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Fish fins as non-lethal surrogates for muscle tissues in freshwater food web studies using stable isotopes

short title: 26 fin-muscle relationships for 14 European freshwater fish species

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Abstract:

RATIONALE: Dorsal white muscle is the standard tissue analysed in fish trophic studies using stable isotope analyses. However, sampling white muscle often implies the sacrifice of fish. Thus, we examined whether the non-lethal sampling of fin tissue can substitute muscle sampling in food web studies.

METHODS: Analysing muscle and fin $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of 466 European freshwater fish (14 species) with an Elemental Analyser coupled with an Isotope Ratio Mass Spectrometer (EA-IRMS), we compared the isotope values of the two tissues. Correlations between fin and muscle isotope ratios were examined for all fish together and specifically for 12 species. We further proposed four methods of assessing muscle from fin isotope ratios and estimated the errors made using these muscle surrogates.

RESULTS: Despite significant differences between isotope values of the two tissues, fin and muscle isotopic signals are strongly correlated. Muscle values, estimated with raw fin isotope ratios (1st method), induce an error of ca 1‰ for both isotopes. In comparison, specific (2nd method) or general (3rd method) correlations provide meaningful corrections of fin isotope ratios (errors < 0.6‰). On the other hand, relationships, established for Australian tropical fish, only give poor muscle estimates (errors > 0.8‰).

CONCLUSIONS: Consequently, there is little chance that a global model can be created. However, the 2nd and 3rd methods of estimating muscle values from fin isotope ratios should induce an acceptable level of error for the studies of European freshwater food web. We thus recommend future studies use fin tissue as a non-lethal surrogate for muscle.

Key words: European freshwater fish, fin clip, trophic relationship, $\delta^{15}\text{N}$ value, $\delta^{13}\text{C}$ value.

Introduction:

Stable isotope analyses (SIA) of nitrogen (N) and carbon (C) are now commonly used in fish feeding ecology. Since the recommendations of Pinnegar and Polunin,^[1] dorsal white muscle is the standard tissue used in fish trophic study. However, the sample mass required for SIA (ca. 0.5 mg of dried mass for one analysis) often corresponds to an appreciable amount of the whole body mass. Thus, sampling white muscle generally implies fish sacrificing, apart from larger fish species for which muscle biopsy is conceivable.^[2] Obviously, this destructive sampling should be discarded to avoid adverse effect on the conservation status of any fish species, especially of threatened ones. In addition, there are epistemological reasons, to limit the impact of sampling on the population under study.^[3] For example, in the case of temporal monitoring, it could be necessary to avoid fish mortality to limit experimental bias particularly for small populations and ecosystems.

To our knowledge, the four potential non-lethal sampling methods for fish consist in sampling blood,^[4-6] mucus,^[7] scales,^[8-11] or fins.^[3, 5, 8] Given that turnover rates in blood and mucus are generally faster than in muscle,^[5-7, 12] they do not seem to be the ideal tissue to serve as a proxy for white muscle. Scales are also problematic, because some fish species^[13] do not have scale or only have a small number of scales, which prevents performing SIA. Currently, the use of fin tissue in fish trophic studies is increasing rapidly (e.g. ^[14, 15] for recent studies). Fins are known to regenerate completely within 1-2 months after sampling.^[16-18] However, researchers need to know how fin tissues compare in isotope values with white muscle, before substituting them.^[8] Because fin and muscle isotope ratios are generally congruent for some temperate^[2, 17, 19, 20] and tropical^[3] fish, Kelly et al.^[8] posit that similar fin/muscle relationships should exist for other fish species but recommend comparing the tissues before extrapolating.

To date, fin/muscle isotope values relationships for freshwater fish mainly concern North American species and specially focus on salmonids. A recent study^[3] developed such relationships for Australian tropical fish, but no fin/muscle relationships are available for European freshwater fish species, except for brown trout (*Salmo trutta*).^[21] To fill this gap, we develop N and C specific relationships for 12 European non-salmonid species. In addition, two general relationships for N and C are established mixing data from 14 European species. Finally, we propose to correct fin isotope values using these relationships or the models obtain for

Australian tropical fish^[3] and discuss how these four different methods of correction affect the error on isotope value assessment.

Material and Methods:

Sampling

Following Jardine et al.^[3], we favoured multiple sites sampling (Figure 1) to obtain a broad range of isotopic values associated with different source baselines. 361 samples of fish were collected between 2009 and 2010 at 21 sites of the Orge and Mauldre river basins (two tributaries of the Seine river, France). In addition, 45 fish individuals were sampled in 2010 at 10 sites in different tributaries of the Seine river belonging to the French RCS, a control- and monitoring network of ONEMA (www.onema.fr) for water quality assessment. We also included previous results from 5 sites of the Orge river basin, 5 sites from the Orgeval river basin (Marne River tributary, France) and 1 site from the Bez river basin (Drôme river tributary), sampled in 2007. Overall, 466 samples of fish from 14 species were collected.

For each species, individuals of similar size were preferentially selected (see Table 1), to avoid confounding effects of size on the relationships. All fish were killed by severing the spinal cord in accordance with recent ethical standards.^[22] All individuals were stored on ice and frozen at the laboratory the day of sampling. White muscle was dissected above the lateral line and was boned using a binocular. Fin tissues were removed from different fins, depending on species and the mass required for SIA (see Table 1) as there is no reason to suspect confounding effect on the results.^[3] All samples were rinsed with distilled water and kept frozen until further handling.

Stable Isotope Analysis

Tissues were freeze-dried (-80 °C, 24h), ground to powder and weighed precisely (300 ± 10 µg for muscle and 500 ± 10 µg for fin) in tin capsules. Samples were analysed in sequences of 96 samples, beginning with 3 empty tin capsules for blank correction, with a Thermo Electron FlashEA 1112 Series elemental analyser (single reactor setup) (Thermo Electron, Bremen, Germany) coupled with a Thermo Scientific DeltaV Plus Isotope Ratio Mass Spectrometer (EA-IRMS) (Thermo Scientific, Bremen, Germany). Following recommendations of Werner & Brand,^[23] three international reference materials (IAEA N1, N2 and CH-7, International Atomic

Energy Agency, Vienna, Austria) were analysed at the beginning and at the end of the sequence for linear normalisation. One internal reference (fish muscle) was analysed every six samples in order to compensate for possible machine drift and as a quality control measure. Linearity correction was carried out to account for differences in peak amplitudes between sample and reference gases (N₂ or CO₂). Resulting isotope ratios R (i.e., ¹⁵N / ¹⁴N and ¹³C / ¹²C) were expressed in conventional delta notation (δ), relative to the international standards, atmospheric air (R = 3678.2 ± 1.5 · 10⁻⁶ [23]) and Pee Dee Belemnite limestone (V-PDB R = 11180.2 ± 2.8 · 10⁻⁶ [23]). Assessed with the standard deviation of our internal reference (fish muscle), the analytical precision associated with our δ¹⁵N and δ¹³C sample runs over the whole SIA period (from Mai 2010 to November 2011) was estimated at 0.21 and 0.15 ‰, respectively. Lipid correction was not carried out, because C:N of both fin and muscle tissue were quite similar and <4, indicating low-lipid content^[24, 25] (Table 1).

Statistical analysis

Overall, δ¹⁵N and δ¹³C values of fin and muscle were available for 466 fish (14 species). First, because of data non-normality (Shapiro – Wilk test) we carried out two paired Wilcoxon tests, to determine whether fin and muscle isotope ratios were significantly different. Then, 50 fish (14 species, ca. 10% of the total of each species, see Table 1) were randomly chosen and kept for fin/muscle relationships validation. With the 416 remaining fish (12 species), we constructed 26 linear models between muscle and fin isotope ratios for both N and C isotopes, first separately for each species (specific models) and then for all fish combined (general models). Linear models were tested a priori because existing relationships for other species showed strong linear correlations.^[2, 3, 8] To investigate the robustness of our models, we compared the model residuals with the ones obtained with the validation dataset, using t-tests, after checking for normality (Shapiro-Wilk test) and for variance homogeneity (Bartlett test).

The suitability of the models, to calculate muscle isotope ratios from fin isotope ratios for both N and C, was assessed using the validation dataset. Four different methods were considered to obtain muscle isotope ratios from fin isotope ratios and the absolute differences between estimated and measured muscle isotope values were calculated for each fish individual. With the first method, muscle isotope values were

directly assessed by fin isotope values, and the corresponding differences constituted errors E (14 species are concerned). With the second method, fin isotope ratios were transformed into estimates of muscle isotope ratios, using the specific linear models and we called the corresponding differences errors ES (12 species). In the third method, absolute differences constituted errors EG (14 species), because muscle isotope values were evaluated from fin isotope values, using the general linear models. The last method used the models developed in Jardine et al.^[3] for all fish (see Table 2), to estimate muscle isotope values from fin ones. In this case, the absolute differences were called errors EJ. Then, the different errors (E, ES, EG and EJ) were compared with Kruskal-Wallis and pairwise Wilcoxon rank sum tests, after checking the data for non-normality (Shapiro – Wilk test). For each concerned species, we calculated the arithmetic mean of each error (E, ES, EG or EJ) and placed it in the context of analytical error to give some correction guidelines to future workers using fin as a proxy of dorsal white muscle.

Results and Discussion:

The differences between muscle and fin isotope ratios for N and C are highly significant (p -value < 0.001 for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values), in accordance with previous results obtained for other species.^[8, 17] However, the magnitude of these differences is higher than currently observed.^[2, 17, 26] In our case, fin tissue is depleted in ^{15}N (-0.8‰ on average), whereas it is enriched in ^{13}C (1.0‰ on average) relative to muscle tissue. This result confirms the need for a correction of fin $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ isotope ratios before replacing white muscle $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ isotope ratios.

Despite these differences, we find very strong correlations between muscle and fin isotope ratios (e.g. see Figure 2a and Figure 2b for the general models), as expected by Kelly et al.^[8]. Except for two species (*Blicca bjoerkna* and *Rhodeus amarus*), all specific linear models are highly significant (p -value < 0.001) with a very high R^2 -coefficient (> 0.9 for half the relationships, Table 2). Similar relationships have been observed for North American species,^[8, 26] salmonids^[2, 17] and recently for Australian tropical fish.^[3] However, we generally find stronger correlations than these studies, especially for $\delta^{15}\text{N}$ isotope ratios. Concerning *Blicca bjoerkna* and *Rhodeus amarus*, the number of sampling sites is reduced (Table 1), inducing a small range in isotopic values (between 2 ‰ for *Blicca bjoerkna* and 2.8 ‰ for *Rhodeus amarus*), which could explain why fin and muscle values are less well correlated. The general models

are also highly significant (p-value <0.001) and are in accordance with the general models of the study on Australian tropical fish^[3] (see Table 2). However, like the specific models, we found a stronger correlation for $\delta^{15}\text{N}$ isotope ratio (R^2 - coefficient of 0.92 instead of 0.56), while it remains similar for $\delta^{13}\text{C}$ isotope ratio (R^2 - coefficient of 0.85 instead of 0.91). Model residuals are not significantly different (p-value > 0.25 for both specific and general models) between calibration dataset (416 fish) and validation dataset (50 fish), revealing a good robustness of our models. Extreme values (fin- $\delta^{15}\text{N}$ values < 5 ‰ or fin- $\delta^{13}\text{C}$ values > -22 ‰) do not strongly change the general models. In the case of the nitrogen model, slope remains very close to one (0.98 instead of 1.01) and R^2 is still very strong (0.88 instead of 0.92) without extreme values. Similarly, slope and R^2 of the carbon model remain quite unchanged without extreme values (0.81 and 0.83 instead of 0.82 and 0.85, respectively for slope and R^2).

In accordance with previous studies,^[2, 8] slopes of the N-models are overall not significantly different from one (z-test, p-value > 0.05 for all models but for *Rhodeus amarus*), indicating that the observed differences between fin and muscle $\delta^{15}\text{N}$ isotope ratios are independent of the $\delta^{15}\text{N}$ value and are mainly due to a constant vertical shift in $\delta^{15}\text{N}$ value (see the general model in Figure 2a). Given that the range of isotopic values (ca. 15‰), covered by the selected fish, is typical of natural variation in stream food webs,^[3] we infer that the differences between the two tissues are independent of the fish trophic position (partly reflected by $\delta^{15}\text{N}$ values) and are more probably due to structural differences in terms of amino acids composition, as reported for other tissues.^[1] On the contrary, slopes of the C-models are significantly different from one for six specific models and for the general relationship (Figure 2b). For C, the range of isotopic values (ca 12‰) only covered half the typical natural variation in stream food webs,^[3] but within this range it seems that fin-muscle isotope ratio differences increase with $\delta^{13}\text{C}$ isotope ratio, suggesting a potential fractionation difference among the two tissues for more ^{13}C -enriched diet. Usually, the hypothesis of higher lipid content in muscle tissue relative to fin tissue is advanced to explain a ^{13}C -depletion in muscle tissue.^[1] However, this cannot apply here because C:N ratios of muscle and fin tissue suggest that lipid content are very similar in both tissue (see Table 1).

Taken together, the four errors (E, ES, EG and EJ) are significantly different (Kruskal-Wallis test, p-value <0.001, for both nitrogen and carbon). Besides, the

pairwise Wilcoxon test for nitrogen reveals that only one difference (between ES and EG) is not significant (p-value =0.234; for all other comparisons p-value <0.027). Concerning carbon, the pairwise Wilcoxon test also shows that the difference between ES and EG is not significant (p-value =0.596). In addition, two other comparisons (between EJ and EG and between EJ and E) are not significant (p-value =0.084 for both). On average, for all species, E (0.8‰ for $\delta^{15}\text{N}$ isotope ratio, 1.1‰ for $\delta^{13}\text{C}$ isotope ratio) is greater than ES (0.4‰ for $\delta^{15}\text{N}$ isotope ratio, 0.5‰ for $\delta^{13}\text{C}$ isotope ratio) and EG (0.6‰ for $\delta^{15}\text{N}$ isotope ratio, 0.6‰ for $\delta^{13}\text{C}$ isotope ratio). On the other hand, EJ is greater than E for $\delta^{15}\text{N}$ isotope ratio (1.4‰, on average) and smaller than E for $\delta^{13}\text{C}$ isotope ratio (0.8‰, on average). In addition, using corrected values generally reduced the maximum error (Figure 3a and 3b), except for the nitrogen correction proposed in Jardine et al.^[3]. Thus, either specific or general models provide relevant corrections, reducing significantly the errors made using fin instead of muscle isotope ratios. On the contrary, models developed for Australian tropical fish^[3] are not appropriate to provide a meaningful correction for our 14 European fish species. Corrections of fin $\delta^{15}\text{N}$ isotope ratio and $\delta^{13}\text{C}$ isotope ratio based on specific models generally give better estimates of muscle isotope ratios than general models. However, this improvement is not significant (cf. pairwise Wilcoxon tests, p-value >0.2). Moreover, general models offer the advantage to propose a general acceptable correction for all species, including potential species not considered for building the models. For instance, E and EG were also calculated for two species (*Abramis Brama* and *Sander lucioperca*) that were not used in our models (because only represented by one individual) and we found similar results, with EG always lower than 0.5‰ (Figure 3a and Figure 3b).

After corrections (specific or general) fin isotope values are not significantly different from muscle $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios (paired Wilcoxon tests with the validation dataset, p>0.25 for N and C). The biases in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios due to the use of corrected fin values instead of muscle isotope ratios remain quite substantial in comparison to analytical precision (more than two folds higher). However, these biases should be negligible for most trophic studies.^[3] For example, using corrected fin $\delta^{15}\text{N}$ isotope ratios generates an additional error of less than 0.2 trophic levels in the calculation of food chain length (FCL, a typical food web metric). This is similar to the error coming from fractionation variation, commonly accepted in most food web studies and estimated at 0.2 trophic levels.^[27] Besides, in mixing models, corrected

fin $\delta^{13}\text{C}$ values introduce a bias of 0.6‰, resulting in an error of 12% for a two-source mixing model having a common difference of 5‰ between end-members (error = $100 \times \Delta \delta^{13}\text{C} / |\delta^{13}\text{C}_{\text{em1}} - \delta^{13}\text{C}_{\text{em2}}|$ ^[25]). These errors are not substantial but could be of ecological importance, depending on the context. In such a case (e.g. to detect extremely subtle differences in $\delta^{15}\text{N}$ values), the choice of a correction based on specific models could be valuable, but would not improve the accuracy of the results as substantially as the use of muscle tissue.

Conclusion:

Previous studies have recommended establishing relationships between fin/muscle isotope ratios every year^[28] and for each species.^[8] However, recent results^[17] show similarity across years, implying less of a need to examine the relationship annually. In this study, we develop two general models, which provide meaningful correction for 14 species commonly living in Europe, suggesting that specific models are not mandatory. Nevertheless, general models developed for Australian tropical fish only provide poor estimations of muscle isotope values for these European species. Thus, the possibility to establish global models is certainly reduced. Nevertheless, the development of regional models can still be relevant. For example, our two general models could constitute the basis of future regional models for all European freshwater fish. For this, further validation would be welcome, especially to increase the number of concerned species and to cover a wider area of Europe. In the meantime, the models, developed in this study, already provide a first correction for European freshwater fish species. Thus, future fish feeding studies in Europe should use these models, which provide estimates of muscle isotope ratios from fin ones, and thereby which avoid the destructive sampling of muscle tissues.

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Table 1: List of the 14 fish species with n = total number of individuals (number of individuals kept for validation), number of sites (number of sites concerned by validation), mean total length (1 s.d.), mean C:N ratios in fin and muscle tissue and type of fin sampled for SIA (A = anal, D = dorsal, C = caudal, Pc = pectorals, PI = pelvics)

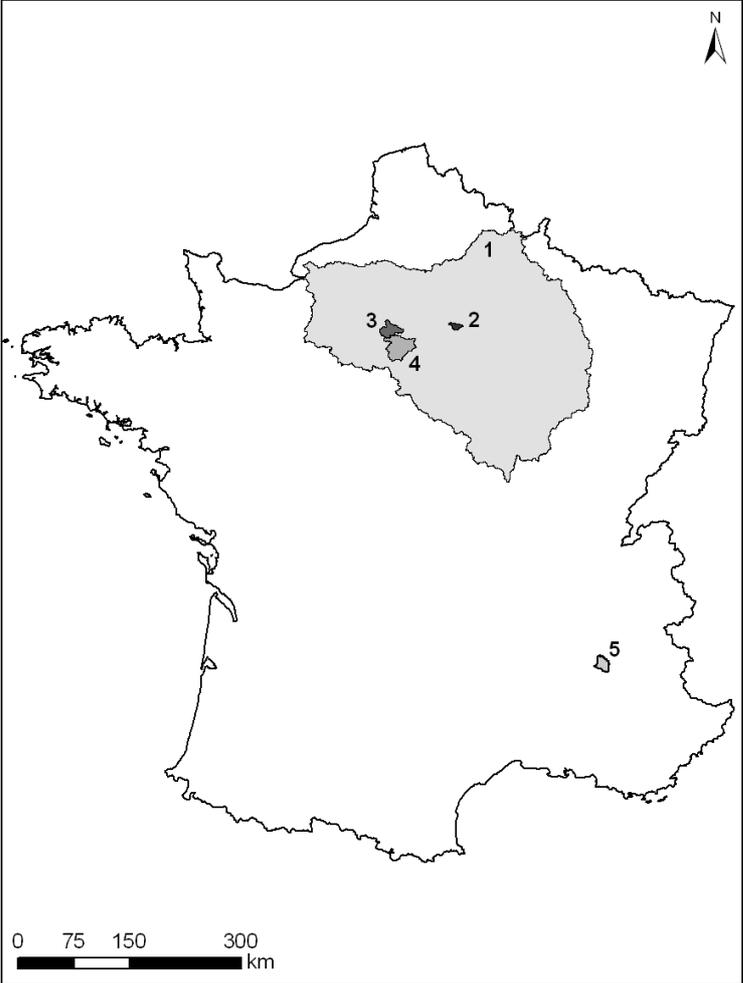
Scientific name	Common name	Species code	n	number of sites	Total length [mm]	C:N in fin	C:N in muscle	Type of fin
<i>Abramis brama</i>	Freshwater bream	Abb	1 (1)	1 (1)	266	3.3	4.0	D, PI
<i>Barbatula barbatula</i>	Stone Loach	Bab	126 (13)	25 (11)	92 (10)	3.8	3.6	D, Pc
<i>Blicca bjoerkna</i>	Silver bream	Blb	14 (1)	4 (1)	164 (74)	3.4	3.6	D, PI
<i>Cottus gobio</i>	Bullhead	Cog	68 (7)	14 (6)	85 (18)	3.2	3.7	2 nd D, Pc
<i>Gobio gobio</i>	Gudgeon	Gog	46 (5)	10 (3)	108 (13)	3.5	3.4	D, Pc
<i>Lepomis gibbosus</i>	Pumpkinseed	Leg	6 (1)	2 (1)	67 (16)	3.4	3.2	A, 2 nd D or C
<i>Leuciscus leuciscus</i>	Common dace	Lel	12 (1)	5 (1)	219 (43)	2.9	3.4	A, D, C or PI
<i>Perca fluviatilis</i>	Perch	Pef	46 (5)	13 (5)	141 (33)	3.1	3.4	A, 2 nd D
<i>Phoxinus phoxinus</i>	Minnow	Php	50 (5)	9 (4)	76 (11)	4.0	3.4	A, D, C or all
<i>Rhodeus amarus</i>	Bitterling	Rha	12 (1)	4 (1)	46 (8)	3.8	3.4	A, D, C or Pc
<i>Rutilus rutilus</i>	Roach	Rur	46 (5)	12 (4)	150 (48)	3.6	3.4	A, D, C or PI
<i>Sander lucioperca</i>	Zander	Sal	1 (1)	1 (1)	228	2.3	3.3	A, 2 nd D
<i>Squalius cephalus</i>	Chub	Sqc	38 (4)	10 (3)	175 (71)	3.5	3.4	A, D, C, Pc or PI

Table 2: Equations of the specific and general linear models between fin and muscle $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios. n indicates the number of fish individuals used to construct the model.

Species code	n	best fit equation	R²	p-value
Bab	113	Muscle $\delta^{15}\text{N} = 0.97 \times \text{fin } \delta^{15}\text{N} + 1.42$	0.94	<0.001
Blb	13	Muscle $\delta^{15}\text{N} = 0.73 \times \text{fin } \delta^{15}\text{N} + 4.84$	0.22	0.107
Cog	61	Muscle $\delta^{15}\text{N} = 1.00 \times \text{fin } \delta^{15}\text{N} - 0.01$	0.99	<0.001
Gog	41	Muscle $\delta^{15}\text{N} = 0.98 \times \text{fin } \delta^{15}\text{N} + 1.35$	0.96	<0.001
Leg	5	Muscle $\delta^{15}\text{N} = 1.17 \times \text{fin } \delta^{15}\text{N} - 1.72$	0.99	0.001
Lel	11	Muscle $\delta^{15}\text{N} = 0.95 \times \text{fin } \delta^{15}\text{N} + 1.27$	0.95	<0.001
Pef	41	Muscle $\delta^{15}\text{N} = 1.02 \times \text{fin } \delta^{15}\text{N} + 0.44$	0.95	<0.001
Php	45	Muscle $\delta^{15}\text{N} = 1.06 \times \text{fin } \delta^{15}\text{N} + 0.74$	0.93	<0.001
Rha	11	Muscle $\delta^{15}\text{N} = 0.66 \times \text{fin } \delta^{15}\text{N} + 6.37$	0.66	0.002
Rur	41	Muscle $\delta^{15}\text{N} = 0.94 \times \text{fin } \delta^{15}\text{N} + 1.60$	0.89	<0.001
Sqc	34	Muscle $\delta^{15}\text{N} = 1.11 \times \text{fin } \delta^{15}\text{N} - 1.27$	0.85	<0.001
All fish	416	Muscle $\delta^{15}\text{N} = 1.01 \times \text{fin } \delta^{15}\text{N} + 0.74$	0.92	<0.001
All fish				
Jardine et al.^[3]	174	Muscle $\delta^{15}\text{N} = 0.81 \times \text{fin } \delta^{15}\text{N} + 1.73$	0.56	<0.001
Bab	113	Muscle $\delta^{13}\text{C} = 0.77 \times \text{fin } \delta^{13}\text{C} - 6.89$	0.86	<0.001
Blb	13	Muscle $\delta^{13}\text{C} = 0.94 \times \text{fin } \delta^{13}\text{C} - 3.02$	0.60	0.002
Cog	61	Muscle $\delta^{13}\text{C} = 0.83 \times \text{fin } \delta^{13}\text{C} - 5.85$	0.90	<0.001
Gog	41	Muscle $\delta^{13}\text{C} = 0.76 \times \text{fin } \delta^{13}\text{C} - 7.73$	0.81	<0.001
Leg	5	Muscle $\delta^{13}\text{C} = 0.84 \times \text{fin } \delta^{13}\text{C} - 5.71$	0.98	0.001
Lel	11	Muscle $\delta^{13}\text{C} = 0.72 \times \text{fin } \delta^{13}\text{C} - 8.38$	0.81	<0.001
Pef	41	Muscle $\delta^{13}\text{C} = 0.96 \times \text{fin } \delta^{13}\text{C} - 1.86$	0.89	<0.001
Php	45	Muscle $\delta^{13}\text{C} = 0.87 \times \text{fin } \delta^{13}\text{C} - 4.38$	0.79	<0.001
Rha	11	Muscle $\delta^{13}\text{C} = 0.57 \times \text{fin } \delta^{13}\text{C} - 12.45$	0.44	0.026
Rur	41	Muscle $\delta^{13}\text{C} = 0.91 \times \text{fin } \delta^{13}\text{C} - 3.72$	0.91	<0.001
Sqc	34	Muscle $\delta^{13}\text{C} = 0.73 \times \text{fin } \delta^{13}\text{C} - 7.90$	0.76	<0.001
All fish	416	Muscle $\delta^{13}\text{C} = 0.82 \times \text{fin } \delta^{13}\text{C} - 5.89$	0.85	<0.001
All fish				
Jardine et al.^[3]	174	Muscle $\delta^{13}\text{C} = 0.89 \times \text{fin } \delta^{13}\text{C} - 3.27$	0.91	<0.001

Figure captions:

Figure 1: Locations of the watershed involved in the sampling of the 466 fish.
1: Seine river basin, 2: Orgeval river basin, 3: Mauldre river basin, 4: Orge river basin
and 5: Bez river basin.



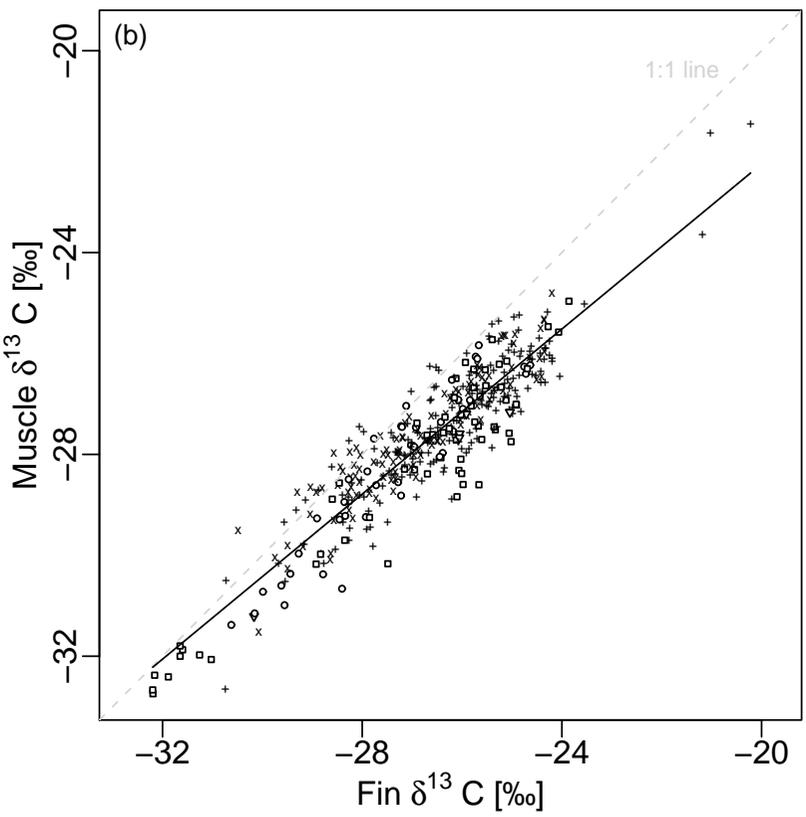
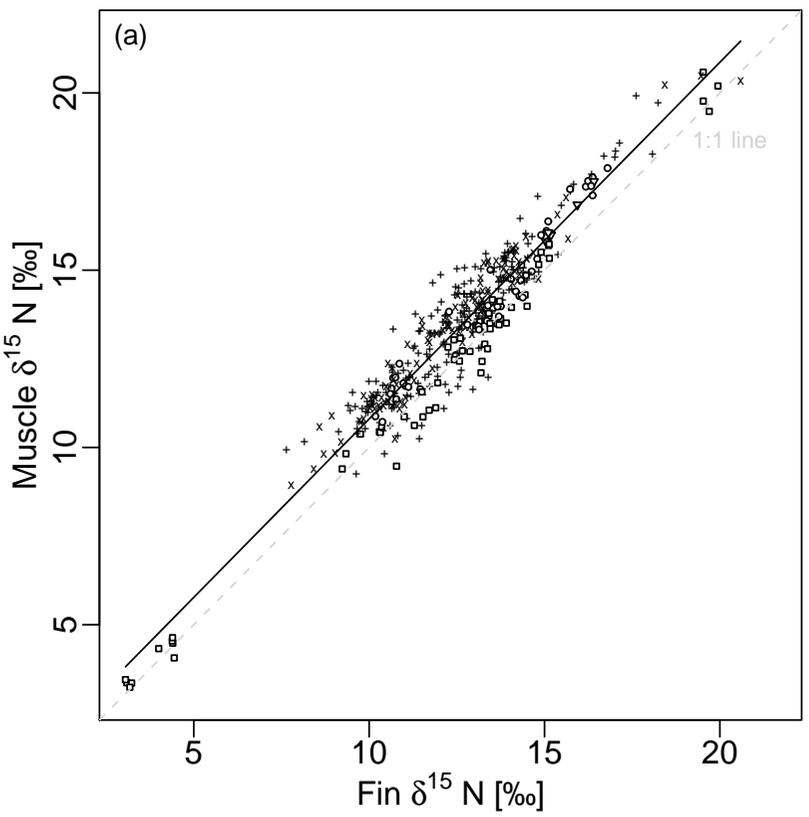


Figure 2: Linear models (black segment) between muscle and fin $\delta^{15}\text{N}$ values (2a) or fin $\delta^{13}\text{C}$ values (2b), constructed with all 1416 fish (+ = cyprinidae, o = percidae, x = Bab, □ = Cog, ▽ = Leg).

Figure 3: Mean values of the four types of errors for each species for $\delta^{15}\text{N}$ values (3a) and for $\delta^{13}\text{C}$ values (3b). Black up-triangles, white squares, black circles and white down-triangles corresponds to E, ES, EG and EJ, respectively. Vertical bars give the range between minimum and maximum values. Horizontal dashed line indicates the level of analytical precision.

