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# Effect of metabolic rate on time-lags change in otolith microchemistry - An experimental approach using *Salmo trutta*

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**Keywords:** Otolith chemistry, LA-ICP-MS, inter-individual variation, Strontium, Barium, Movement patterns.

**Summary statement:** Individuals with the highest metabolic rate are more likely to record detailed temporal changes in their otoliths

## Abstract

Over decades ecologists have long been interested in relevant techniques to track the field movement patterns of fish. Elemental composition of otolith represents a permanent record of the growing habitats experienced by the fish throughout its entire lifetime that is increasingly used in the literature. The lack of a predictive and mechanistic understanding of the individual kinematics underlying ion incorporation/depletion still limits our fine-scale temporal interpretation of the chemical signal recorded in the otolith. In particular, the rate at which elements are incorporated into otoliths is hypothesized to depend on fish physiology. However, to date, time-lags have mostly been quantified at a population scale. Here, we report results from controlled experiments (translocation and artificially enriched environment) on individual trace element incorporation/depletion rates in *Salmo trutta* (Salmonidae). We reported significant lags (i.e. weeks to months) between changes in water chemistry and subsequent change in otolith composition and highlighted substantial inter-individual variations in the timing and magnitude of Sr/Ca and Ba/Ca responses. These

differences are partially linked to the energetic status (i.e. metabolic rate) of the individuals. It, therefore, appears that individuals with the highest metabolic rate are more likely to record detailed (i.e. brief) temporal changes than individuals having lower metabolic values. The time taken for environmental changes to be reflected in the growing otolith thus can no longer be assumed to remain a constant within populations. Results from the current study are a step towards fine reconstructing environmental histories in dynamic environments.

## Introduction

Knowledge of population structure is fundamental to effective management and conservation. Patterns of connectivity, or the exchange of individuals among subpopulations of a species, are of primary importance, as well as a robust understanding of migration patterns. In this context, the field of movement ecology has grown rapidly in the last decade and is now providing increasingly detailed spatio-temporal data for a wide variety of taxa (Allen and Singh, 2016; Fraser et al., 2018). In particular, ecologists have paid a great deal of attention to the proper techniques to track fish movement in the wild since accurately resolving fish habitat use (i.e. movement and migration patterns) is essential to the development of effective management and conservation plans (Cooke et al., 2016; Fromentin et al., 2009).

Historically, telemetry-derived methods (especially radiotelemetry, acoustic telemetry, and passive integrated transponders [PIT]) have massively improved our capacity to collect fine-scale spatiotemporal information on fish (Cooke et al., 2013; Lucas and Baras, 2000). Subsequently, significant developments were largely based on the technological/analytical advances in extracting information from the calcified structures of fish (Begg et al., 2005) which has provided some of the best examples of biochronologies in the animal kingdom (Campana and Thorrold, 2001). More specifically, chemical signatures of otoliths (fish ear stones, a bone in the inner ear) have proved to be valuable tools to reconstruct environmental histories and migratory patterns of individual fish and have become widespread in both marine and freshwater fishery research (Carlson et al., 2017). These applications are possible due to how otoliths are formed. They grow continuously throughout the life of the fish, incorporate some elements and their isotopes in proportion to their ambient abundance and accrete calcium carbonate in sequential layers that preserve the timing of deposition (Campana, 1999). Given that carbonates are metabolically inert in otoliths, they act as permanent fingerprints recording environmental signatures of body waters sequentially encountered by fishes (Campana, 1999; Elsdon et al., 2008). The complete sequence of

habitats experienced by a fish throughout its lifetime can therefore be assessed using the elemental composition along a transect ranging from the *primordium* to the otolith's edge.

Currently, otolith chemistry is used to address various issues relating to the ecology of fish, ranging from studies estimating population structure and discriminating stocks (reviewed in Tanner et al., 2015; Thresher, 1999), migration patterns and philopatric behavior (e.g. Bradbury et al., 2008; Elsdon and Gillanders, 2003a; Walther et al., 2011), site residency (e.g. Lord et al., 2011; Teichert et al., 2018), nursery habitats (e.g. Brennan et al., 2015; Marklevitz et al., 2016), environmental histories of fish (Limburg, 1995; Walther et al., 2011), and finally investigating connectivity, by retrospectively assigning adults to their areas of origin (e.g. Gillanders, 2005; Thorrold et al., 2001). In all these contexts, applications rely on the concentration of elements in otoliths changing in predictable manners in relation to changes in environmental settings. The underlying hypothesis is that the duration of elemental and isotopic singular signatures are linked to the amount of time spent by fishes in a particular water body.

Initial studies mostly focused on movement patterns through chemically distinct bodies of water over long periods. In this context, diadromous migrations have been intensively studied (Walther and Limburg, 2012). Until recently, only a few elements (Sr, Mg, Mn, and Ba) were routinely measured in otoliths. For example, in the case of diadromous migrations, Sr and Ba can be used alone as environmental recorders (Sturrock et al., 2012; Walther and Limburg, 2012). The recent use of laser ablation and/or microprobe analysis on otoliths allows the simultaneous collection of various elements that are more likely to discriminate among water masses experienced by fish (Mercier et al., 2011). Multi-proxy approaches may therefore allow investigating movement patterns in the wild at remarkably fine geographical scales (e.g. Gemperline et al., 2002; Gillanders and Kingsford, 2003; Svedäng et al., 2010). In the meantime, the rapid improvements in the analytical capabilities of sampling equipment raised expectations of unraveling environmental and migratory histories with increasingly fine temporal resolutions. Analyses have indeed shifted at time scales ranging from inter-annual to seasonal or beyond (Brennan et al., 2015; Elsdon and Gillanders, 2005a; Kennedy et al., 2000; Macdonald and Crook, 2010; Weidel et al., 2007). On a theoretical basis, it may even be possible to detect brief incursion/residence in adjacent habitats having contrasted geochemical signatures (e.g. among freshwater drainages/tributaries). However, the fine-scale analytical resolution of equipments sharply contrasts with the physiological temporal resolution of elemental incorporation in otoliths.

Several investigations have reported the existence of substantial time-lags (i.e. weeks to months) between a fish's exposure to changed environmental conditions and when elemental concentrations stabilize to reflect these new conditions (Lowe et al., 2009; Macdonald and Crook, 2010; Miller, 2011). Otoliths are not in direct contact with the surrounding water and elements must pass through several tissue and cell boundaries (i.e. gill epithelium, gut membrane and endolymphatic epithelium, Hussy et al., 2020) before being incorporated into the matrix of calcified structures. Consequently, changes in environmental variables would not result in instantaneous changes in elements within calcified structures. This temporal lag is a critical aspect that limits the spatial and temporal resolution attainable for multi-elemental otolith analyses and would affect the interpretation of environmental histories (Chang and Geffen, 2013; Sturrock et al., 2012).

Retracing movements of fishes using otolith chemistry analysis is not straightforward and also requires detailed knowledge on the predictable relationships between otolith and water chemistry (through otolith elemental ratios are some fraction of those present in the ambient environment). In this context, considerable research effort was focused on the exogenous factors (i.e. salinity, temperature, ambient water concentrations) that can independently, or interactively, influence the uptake and incorporation of trace elements into the otolith. In contrast, endogenous factors (i.e. physiological factors such as growth rates or stress) have received relatively less attention (Walther et al., 2010) despite the long recognition of their importance (e.g. Kalish, 1989; Kalish, 1991; Kalish, 1992; Radtke and Shafer, 1992). The experimental interaction between exogenous and physiological factors is even more rarely considered in the literature. Most recent advances in the field highlighted that, contrary to a long-standing assumption, physiological components can considerably influence elemental uptake and processing mechanisms (Sturrock et al., 2015; Sturrock et al., 2014). However, such rigorous investigations only focus on elemental concentrations (i.e. partition coefficients) with no specific consideration for the time taken for environmental changes to be reflected in the growing otolith (i.e. kinematics). Physiological effects such as metabolic rate are frequently suspected, otherwise hypothesized to explain inter-individual differences. On a theoretical basis, biophysical factors influencing the kinematics of both uptake and depletion of ions in the endolymph are mediated by the transport of these ions through cell membranes, which in turn may be related to some extent to metabolic rate. Nevertheless, the potential effect of individual metabolic rate on time-lag has, to the best of our knowledge, never been investigated. This has been identified as an area urgently requiring

further investigation (Sturrock et al., 2012; Tanner et al., 2015) that would be hugely beneficial (Walther *et al.*, 2010).

The present study addressed this issue by examining chemical signatures in the otoliths of juvenile brown trout (*Salmo trutta*, Linnaeus 1758) following experimental changes in environmental conditions reproducing the range of concentration typically encountered by the species between different tributaries in the same watershed. More precisely, our specific aims were to (1) precisely quantify the kinematics of both incorporation and depletion of Sr and Ba in otoliths following habitat change, (2) examine inter-individual times taken for environmental changes to be reflected in the growing otolith, and (3) examine whether inter-individual differences in time lag is related to individual metabolic rates (i.e. as revealed by oxygen consumption), with the prediction that individuals with the highest metabolic rate have the finest micro-chemical resolution (i.e. shortest time-lag).

A variety of chemical tracers in otoliths have been used to reconstruct movements pattern. In particular, elemental ratios such as Sr/Ca and Ba/Ca are well documented and widely used in the literature. Apart from their use to track diadromous migrations (Walther and Limburg, 2012), these elements can also vary significantly in freshwater systems between or even within rivers depending on streambed geological composition, (Ramsay et al., 2011; Walther et al., 2011; Zitek et al., 2010) and have specifically proved to be useful when studying movement patterns of salmonids species between tributaries in southwestern France (Martin et al., 2013a; Martin et al., 2013b). Here, we exclusively focused on Sr/Ca and Ba/Ca because among elements used in otolith microchemistry strong positive relationships between waters and otoliths in freshwater systems were mainly found for these two elements (see the review of Brown and Severin, 2009; Hussy et al., 2020).

## **Material and Method**

### **Site, fish collection and experimental design**

For this experiment, a full-sibling group of juvenile brown trout (*Salmo trutta* Linnaeus, 1758) spawned from captive parents (43 females and 15 males) in the Lees-Athas fish hatchery facilities, southwestern France (42°57'57"N, 0°36'50"W) were used. After fertilization, eggs were incubated and individuals were maintained in natural temperature conditions (water supplied by spring water with a constant temperature of 11.6±0.2°C during

the 2015–2016 winter). On April 15<sup>th</sup> 2016, three-month post-emergence (i.e., alevins live in the gravel, feeding off the remaining yolk that is attached to their body, then they emerge out of the gravel as fry, set up territories, and start exogenous feeding), 300 individuals were transferred by cars in large oxygenated coolers maintained at 12°C and reared in controlled experimental facilities (ECP, 2018) with water derived from the neighboring Nivelles River (southwestern France 43°21'14"N, 1°33'54"W). Such water has comparatively lower barium and higher strontium concentrations compared to previous conditions. While transferred, fishes were marked by immersion in Alizarin Red S solution (100 mg L<sup>-1</sup>, 3h in a well-aerated 20 L tank maintained at 12°C) for obtaining *a posteriori* time-resolved elemental signals (Caudron and Champigneulle, 2006). Throughout the entire experiment (4.5 months, from 145 to 153 days according to individuals), all fishes were maintained under the same environmental conditions in a single tank (750 L), with water maintained at 12.0±0.3°C and *ad-libitum* feeding conditions.

After two months (66 days post translocation), experimental tanks were artificially enriched in Barium (fourfold increase, from about 10µg L<sup>-1</sup> to 40µg L<sup>-1</sup>, for empirical, see Table 1) by adding the appropriate amount of BaCO<sub>3</sub> (Sigma-Aldrich) powder, reproducing the range of concentration naturally encountered by the species between different tributaries in the same watershed in southwestern France (Martin et al., 2013a). The strontium concentration remained unchanged. On the first day of barium enrichment (June 20<sup>th</sup> 2016) fishes were collectively marked a second time by immersion in Alizarin Red S (same conditions as before). About 10% of the water volume was replaced every week in the experimental tank, with each time the addition of the appropriate amounts of barium. Water samples were collected at approximately 15-days intervals for *a posteriori* verification of the enrichment (Table 1). After additional rearing months in these enriched conditions (i.e. 79-87 days according to individuals), 51 individuals were randomly selected for measuring oxygen consumption (as a proxy of metabolism, see after). They were subsequently euthanized, measured (standard length, nearest mm), weighted (1 mg), and prepared for otolith extraction. Given that transferred individuals were initially of homogeneous size (based on a randomly selected subset of 101 fishes, mean ± sd. of 33.7 ± 1.5 mm), fish length at the end of the experiment is considered as a proxy of somatic growth. During the rearing period, Sr/Ca and Ba/Ca ratios are the only noteworthy changed component through time. The complete experimental design is summarized in Fig. 1. Experimental design and maintaining conditions were both approved by National Ethic Committee for Fishes and Birds (CE73), with respect to the national chart.



## Metabolism

We needed to quantify individual metabolic rates (i.e. the total amount of energy used by the animal at a time interval). In fishes, standard metabolic rate (*SMR*, the rate of energy expenditure by the body during complete physical, mental, and digestive rest) and routine metabolic rate (*RMR*) are usually measured. Both measures have been considered and provide complementary information. Since the metabolic rate varies with activity level, more active animals have a higher metabolic rate than less active animals. *RMR* thus integrates the intrinsic/constitutive swimming mobility of individuals that should be fully considered as a significant part of their energy budgets.

Measures of metabolic rate were obtained through the determination of oxygen consumption. 51 juveniles were retrieved for oxygen consumption determination for 7 days (September 7<sup>th</sup> to 11<sup>th</sup>, and then 14<sup>th</sup> to 15<sup>th</sup> 2016). Briefly each day, 8 juveniles (only 3 juveniles the last day) were starved during 36h before analysis. Then, they were transferred to 8 respirometric chambers (diameter: 45mm, length: 180mm) of an intermittent flow respirometer as described by Régnier et al. (2010). Juveniles were introduced into the chambers at 3 p.m. and oxygen consumption was recorded continuously every minute until 10 a.m. the next day. The closed/open phase of the system was 30 min/15 min and the duration of the closed phase was determined in order that the oxygen level in the chamber was always kept above 80% O<sub>2</sub> saturation. Each 30 min oxygen consumption was approximated using a simple linear regression from which the regression slope was extracted. After that time, the background respiration, i.e. oxygen consumption within the respirometer due to microbial respiration, was estimated by measuring the oxygen consumption rate in the respirometer without a fish (i.e. blank run for two hours). Temperature and photoperiod used for the acclimatization phase and oxygen measurement were similar to those used all along within experimental facilities. To estimate the *SMR*, the first 5 hours were considered as a period of acclimation and the average oxygen consumption was calculated using the three slightest consecutive slopes (using an exhaustive search within the 8 p.m.- 8 a.m period). As some individuals may be more or less restless in the respirometric chambers, the *RMR* was also estimated by considering all slopes after the acclimation period. After their maintain in respirometric chambers, individuals were immediately euthanised by immersion in buffered benzocaine solution (preliminary anaesthesia at 4.0 ml L<sup>-1</sup> followed by euthanasia at 12.0 ml L<sup>-1</sup>; 11–12°C).



## Water and otolith microchemical analysis

Water samples were collected at approximately 15-days intervals from experimental tanks and were processed according to the procedure given in Martin et al. (2013). Briefly, using polypropylene syringes samples were filtered through 0.45µmPVDF Whatmann filters into acid-washed low-density polyethylene (LDPE) bottles and acidified to 2% with 70% ultrapure nitric acid (J.T. Baker, Ultrex II) to fix the sample and enable storage. Analysis of water samples was carried out on undiluted samples using solution-based ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry - ACTIVA, *Jobin Yvon*). Internal standard of Indium ( $500 \mu\text{g L}^{-1}$ ) was used to correct for instrument drift. Blanks were regularly performed, using the same protocol as for the samples, with 18.2 MΩ MQ water (Millipore). Samples of known concentration, certified reference freshwater (SLRS-6, NRCC; [www.nrc-cnrc.gc.ca](http://www.nrc-cnrc.gc.ca)), were used to estimate accuracy and precision; measured concentrations were within 93 to 103% of reported values for all elements and external precision lesser than 4% for Ca and Sr and about 5% for the Ba.

The exact same individuals (N=51) were used for both oxygen consumption and microchemistry. Sagittal otoliths were removed with acid-washed plastic forceps, gently cleaned in pure water, dried, and stored in acid-washed plastic vials prior to embedding in epoxy resin (Araldite 2020, Huntsmann). Otolith lengths (from rostrum to antirostrum) were recorded as a rough proxy of otolith growth. Embedded otoliths were ground with decreasing size abrasive paper (grits 1200, 2500, and 4000) until the otolith core was visible, polished with a polycrystalline diamond suspension (1 µm), and carefully rinsed with ultrapure water. Otolith Sr/Ca and Ba/Ca were quantified using a 1030nm femtosecond laser ablation system (Alfamet-Novalase, France) coupled with an inductively coupled plasma mass spectrometer (DRCII, Perkin Elmer). The laser was set at a pulse rate of 50Hz with a 12-µm ablation spot and traveled at  $5\mu\text{m s}^{-1}$ . Analyzed isotopes were  $^{86}\text{Sr}$ ,  $^{138}\text{Ba}$  and  $^{43}\text{Ca}$  used as an internal standard to account for any variations in the ablation yield. External calibrations were performed using synthetic certified reference glasses NIST614, NIST612, and NIST610 (NIST, USA). Analytical accuracy was achieved with the fish otolith FEBS-1 (NRCC, Canada; Sturgeon et al., 2005) and NIES22 (NIES, Japan; Yoshinaga et al. 2000). Limits of detection were calculated based on a  $3\sigma$  criterion, where  $\sigma$  is the standard deviation for 20 seconds measurements of the blank signal. Limits of detection (ppm) were:  $^{86}\text{Sr}$ =1.85 ppm, and  $^{138}\text{Ba}$ =0.06 ppm. Accepted recovery of reference materials for both elements ranged from 94% to 109%. Strontium and barium compositions were expressed as mass elemental ratios

(i.e. Sr/Ca, Ba/Ca) on the basis of the stoichiometry of Ca carbonate ( $380,000 \mu\text{gCa g}^{-1}$  otolith) (Campana, 1999).

Otolith elemental data were collected through a bipartite transect using the core as a bifurcation point (Fig. 2). Two chronologies of different lengths were therefore obtained for each fish. They were arbitrarily categorized into two groups: "short" transects (located on the dorsal part of the otolith, Aymes et al., 2016)) and "long" transects (comparatively located on the anteroventral part) (Fig. 2). The starting and ending points were both located at  $50 \mu\text{m}$  from the outermost edge of the otolith. All transect parts were located perpendicular to daily increments to generate a time series of elemental composition during the experiment.

Photographs were taken under unfiltered white light and fluorescent light (filter model: RFP1, excitation 530–550 nm/emission 575-nm, Olympus SZX-16 stereomicroscope, Olympus DP72 camera), which was used to detect the Alizarin marks. For each otolith two calibrated images were superimposed, one with transmitted light and the other with fluorescent light (Olympus Cell A software) for simultaneous observation of the transect, otolith edges, and ARS mark (Fig. 2b). The position of these marks along the transect was used to identify the three main experimental periods and to proceed with a signal standardization, converting distance (in  $\mu\text{m}$ ) into time (months) using a linear intrapolation assuming constant growth (during this period, fish growth is maximized, in absence of sharp thermal difference, Pearson's correlation coefficient between time and otolith increment width of 0.88, Supplementary file 1, Fig. S1). Time series for otolith Sr/Ca and Ba/Ca were therefore plotted against otolith distance during the experimental period.

### Statistical analysis

The change in concentrations over time along each transect was modeled using a non-linear mixed-model (implemented using the *nlme* package (version 3.1) in R version 3.2.2) applied to raw instrumental data. For convenience, we used a re-parameterized analogue of the von Bertalanffy model in which both the initial and asymptotic values are explicitly inferred. Distinct formulae were used for decreasing trends (initial Ba/Ca, Eqn.1) and increasing trends (Sr/Ca and enriched Ba/Ca, Eqn.2).

$$\text{Eqn.1} \quad \text{Me/Ca} = (L - Z)(1 - e^{-k\text{Time}}) + Z$$

$$\text{Eqn.2} \quad \text{Me/Ca} = \frac{L}{1 + \left(\frac{L-Z}{Z}\right) e^{-k\text{Time}}}$$

With Me/Ca standing to metal-to-calcium ratios, *Time* the relative time (in months) along the transect, *k* the kinematic parameter (increasing/decreasing rate), *L* the asymptotic concentration, and *Z* the explicit initial concentration. In the mixed-model, ‘*Time*’ was used as the main independent variable, with ‘*Individuals*’ (51 individuals) as a random effect that allows for the three parameters (*k*, *L* and *Z*) to be estimated for each fish. Due to the anisotropic growth of the otolith, the two transects obtained for each individual (*Replicates*) are of different lengths and may exhibit distinct signals. We tested for a possible effect of these "Replicates" by comparing a model with and without such effect. When considered, transects (treated as a dual-factor: *short* vs. *long*) were primarily included in the statistical analysis as a fixed source of intra-individual variance (i.e. fixed effect term in mixed models) with individuals nested within each transects group. Alternatively, the *Replicates* term was discarded in a second model, pooling all data per individual. For comparing the two models having distinct fixed-effects, the maximum likelihood estimator was preferred for fitting models. Akaike weight was used for model selection (supplementary file 2, Table S1). As the model taking into account *Replicates* is far less likely than the alternative model, we further pooled for each individual data from the two transects. We also accounted for autocorrelation in the residuals due to the underfitting of the model by including an AR1 model (i.e. first-order autoregressive model).

Global kinematic trends were estimated using the fixed effects of the above-mentioned non-linear mixed-model. Moreover, at a global scale, fitted data were analyzed using a quantile regression approach (implemented using the *mqqam* function from the *qqam* package (version 1.3.2) in R version 3.2.2)(Fasiolo et al., 2020) to quantify time-lag in incorporation/depletion. We specifically quantified 5<sup>th</sup> and 95<sup>th</sup> time-percentiles for which individuals exhibited 90% change in their concentration values. To exclusively focus on time-lag by accounting for inter-individual differences in initial and asymptotic values (expressed respectively by the *Z* and *L* random terms in the mixed-model approach), fitted values were standardized [0-1] between these two limits. At the individual scale, we have identified the minimum period of exposure time required for otoliths to incorporate/deplete 90% of Sr and Ba from initial to asymptotic modeled value (referred hereafter as *T90*).

Pearson's correlation coefficient for repeated measures was used to quantify the strength of association between individuals' kinematic parameters ( $k$ ,  $L$ , and  $Z$ ) and either standard metabolic rate ( $SMR$ ), routine metabolic rate ( $RMR$ ), or 90% time-lag ( $T90$ ). Somatic growth (fish length at the end of the experiment) and otolithic growth (otolith weight) were similarly compared to  $SMR$  and  $RMR$ .

## Results

Observed elemental trends in otoliths (Sr/Ca and Ba/Ca ratios) are consistent with environmental conditions experienced by fish during the experiment (transfer between rivers and experimental enrichment, Fig. 3). In particular, abrupt changes in geochemical signatures (both uptake and depletion) are well noticeable on otoliths. Uptake of strontium and uptake/depletion of barium followed the expected nonlinear trend, as modeled by re-parameterized analogues of the von Bertalanffy model. The overall mean response exhibits a lag response of several weeks, before reaching equilibrium (i.e. asymptotic value). Beyond this global trend and despite the controlled chemical environment, stable temperature regime, and standardized diet, data exhibit substantial inter-individual variations in the timing and magnitude of Sr/Ca and Ba/Ca responses, with a large portfolio of elemental composition at equilibrium (initial  $Z$  and asymptotic  $L$  values) and kinematics ( $k$ ).

When focusing on the kinematics by accounting for equilibrium values, our results emphasize at the individual scale a highly variable minimum period of time required for otoliths to reach 90% of predicted equilibrium (Fig. 4). Overall, this time-lag is at least two time longer for individuals with low uptake/depletion rate, compared to those with more rapid kinematics. More specifically, for strontium uptake, 90% of predicted equilibrium is reached after only 1.1 months for the 5<sup>th</sup> percentile, while it takes 2.4 months for the 95<sup>th</sup> percentile. Similarly, for barium uptake, a distinct time-lag was apparent among individuals before its concentration reached a stable level. Saturation occurred after 0.3 and 0.9 month respectively for 5<sup>th</sup> and 95<sup>th</sup> percentiles. Ultimately, for barium depletion, a stable Ba/Ca ratio is obtained within only 0.6 month for several individuals while it requests 1.1 months for others.

Given that  $SMR$  and  $RMR$  are highly correlated ( $\rho=0.81$ ,  $p\text{-value}=2.8 \cdot 10^{-24}$ ) in our data and behave similarly, we subsequently only focused on  $RMR$ . As the main result of our experiment, the energetic status of individuals (as measured by oxygen consumption) correlates with shift characteristics of chemical data (Fig. 5, supplementary file 3, Fig. S2). This is particularly true for the kinematics of barium depletion and its relative time-lag ( $k$ ,

$\rho=0.64$ ,  $p\text{-value}=5.9 \cdot 10^{-13}$ ;  $T90$ ,  $\rho=0.61$ ,  $p\text{-value}=1.9 \cdot 10^{-11}$ ) and to a lesser extent to Ba values at equilibrium ( $Z$ ,  $\rho=0.22$ ,  $p\text{-value}=2.7 \cdot 10^{-2}$ ;  $L$ ,  $\rho=0.42$ ,  $p\text{-value}=1.3 \cdot 10^{-5}$ ). It is also less well marked for barium uptake ( $Z$ ,  $\rho=0.31$ ,  $p\text{-value}=1.7 \cdot 10^{-3}$ ;  $L$ ,  $\rho=0.09$ ,  $p\text{-value}=0.36$ ,  $k$ ,  $\rho=0.43$ ,  $p\text{-value}=5.8 \cdot 10^{-8}$ ;  $T90$ ,  $\rho=0.35$ ,  $p\text{-value}=3.6 \cdot 10^{-4}$ ). In contrast for strontium uptake, *RMR* poorly correlates with elemental composition at equilibrium ( $Z$ ,  $\rho=0.25$ ,  $p\text{-value}=1.3 \cdot 10^{-2}$ ;  $L$ ,  $\rho=0.28$ ,  $p\text{-value}=3.4 \cdot 10^{-3}$ ), kinematics ( $k$ ,  $\rho=0.02$ ,  $p\text{-value}=0.83$ ) and time-lag ( $T90$ ,  $\rho=0.09$ ,  $p\text{-value}=0.39$ ). For a more detailed review of the results, the exhaustive list of repeated-measures correlations (and associate p-values) are provided in the Supplementary file 3, Fig. S2. Ultimately, both somatic and otolithic growth poorly correlate with *SMR* ( $\rho=-0.31$ ,  $p\text{-value}=2.4 \cdot 10^{-2}$ ;  $\rho=-0.17$ ,  $p\text{-value}=2.1 \cdot 10^{-1}$  respectively) and *RMR* ( $\rho=-0.29$ ,  $p\text{-value}=3.4 \cdot 10^{-2}$ ;  $\rho=-0.27$ ,  $p\text{-value}=5.1 \cdot 10^{-2}$  respectively).

## Discussion

Overall, our data exhibited substantial inter-individual variations in the time-lag response of Sr/Ca and Ba/Ca signals, ranging from a few to several weeks, up to months. Individuals with the highest metabolic rate are more likely to record detailed (i.e. brief) temporal changes than individuals having lower metabolic values.

### Time-lags change in otolith microchemistry

The hard acid elements such as Sr and Ba (Walther and Thorrold, 2006). are widely used in combination to unravel anadromous movements (Sturrock et al., 2012; Walther and Limburg, 2012) but also exhibit regional variations in freshwater depending on streambed geological composition and have emerged as useful to track movement patterns at a finer spatio-temporal scale. The usefulness of these markers mostly depends on their predictable spatio-temporal distribution as well as the comprehension of the regulatory mechanisms affecting their incorporation. Although prior works have comprehensively addressed the spatial and temporal variability in these chemical tracers (e.g. Walther and Thorrold, 2009), the temporal resolution has not been thoroughly investigated for these systems.

Ambient change in chemical composition can result from a shift in habitat use (i.e. movement between water masses of distinct composition). Alternatively, fishes can remain spatially static over time in complex and temporally dynamic environments with changes in water chemistry. This should be particularly relevant in estuaries with varying physical and chemical properties as short as a tidal cycle, due to the mixing of marine and freshwater

inputs (Dorval and Jones, 2005; Elsdon and Gillanders, 2006). In either case, for fish that undertake rapid movements between adjacent tributaries having contrasted geochemical signatures, the presence of time-lags indicates that the residence time in a given tributary may be too short for the environmental chemical signature to be properly recorded in the otolith. Consequently, particular Sr/Ca or Ba/Ca values could either represent equilibrium with the ambient water or transitional values at some unknown stage within a time-lag period (Macdonald and Crook, 2010). Likewise, analyses that incorporate otolith material laid down during weeks following habitat change will underestimate elemental concentration in the otolith (Elsdon and Gillanders, 2005b). The presence of time-lag limits the spatial and temporal resolution achievable (Chang and Geffen, 2013; Macdonald and Crook, 2010; Sturrock et al., 2012) and poses substantial challenges to reconstruct environmental histories of fish at the relevant temporal scale. It is therefore critical to determine how rapidly otolith composition changes in response to variation in water composition.

To date, though many studies have used otolith chemistry to examine movement patterns, few manipulative experiments have specifically attempted to resolve the precise temporal resolution of their methods. In addition, publications are strongly biased toward manipulative experiments in which elemental concentrations are artificially increased. The opposite (*i.e.* depletion) is rarely shown but of equal interest. Here, both otolithic signals were jointly investigated and rapidly reacted to changes in environmental concentrations but otolith composition did not stabilize for weeks. The decrease/increase in concentration in our time-resolved signal was always observed after only a few sampling time points or no delay. Given the analytical parameters or the femtosecond laser equipment, the estimated delay between two consecutive measures encompass only a few days (depending on individuals and transect length), indicating that the otolithic signal rapidly reacts to changes in environmental concentrations. Habitat transitions should therefore be discernible after a relatively moderate/brief residence time but the otolith may not reflect ambient water levels until weeks. On a practical basis, even in case of the instantaneous change in otolith composition along a transect, any laser beam of a given diameter would average in a mixed proportion material on either side of the center, introducing an apparent lag (Warburton et al. 2017). The importance of this lag is defined by growth increment widths relative to the laser diameter. Similarly, given the concentric three-dimensional nature of otoliths, depth-profiling laser ablation collects data through multiple vertical layers, potentially leading to time-lag (Hoover and Jones 2013, Warburton et al. 2017). In addition, washout/purge of the ablation cell (a sealed chamber) also plays a role in avoiding contamination from previous ablations. Overall,



operating parameters of LA-ICP-MS (including spot size, ablation depth, laser speed, and washout rate) can artefactually affect time-lag response and define the absolute minimum limitation on the temporal resolution that could be identified. In our analysis, we quantified the artefactual (analytically-related) time-lag that is around 20  $\mu\text{m}$  (Supplementary file 4, Fig. S3), corresponding to about 6 days (assuming a constant growth, Supplementary file 1), an order of magnitude below the one related to inter-individual variations. In addition to time-lags, our data emphasize that even if fish remain in a given environment long enough to reach equilibrium, individual variations in the kinematics may hinder accurate reconstructions of environmental histories (Macdonald and Crook, 2010).

On a theoretical basis, elements with no major structural or physiological role (e.g. Ba) may depure (i.e. depletion of ions in the endolymph) more rapidly than those being constituent parts of functioning enzymes or structural tissues (e.g. Sr), resulting in short turnover times (Sturrock et al., 2012). While the comparison between Sr and Ba in terms of depletion kinematics was not possible, the decrease in Ba was pretty rapid, emphasizing that this element (with short turnover time) is well suitable for studying detailed temporal changes. However, experiments were conducted using fish exposed to constant environments before and after abrupt changes. For analysis aiming at reconstructing dynamic change in the environment using chemical proxies, further work is required to fully characterize the response of otolith chemistry to brief/intermittent environmental variations. Such pulses of ambient Ba/Ca simulate either upwelling or flood conditions in marine systems or riverine discharge in freshwater systems.

Various studies have also reported substantial time-lags of the same magnitude in elemental uptake for other diadromous species: 30-40 days, (Ba/Ca and Sr/Ca respectively) for the Australian bass *Macquaria novemaculeata* across a salinity gradient (Macdonald and Crook, 2010), 20d (Sr/Ca) in juvenile black bream *Acanthopagrus butcheri* (Elsdon and Gillanders, 2005b), 21d (Sr/Ca) for juvenile largemouth bass *Micropterus salmoides* (Lowe et al., 2009), over 10d (Ba/Ca, Sr/Ca) in juvenile Chinook salmon *Oncorhynchus tshawytscha* (Miller, 2011), over weeks for juvenile striped bass *Morone saxatilis* (Secor et al., 1995), more than 50d for juveniles pike *Esox lucius* (Engstedt et al., 2012). A brief overview of previously cited time-lag records obtained in juveniles does not exhibit clear analytical bias (see Supplementary file 5, Table S2 for operating parameters of LA-ICP-MS and recorded time-lags), but rather highlights inter-specific differences, depending on local conditions. Slight differences between studies may concomitantly rely on species-specific physiology



(reviewed in Chang and Geffen, 2013), as well as ambient concentration. All these studies have emphasized that otolith Sr/Ca and Ba/Ca are linked to ambient concentrations, covering a wide range of concentrations, sometimes encompassing freshwater to marine values, with no evidence of saturation (Bath et al., 2000; Elsdon and Gillanders, 2003b; Reis-Santos et al., 2013; Secor et al., 1995). Hence, the complete time-lag before equilibrium is likely related to both absolute and relative change in water concentration, in a potentially non-proportional manner, with potential interaction with salinity and temperature (Bath et al., 2000; Elsdon and Gillanders, 2003b; Miller, 2009). Since diet can also influence elemental uptake (Doubleday et al., 2013; Jaecks et al., 2016; Lin et al., 2007; Marohn et al., 2009; Milton and Chenery, 2001; Walther et al., 2010; Woodcock et al., 2012), concomitant change in both alimentation and environmental conditions remain to be investigated. In the meantime, because relative growth asymptotically decreases through time for many teleost fishes (Jones, 2000), the temporal resolution attainable should intrinsically change over time for any given analytical resolution (*e.g.* constant speed of ICP-MS beam). A finer temporal resolution in chemical transects is therefore expected compared to older stages. Moreover, artefactual (analytically-related) lags may be apparently ‘longer’ in older fish that grow more slowly. As a consequence, generalizations about the time-lag response of elemental incorporation is *a priori* difficult and investigations remain to be conducted in a species-specific manner (species-specific elemental incorporation reviewed in Chang and Geffen, 2013), at the relevant life history stages and on a case-by-case basis.

### **Metabolic influence on kinematics**

While the concentration of trace elements predictably reflects environmental conditions, the interpretation of elemental signals is not straightforward since several confounding factors can act concomitantly in an interactive manner. Otolith composition is indeed a multifactorial component that is simultaneously influenced by the composition of the surrounding water and various physiological processes that can mediate the incorporation of available elements into the otolith. While specific regulatory processes are not fully elucidated, several iono-regulatory processes can be involved since depletion and element uptake into the otolith matrix from surrounding water and food involves the transport of dissolved ions over several membranes (Campana, 1999; Kalish, 1989; Kalish, 1991; Melancon et al., 2009; Payan et al., 2004). It includes modulation uptake in the blood, binding of metal elements by circulating plasma proteins that change ion availability (at least for soft

acid ions that are more strongly associated with blood proteins, GrønkJær, 2016), and mechanisms of ion transport from external plasma across the saccular macula into the endolymph (Payan et al., 2004). Finally, the incorporation of an element into the otolith depends on its compatibility to bind directly into the crystal lattice, within interstitial spaces, or to the organic matrix (Campana, 1999; Sturrock et al., 2012). In this context, otolith is bathed in a supersaturated endolymphatic solution which contains all the ionic precursors and proteins necessary for otolith accretion (Hüssy et al., 2020). On a theoretical basis, the secretion of most of these precursors are energy-demanding processes, and therefore linked to the bioenergetic status of the fish, that in turn can explain the (indirect) link between uptake/depletion kinematics and SMR. On a practical basis, most studies concluding to metabolic regulation are however based on indirect evidence (e.g. Hüssy and Mosegaard, 2004; Kalish, 1989; Wright et al., 2001). For example, Hüssy and Mosegaard (2004), observed that otolith growth continues during a period of starvation while fish lose weight, suggesting that otolith accretion is more linked to the standard metabolic rate of the fish, independent of somatic growth. Most recent experiments in which blood samples, physiological data and water chemistry were sampled over the course of a year directly highlight physiological influences for thiophilic elements with possible metabolic implication (Sturrock et al., 2015; Sturrock et al., 2014). In a distinct but related context, isotopic composition in otoliths (proxy of trophic level) is known to depend on prey composition, with a strong physiological modulation (Lueders-Dumont et al., 2020). This study is one of the few to experimentally address the endogeneous effect of metabolism on the kinematics of elemental incorporation/depletion. Results are unambiguous and fully in line with the growing evidence of physiological effects on otolith microchemistry.

Metabolic costs are central to individual energy budgets, making estimates of metabolic rate fundamental to understand how an organism interacts with its environment (Treberg et al., 2016). Modeling otolith growth using a bioenergetic model in the framework of the dynamic energy budget (DEB, i.e. treating otoliths as “metabolic products” of organism growth and maintenance processes) indeed provided insights into otolith growth (Fablet et al., 2011; Pecquerie et al., 2012). Interestingly both incorporation and depletion are related to SMR, suggesting that the roughly same pathways are used and regulated for these two components, although differences in kinematic exist between the two elements. More generally, the observed kinematics derives from normal metabolic processes in tissues including synthesis and degradation. Surprisingly, initial ( $Z$  parameter) and asymptotic ( $L$

parameter) elemental ratios are less well related to SMR compared to the kinematics of incorporation/depletion. The reasons for such incongruence between elemental composition and kinematics is unclear. On a theoretical basis, the two should be linked, with individuals having the highest metabolic activity that should have more active ionocytes (Limburg et al., 2018), leading to higher transport/availability of the element to be incorporated in the otoliths. This in turn would increase both elemental composition and kinematics of incorporation/depletion. Previous works have already emphasized that the partition coefficients of a given element can be related to the bioenergetic status of the fish (Kalish, 1989; Kalish, 1991; Sturrock et al., 2015; Sturrock et al., 2014), but in absence of additional kinematic data, this discrepancy remains to be further investigated.

### **Implication for individual reconstruction**

While experimental investigation should be carried out at the individual level to provide an appropriate framework for future field applications and movement reconstructions (Fromentin et al., 2009; Grønkvær, 2016), manipulative experiments have largely over-neglected inter-individual differences. In general, a fish cohort experiencing similar migration pathways with identical duration is expected to record the same detailed temporal changes in their otoliths. Our data strongly support that it is not the case with the temporal resolution being highly variable on an individual basis. In particular, it appears that individuals with the highest metabolic rate are more likely to record detailed (*i.e.* brief) temporal changes than individuals having lower metabolic values. From a biological point of view, many animals perform extensive niche shifts because of their ability to utilize resources and avoid predation changes through their ontogeny (L'Abée-Lund et al., 1993). While the proximate mechanisms controlling the niche shift in fish remain partially unresolved, several studies have highlighted that movement patterns may be conditioned by the energetic status of the individuals, possibly in relation to metabolic activity (Dodson et al., 2013; Nevoux et al., 2019; Pettersson and Hedenström, 2000). Fast-growing individuals that maintain higher metabolic rates are energetically constrained at a younger age by limited food resources than slow growers (e.g. Metcalfe et al., 1989; Metcalfe et al., 1992; Økland et al., 1993). For example, in *Salmo trutta*, the food consumption and the energy budgets for early migrants were more than four times higher than those of the stream residents (Forseth et al., 1999). As a consequence, the finest time resolution would be available for fast-growing and more active individuals. This clearly can bias investigation conducted at the population level, although we recognize that the

degree of bias remains elusive. A time series composed of juvenile transects stitched together from fast-growing and low-growing in an unknown proportion may lead to an overall underestimate of movement patterns if movements are only detected for fast-growing individuals.

Whatever the iono-regulatory explanation, inter-individual differences in physiologically-related kinematics of elemental incorporation/depletion in otoliths have practical implications for the reconstruction of movement patterns, especially at a fine temporal scale (as illustrated in the Fig. 6). In Fig. 6 we used data from two individual fishes with high or low kinematics to illustrate the marked differences in otolith signature over time and reconstructed environmental history when fish follow the exact same environmental sequence between habitats with sharply contrasted barium concentration. Time-resolved chemical profiles from otoliths are conventionally analyzed using zoning or regime-shift algorithms that quantitatively classify otolith sequences into fish environmental histories (Fablet et al., 2007; Hedger et al., 2008; Vignon, 2015). While globally correct (i.e. at the population level), using a mean temporal scale in these algorithms will confuse noise signal with a brief incursion between contiguous habitats having contrasted geochemical signatures for some individuals. Alternatively, algorithms should no longer be completely naïve (i.e. unsupervised, using original untagged data with no supplementary information, Fablet et al., 2007) but rather would be supervised by accommodating individual covariables. Comprehensive knowledge of inter-individual differences in the kinematics of elemental incorporation/depletion and of factors that can readily be used as a proxy will enable us to determine realistic and appropriate supervised statistical methods (for statistical discussion, see Vignon, 2015).

### **Physiological proxy through lifetime**

While the incorporation/depletion kinematics is linked to physiological conditions of the fish, this component is not a steady-state condition and metabolic rate can change drastically throughout the lifespan of individuals (Treberg et al., 2016). In particular, osmoregulation can change over ontogeny (Tanner et al., 2015; Varsamos et al., 2001; Walther et al., 2010), leading to changes in plasma and endolymph composition that contribute to ontogenetic shifts in elemental composition (Fuiman and Hoff, 1995; Gallahar and Kingsford, 1992; Hüseyin, 2008; Morales-Nin et al., 2005; Tomas et al., 2006; Tzeng, 1996). This could be particularly visible for some species at metamorphosis or settlement when most of these variables change (e.g. Chen et al., 2008; de Pontual et al., 2003; Otake et

al., 1997; Sandin et al., 2005). Here, we can't completely assume that punctually-measured metabolic status remains stable over the complete course of the experiment (neither they are intrinsic, potentially life-long, characteristic of the individual). Such potential change through ontogeny may partly explain the overall relatively low correlations between physiological and chemical components. As in most experimental studies dealing with otolith microchemistry, our results were obtained from juvenile fishes. Considering age-related changes in elemental incorporation dynamics, however, interpretation of otolith signals in adult fishes based on observations from young life-history stages should be treated with caution (Sturrock et al., 2012; Walther et al., 2010). In addition, several other variables can concomitantly affect deposition of otolith carbonate during lifetime, including growth rate, stress, availability of nourishment, gonadal development, reproductive investment, ambient temperature, diurnal and seasonal cycles, and activity levels of individual fish (Friedland et al., 1998; Kalish, 1989; Kalish, 1991; Radtke and Shafer, 1992; Walther et al., 2010). All these changing variables often play a substantive role and have the potential to complicate attempts to retrospectively identify individual fish movement at the relevant temporal scale through lifespan.

In this context, the individual interpretation of otolith signal using the relevant temporal scale would greatly benefit from a retrospective tracking of change in the bioenergetic status of individuals over time. In particular, otolith growth may be used as a metabolic proxy. While numerous studies have experimentally linked standard metabolic rate to otolith growth (e.g. Wright, 1991; Wright et al., 2001; Yamamoto et al., 1998), the relationship may not be that simple in the field due to the potential presence of fitness-related costs associated with fast growth (Álvarez and Nicieza, 2005; Bang et al., 2007; Bochdansky et al., 2005). In addition, a high SMR may be a prerequisite for fast growth, but does not necessarily result in fast growth, revealing complex relationships between these two components. Several observations further suggested that processes associated with the secretory capacity and thus the metabolic rate of the endolymphatic epithelium are the driving forces behind otolith growth (Hüssy, 2008). While metabolism is known to be correlated among various tissues from the same organism (Houlihan et al., 1986), the epithelium of fast-growing otoliths would have had to increase secretory activity, thereby increasing epithelium metabolism, which would lead to an uncoupling from global metabolic rate (as measured using oxygen consumption) (Hüssy, 2008). Such decoupling may partly explain the significant but relatively moderate relationship between SMR and the observed kinematics. Unfortunately, information on the growth dynamics of the secretory area and the saccular epithelium are virtually non-existent (Hüssy, 2008), and in absence of any other alternative

several authors have preferred using otolith growth or even fish length as a proxy of metabolism. Unfortunately, otolith growth depends on metabolic activity, regardless of whether that activity goes into somatic or reproductive growth, movement, foraging, or maintenance (Limburg et al., 2018). In this context, several studies have emphasized the absence of a direct relationship between otolith and somatic growth (Baumann et al., 2005; Francis et al., 1992; Mosegaard et al., 1988; Wright, 1991), precluding the direct and reliable use of fish length as a proxy of metabolism. Our data indeed indicate a poor correlation between these components (i.e. all  $|p| < 0.32$ ) and also emphasized that incorporation/depletion kinematics are much more related to metabolism itself than otolith/somatic growth. Attempt to use otolith to unravel metabolic components may poorly mirror quantification made based on direct physiological measurement. However, recent attempts have been focused on the direct use of otolith isotopic composition (e.g.  $\delta^{13}\text{C}$  and  $^{26/24}\text{Mg}$  ratio in aragonite) to infer time-resolved field metabolic rates (reviewed in Grønkvær, 2016; Limburg et al., 2018; Chung et al., 2019). This suggests promising work in the near future.

### Conclusion

Contrary to the use of otoliths as group-specific natural tags for stock discrimination, the reconstruction of environmental histories of individual fish depends on effectively coupling environmental changes with otolith chemistry (Elsdon et al., 2008). Beyond assessing anadromous movement at a seasonal/annual scale, untangling movement and habitat use patterns at finer temporal scales still pose a greater challenge. In particular, the lack of a predictive and mechanistic understanding of the individual kinematics underlying ion incorporation/depletion still limits our fine-scale temporal interpretation of the chemical signal recorded in the otolith. Further kinetics knowledge is fundamental, whilst requested since it ultimately determines the spatio-temporal scope and the ecological questions that can be adequately resolved. Such consideration is also of primary importance since accurate identification of migratory pathways and habitat use underpins the effective management of exploited populations.

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- **Competing interests**

The authors declare no competing or financial interests.

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- **Data availability**

Data are available from institutional *Data INRAE* Digital Repository (<https://entrepot.recherche.data.gouv.fr/dataverse/inrae>): DOI at the following address: 10.57745/1VSDOG

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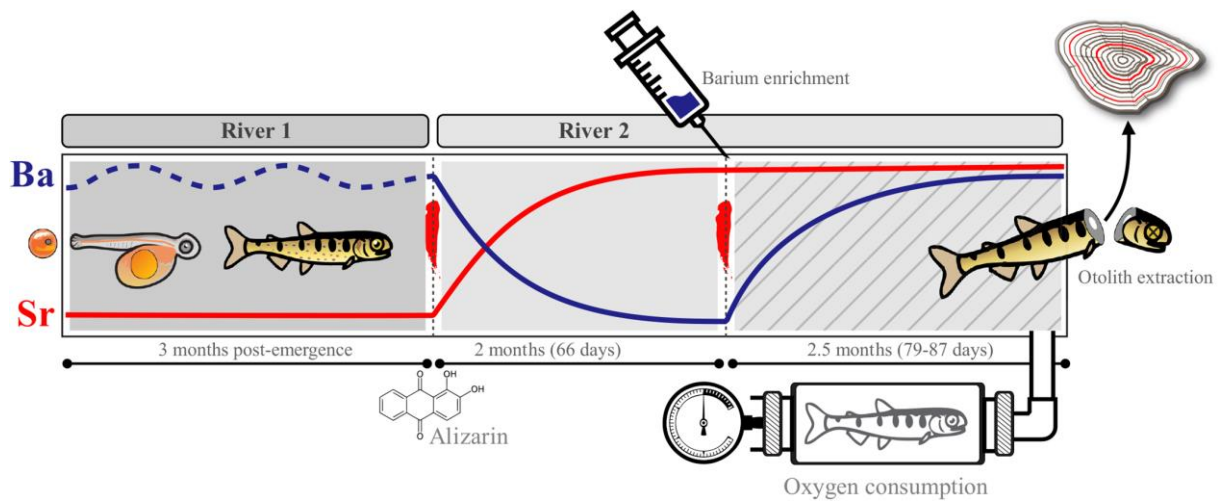
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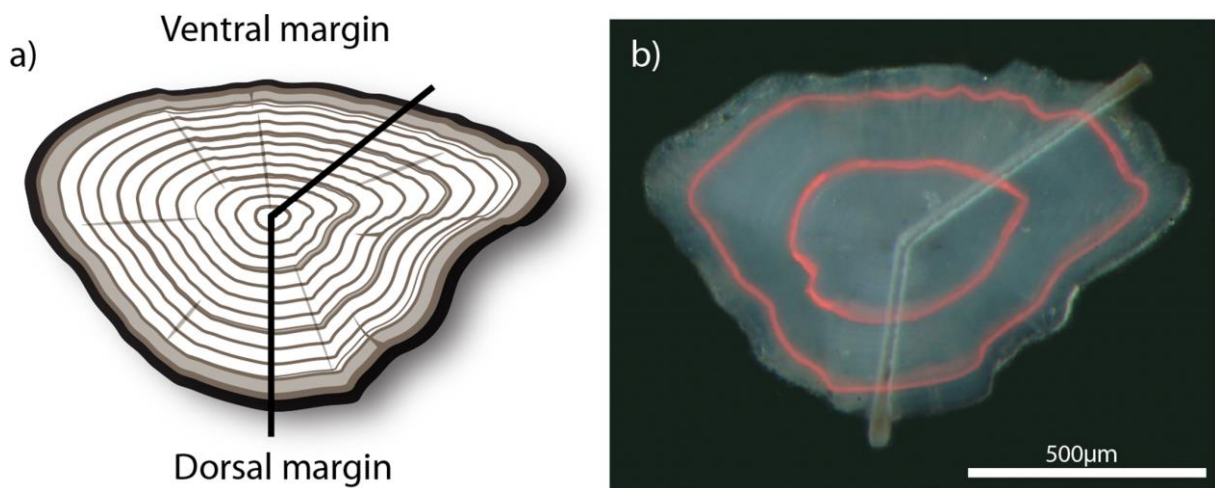
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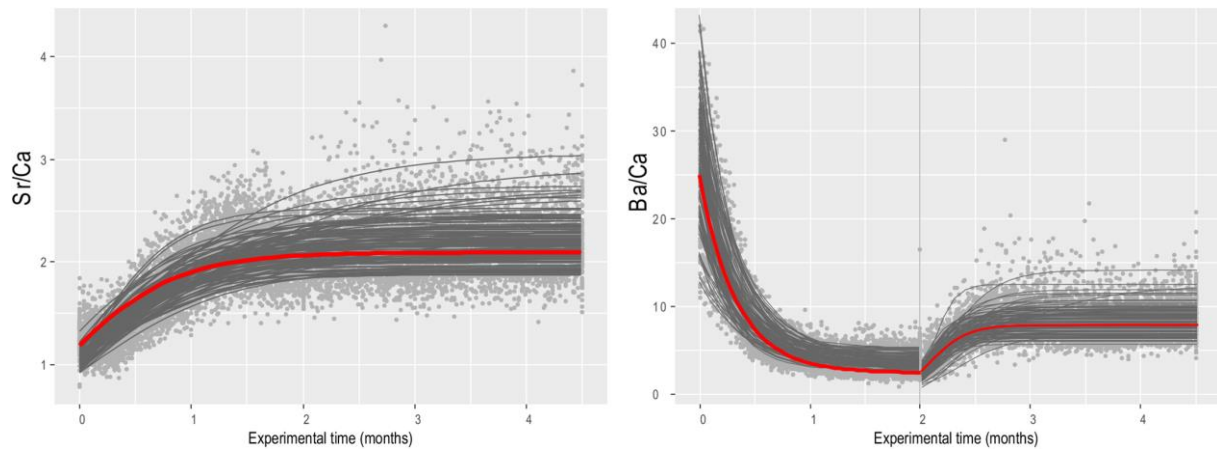
## Figures and Table



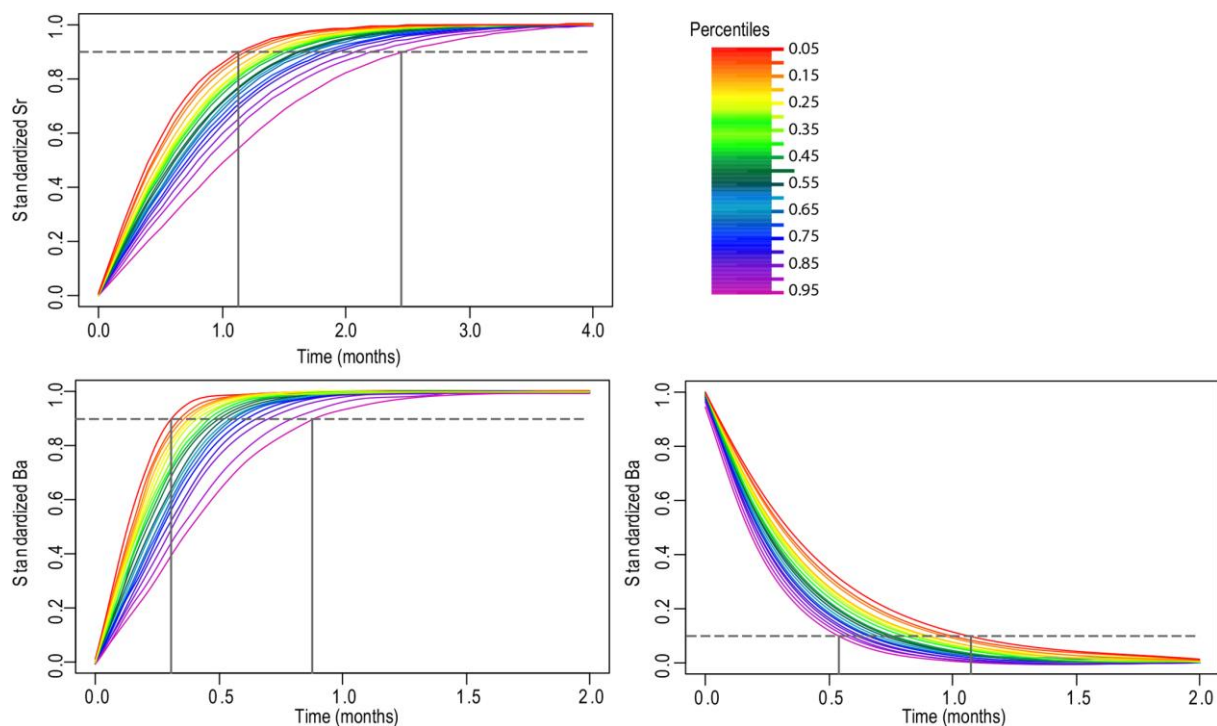
**Fig. 1** Schematic chronology of the main steps in the experimental design.



**Fig. 2** Position of the transects A) Typical location on the otolith. B) Composite image (white light and TXR filter) allowing us to relate the position of the otolith core and Alizarin mark to the corresponding time-resolved elemental concentrations along the transects.

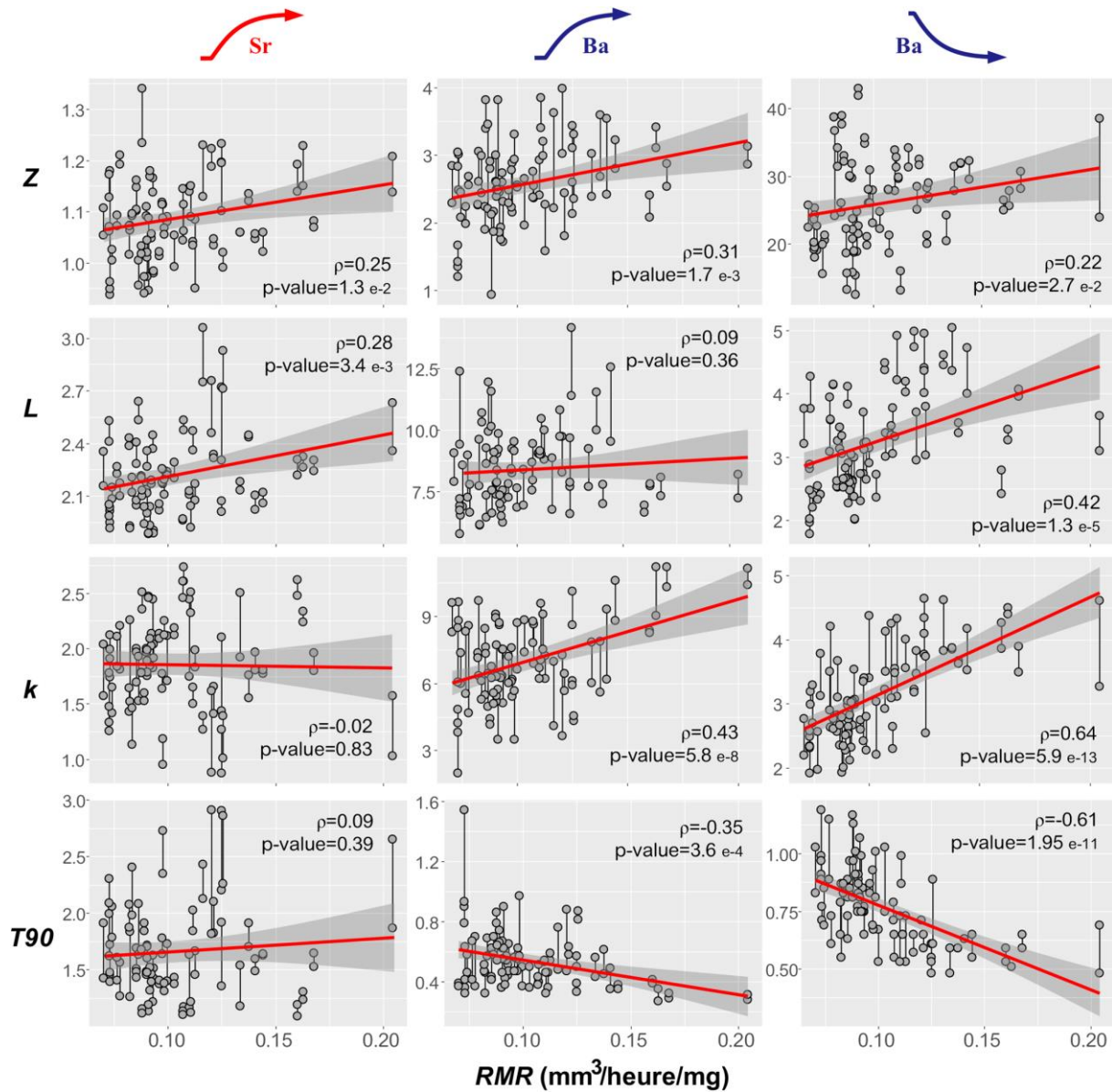


**Fig. 3. Trends in microchemical concentrations through time expressed in months.** Raw data as dots, predicted individual values as grey lines ( $N=51$ ), and mean trend (fixed effect in the mixed-model) in red. Experimental time started after the first Alizarin Red S mark (3 months post-emergence).



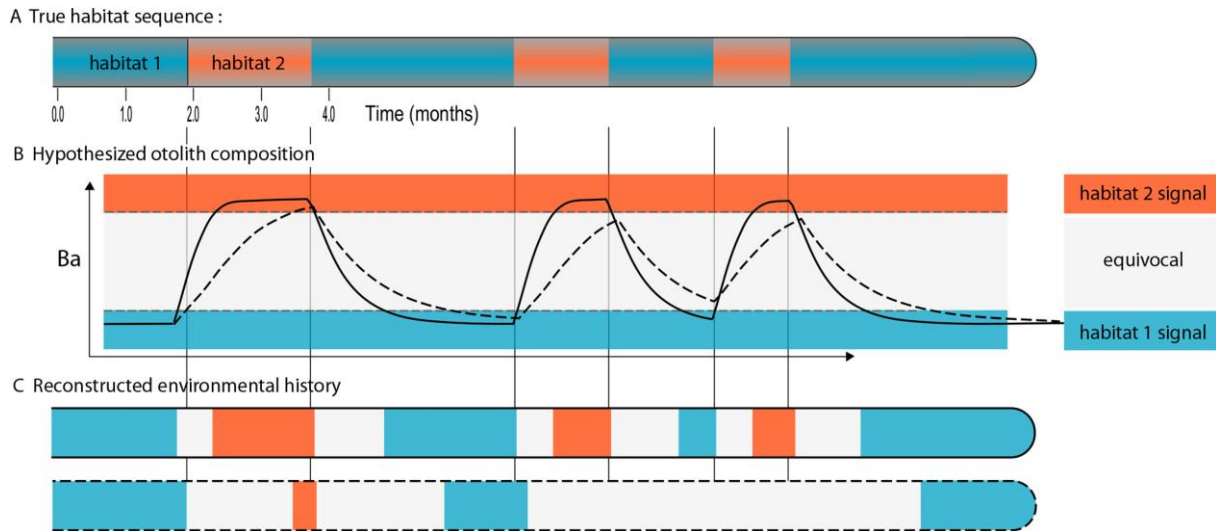
**Fig. 4. Quantile regression on predicted individual responses.** Values were standardized [0-1] between initial and asymptotic values. Relative times requested to reach 90% of complete equilibrium (dashed line) are depicted for 5<sup>th</sup> and 95<sup>th</sup> percentiles.





**Fig. 5. Repeated-measures correlation between metabolic (*RMR* only) and chemical components** (composition at equilibrium: *Z* and *L*, kinematics: *k* and time-lag: *T90*). Linear least-squares regressions in red. Strontium increase (first column), Barium increase (second column) and Barium decrease (third columns). *N*=51 individuals.

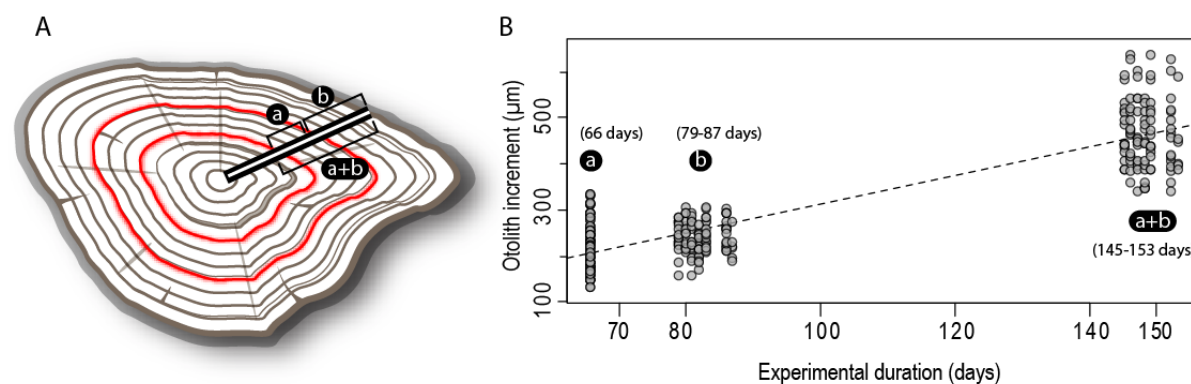




**Fig. 6 Simplified reconstruction of environmental history depending on individual's kinematics.** A) Here two fishes are hypothesized to follow the exact same environmental sequence (on top, using only two habitats with sharply contrasted barium concentration). B) Theoretical curves from the otolith are extracted from the figure 4, using two individuals with contrasted kinematics (i.e. one with high kinematics in plain line, and the second with low kinematics in dashed line, corresponding to 5<sup>th</sup> and 95<sup>th</sup> percentiles). Two thresholds are reported to discriminate areas with clear assignation (below which the signal typically corresponds to habitat 1, and under which the signal typically corresponds to habitat 2). C) Many methodologies/algorithms exist to reconstruct environmental history and for this purpose, we used the threshold-based zonation algorithm with equivocal area from Vignon (2015). While the figure is over simplistic, it clearly illustrates that individual variability in kinematics can affect reconstruction both in term of habitat-sequence and duration. For example, signal in the otolith for the last two incursions within the habitat with high barium concentration is under the detection limit and therefore completely neglected for the individual with the lowest kinematics.

**Table 1.** Monitoring of the elemental composition of water throughout the experiment. Sr/Ca and Ba/Ca ratios  $\pm$  1 sd. of the successive environments encountered by fishes.

	Lees-Athas	Nivelle	Nivelle (enriched)
Sr/Ca (mmol.mol <sup>-1</sup> )	0.89 $\pm$ 0.02	2.8 $\pm$ 0.3	2.8 $\pm$ 0.1
Ba/Ca ( $\mu$ mol.mol <sup>-1</sup> )	36 $\pm$ 1	149 $\pm$ 26	458 $\pm$ 65



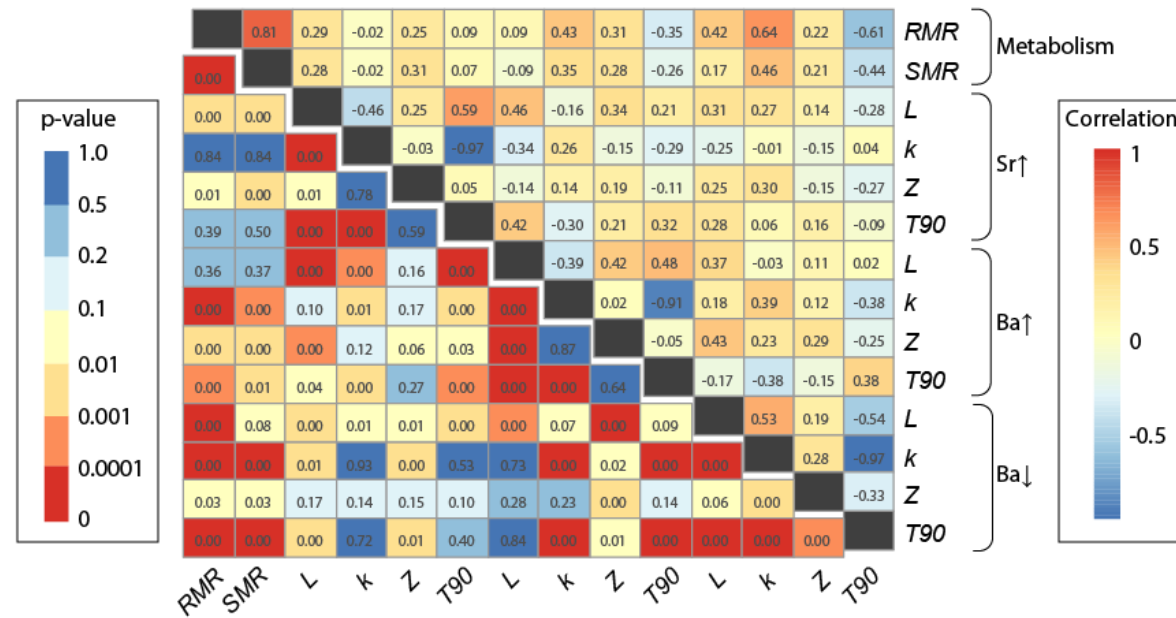
**Fig. S1. Otolith growth during the experiment.** **A)** Schematic otolith depicting the transect and the two Alizarin marks, separating the two phases of the experiment. The *a* area corresponds to the period between the initial translocation and Ba increase (66 days). The *b* area extends from Ba increase to the outermost edge of the otolith (from 79 to 87 days according to individuals). **B)** Correlation between otolith increment ( $\mu\text{m}$ ) and experimental duration (days). Linear regression through the origin using a dashed line (Pearson's correlation coefficient  $\rho=0.88$ ). In these conditions, we assumed constant growth during the experiment.

Due to the anisotropic growth of the otolith, the two transects of different lengths may exhibit distinct signals per individual. To test for this possible effect, transects (treated as a dual-factor: *short* vs. *long*) were primarily included in the statistical analysis as a fixed source of intra-individual variance (i.e. fixed effect term in mixed models) with individuals nested within each transects group. Alternatively, the *Replicates* term was discarded in another model, pooling all data per individual. For comparing the two models having distinct fixed-effects, the maximum likelihood estimator was preferred for fitting models. *Akaike weight* was used for model selection.

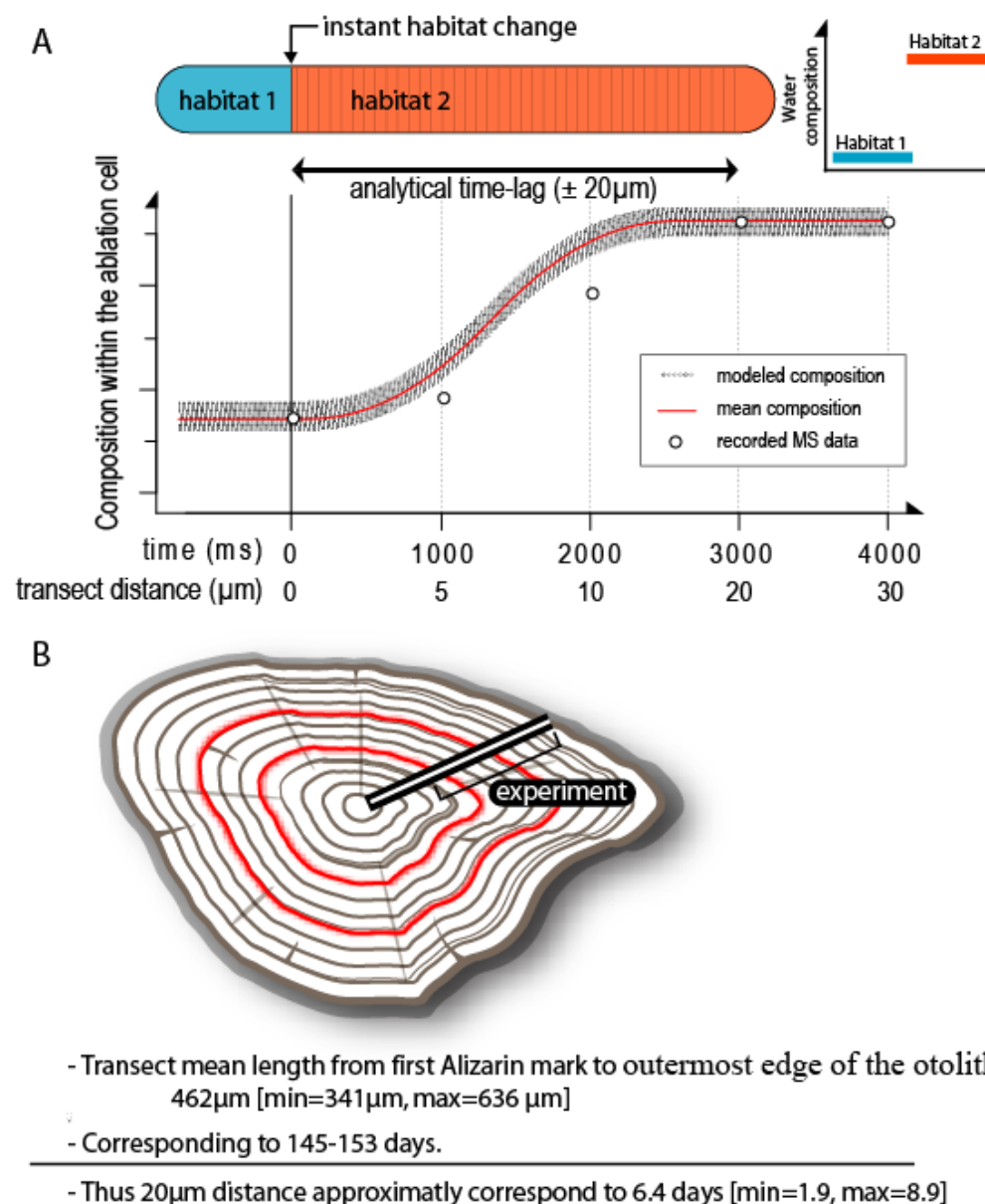
**Table S1.**

<i>Replicates</i>	<b>AIC</b>	<b>df</b>	<b>Akaike weight</b>
- discarded	20989.5	16	0.83
- as a fixed effect	20992.8	19	0.17

Akaike weights represent the relative likelihood of a model. The Akaike weight is a value between 0 and 1, with the sum of Akaike weights of all models in the candidate set being 1, and can be considered as analogous to the probability that a given model is the best approximating model. As the model treating transects as a fixed effect is far less likely than the alternative model, we further omitted this term (pooling all data per individual.). Whilst direct examination of this factor was beyond the scope of the present study, it is noteworthy that the chemical profiles were consistent for the two palindromic parts of the transect.



**Fig. S2. Pairwise (repeated) measures correlations (upper triangle) and associate p-values (lower triangle) among main variables.** *SMR*: standard metabolic rate, *RMR*: routine metabolic rate, *L*: asymptotic concentration, *k*: kinematic parameter (increasing/decreasing rate), *Z*: initial concentration, *T90*: minimum period of exposure time required for otoliths to incorporate/deplete 90% of Sr and Ba from initial to asymptotic modeled value.



**Fig. S3. Analytical time-lag** A) Given the specification of the ICP-MS (spot size of 12 µm in diameter, constant speed of ICP-MS beam of 5 µm.s<sup>-1</sup>, pulse rate of 50Hz, one raw instrumental data every 5 µm, washed out of the ablation cell in a continuous flow using an halfnormal distribution with 90% clearance within 150 ms, 99% clearance within 300 ms) and considering an instantaneous change in otolith composition between two habitats with increased elemental concentration, we have modeled the composition within the ablation cell and the mass spectroscopy (MS) data. We have then deduced the artefactual time-lag related to our analytical analysis, which is about 20 µm. B) The transect length corresponding to the experiment (145-153 days) can be extracted from the Alizarin marks, with a mean length of 462 µm among individuals [min=341 µm, max=636 µm]. This all leads to an estimated artefactual time-lag of about 6.4 days [min=1.9, max=8.9 days].

**Table S2. Operating parameters of LA-ICP-MS from cited works that quantified time-lag in juvenile fishes.** Data does not exhibit clear relationship between these parameters and time lags, suggesting the absence of prominent analytical bias between these studies.

References	Family	Time-lag (days)	Spot size ( $\mu\text{m}$ )	Frequency (Hz)	Speed ( $\mu\text{m s}^{-1}$ )	ablation depth ( $\mu\text{m}$ )
Macdonald and Crook (2010)	Percichthyidae	30 (Ba/Ca) 40 (Sr/Ca)	6×100 rectangular	10	2	3
Elsdon and Gillanders (2005b)	Sparidae	20 (Sr/Ca)	30 $\emptyset$	6	1.3	
Lowe <i>et al.</i> (2009)	Centrarchidae	21 (Sr/Ca)	30-35 $\emptyset$	16	5	
Miller (2011)	Salmonidae	10 (Ba/Ca) 10 (Sr/Ca)	40 $\emptyset$	8	1	
Secor <i>et al.</i> (1995)	Moronidae	over weeks (Sr/Ca)	5 $\emptyset$		13.5 to 14 intervals	
Engstedt <i>et al.</i> (2012)	Esocidae	Over 50 (Sr/aCa)	map scan		6 intervals	