution of growth (between 56 and 79%) to ^{13}C incorporation was comparable with what is reported in the literature. A similar study on cold-water red drum larvae (*Sciaenops ocellatus*)[16] evaluated the growth contribution at 90%, but this trend could be inverted for larger adult fishes such as shark.[38]

Chub allocated more energy to growth than roach, which had a higher metabolic rate (Fig. 3). Chub tissue production accounted for 61% in the $\delta^{13}C$ incorporation during the first part of the experiment and for 79% after the diet shift, while the roach values were slightly lower (56 and 71%, respectively). In both cases, the ratio was higher when organisms were fed using the second rather than the first diet. The Chironomidae diet facilitated assimilation and reduced metabolic processes for both studied species (Fig. 3). This diet benefited both fish larvae, although the Chironomidae diet was more in line with the natural food of chub, whose juvenile mainly consume macroinvertebrates, than that of roach, which are omnivorous.[42] In a natural environment, a Chironomidae-based diet would be more profitable to chub, and the high associated growth rate could increase the development of the fish larvae and their chances of survival.[43]

CONCLUSION AND CAVEATS

This experimental study evaluated the isotopic HL and TEF of two freshwater cyprinid larvae fed using two different regimes close to their natural diets. For both species, the HL was short and isotopic incorporation rates were mostly due to the prompt production of new tissue during the early stage of life. The carbon and nitrogen TEFs were in the range of those reported in the literature. While they differed slightly according to consumer species, due to analogous requirements (i.e. larval growth phase) and their taxonomic proximity, the $\Delta^{13}C$ observed were very different between the two experimental diets. The metabolic rate seems lower for chub than for roach, especially when they were fed with Chironomidae, which can benefit chub in its natural environment. Based on these results, we can make some recommendations to open field researchers for increasing the accuracy and realism of their models. First, it is important to precisely determine the developmental stage of the consumers, because the HL of larvae is shorter than that of adults and the HL observed may not follow the modelled curves from the literature.[40] Secondly, open field researchers have to use a TEF that is estimated on the studied species and their potential food sources that can be found in the natural environment. In the same way, the HL has to be evaluated under experimental conditions close to the natural environment, e.g., for ectothermic species in which metabolic activities are directly linked with temperature.[17] Thirdly, laboratory measurements must be taken with caution; consumers are generally located in an ideal position in terms of food availability and environmental stability. In an open field, life conditions might be more difficult with less food available, which leads to a lower growth rate and slower HL than in the laboratory.

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