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1 **Freshwater fish otoliths record signals from both water and physiological**
2 **processes: new insights from Sr/Ca and Ba/Ca ratios**

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4 G. Bareille^{1*}, M. Vignon², A. Chappaz³, A. Fontaine¹, H. Tabouret¹, F. Morat⁴, J. Martin^{1,§},
5 J.C. Aymes², F. Daverat², C. Pécheyrán¹, O. Donard¹

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7
8 ¹ Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, MIRA, Pau, France,
9 gilles.bareille@univ-pau.fr, anthony.fontaine@univ-pau.fr; helene.tabouret@univ-pau.fr,
10 christophe.pecheyrán@univ-pau.fr, olivier.donard@univ-pau.fr,

11
12 ² Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-
13 Nivelles, France. matthias.vignon@inrae.fr, Jean-Christophe.Aymes@inrae.fr,
14 francoise.daverat@inrae.fr.

15
16 ³ STARLAB, Department of Earth and Atmospheric Sciences, Central Michigan University,
17 MI 48859, USA. anthony.c@cmich.edu

18
19 ⁴ PSL Université Paris : EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan,
20 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France; Laboratoire d'Excellence «
21 CORAIL ». fabien.morat@univ-perp.fr

22
23 [§] Current address : Fédération Départementale de Pêche et de Protection du Milieu Aquatique
24 33 – 10 ZA du Lapin – 33750 Beychac-et-Caillau. jean.martin@peche33.com
25

26 * Corresponding author. Email: gilles.bareille@univ-pau.fr

28 **ABSTRACT**

29 Using strontium and barium in otoliths to determine natal origins and understand patterns of
30 fish movements is based on the fundamental assumption that otoliths record water chemistry
31 signals without any major alterations. Although prior studies highlighted that fish physiology
32 can modify the water signal in otolith, studies for freshwater fish are scarce. We exposed
33 different group of Atlantic salmon parr *Salmo salar* to different scenarios of ambient-level
34 variations in Sr/Ca and Ba/Ca ratios and then combined otolith chemical profiles with
35 environmental data (water chemistry, temperature), Fulton's index and otolith growth rates to
36 assess what factors explain/influence the elemental ratios of Sr and Ba in otoliths. Generalized
37 additive mixed-models (GAMMs) using water-based otolith composition, temperature,
38 Fulton's index and 'individual' as explanatory variables, allow to demonstrate that water
39 chemistry alone cannot fully explain measured ratios in otoliths, except in scenarios involving
40 significant changes in water chemistry. Others factors (physiological effects) should be
41 accounted for reproducing short and minimal seasonal variations in water composition and
42 considering that inter-individual variability contributed quite significantly in most scenarios.

43

44 **Keywords:** Otolith chemistry; Otolith growth rate; Atlantic salmon; Freshwaters; Field and
45 experimental studies.

46

47 **INTRODUCTION**

48 Examining otolith elemental records for reconstructing fish population structure,
49 connectivity and movement patterns, has emerged as a reliable method complementary to
50 tagging and genetic studies (Walther et al., 2010). Otoliths are composed of calcium (Ca)
51 carbonate structures that grow throughout the life of the fish, and incorporate chemical elements
52 derived from both extrinsic (environmental) and intrinsic (physiological dynamics) sources

53 (Campana et al. 1985; Campana, 1999; Woodcock et al., 2012; Sturrock et al., 2012, 2014,
54 2015). As otolith expand, new Ca carbonate layers are formed. Lattice vacancies present in any
55 crystal structure are then being filled with trace elements via extrinsic and intrinsic inputs. This
56 unique feature constitutes the basis for reconstructing fish life-histories. However, to achieve a
57 reliable reconstruction across habitats, we need to associate changes in otolith elemental records
58 with either signals induced by environmental conditions (temperature, ambient water
59 composition) and/or signatures resulting from biological processes (growth rate, diet,
60 metabolism) (Campana, 1999; Campana and Thorrold, 2001; Walther, 2019). In addition, this
61 approach must be reliable throughout the entire fish life and applicable for all locations visited
62 by the fish. Unfortunately, disentangling between environmental and biological influences on
63 the element-otolith association remains challenging, particularly when examining fish otoliths
64 from freshwater systems. Prior studies characterized the factors controlling elemental
65 incorporation into otoliths mostly by focusing on fish from estuarine and marine environments
66 through experimental approaches (Sturrock et al., 2012; Hüsey et al., 2020). Taken together,
67 the main finding is the complexity of the system as several mechanisms are involved. Otolith
68 records a combination of chemical (water salinity), physical (water temperature) and biological
69 (growth rate) factors, as well as specific binding pathways related to each species (Eldson and
70 Gillanders, 2003; Barnes and Gillanders, 2013; Sturrock et al., 2014, 2015; Stanley et al., 2015;
71 Izzo et al., 2018). Surprisingly, most experiments were conducted with fish exposed either to
72 constant water chemical and physical parameters or to unrealistic transitions (Sturrock et al.,
73 2012), making the data collected difficult to interpret (Sturrock et al., 2015; Izzo et al., 2018).
74 This may be particularly problematic for studies conducted in freshwater systems where we can
75 expect the water chemical composition to display a stronger heterogeneity than marine waters.
76 Previous works found contrasted outcomes on how temperature affects the elemental uptake of
77 marine fish otoliths. A first group of studies reported an increase of the Sr/Ca ratio with

78 temperature (Martin et al., 2004; Collingsworth et al., 2010; Miller, 2011; Webb et al., 2012;
79 Reis-Santos et al., 2013; Walsh and Gillanders, 2018), a second group observed an opposite
80 trend with the Sr/Ca ratio decreasing as temperature rises (Radtke, 1989; Radtke et al., 1990;
81 Townsend et al., 1995; Martin and Wuenschel, 2006; DiMaria et al., 2010; Marohn et al., 2011)
82 while no significant effect of temperature on the Sr/Ca and Ba/Ca ratios could be found by a
83 third group of studies (Gallahar and Kingsford, 1996; Gaudie, 1996; Chesney et al., 1998;
84 Walther et al., 2010; Miller and Hurst, 2020). The diet composition may affect the Sr/Ca and
85 Ba/Ca ratios in otoliths yet there is still no consensus regarding the direction (Gallahar and
86 Kingsford, 1996; Hoff and Fuiman, 1995; Collingsworth et al., 2010; Webb et al., 2012;
87 Doubleday et al., 2013; Miller and Hurst, 2020). Additionally, a certain inertia between
88 variations in the chemical water composition and subsequent changes in otolith microchemistry
89 were documented suggesting a potential metabolic influence (Elsdon and Gillanders, 2005;
90 Macdonald and Crook, 2010; Yokouchi et al., 2011; Miller, 2011). Finally, ontogenetic
91 variations are likely to affect otolith chemistry regardless of environmental conditions (De
92 Pontual et al., 2003; Kalish, 1989).

93 The Sr/Ca and Ba/Ca ratios have been used to determine the displacement or natal
94 origins of anadromous and freshwater fish (Wells et al., 2003; Brown and Severin, 2009;
95 Doubleday et al., 2013; Martin et al., 2013a; Teichert et al., 2018). However, despite possible
96 confounding effects, the influence of temperature, diet and growth rate on the incorporation of
97 Sr and Ba were rarely investigated in freshwater environments (Collingsworth et al., 2010;
98 Miller, 2011; Doubleday et al., 2013). When examined for freshwater systems, relationships
99 between water chemical composition and elemental otolith records were only investigated for
100 short time-periods (Teichert et al., 2018) or over extended periods of life (Martin et al., 2013a).
101 The fundamental assumption made when using Sr/Ca and Ba/Ca ratios is that otoliths record
102 water chemistry signals without any major alterations. Although prior studies focusing on

103 marine systems highlighted that fish physiology can modify the water signal in otolith, similar
104 evidences for freshwater fish are scarce. Thus, it is crucial to properly assess how the water
105 chemistry affects the otolith capacity to record environmental changes in freshwater systems.

106 The two aims of our study were i) to assess whether Sr/Ca and Ba/Ca ratios in otoliths
107 can reliably reflect different life-history scenarios in salmon parr and ii) to verify if other
108 variables (i.e. temperature, fish condition factor and individual variability) were able to
109 explain/influence deviations between observed and water-based predicted Sr/Ca and Ba/Ca
110 ratios. Several scenarios simulating an array of chemical changes, including steep decrease or
111 increase, transient increase and slight seasonal oscillations in water Sr/Ca and Ba/Ca ratios,
112 were tested using generalized additive mixed-models (GAMMs, Lin and Zhang, 1999) and
113 correlation tests (Table 1).

114

115 **MATERIALS AND METHODS**

116 **Otolith sampling**

117 A first group of twenty-nine otoliths were selected from fish that spent their early life in
118 two hatcheries (Medous and Sassis) during fry stage (Table 1). Fish were collected in 2006,
119 2009, and 2010 on sites along the Gave de Pau River (Table 1, Figure S1) using a standardized
120 electrofishing method. Twenty-two parr were classified as age-0⁺ and seven as age-1⁺ years.
121 Fry held at a relatively constant water temperature (8-10°C) in the Medous hatchery were
122 released April 15th of 2005, 2006, 2009 and 2010 in the Gave de Pau River — 2 weeks after
123 yolk-sac resorption. Fry at the Sassis hatchery were kept at constant temperature (12°C) for a
124 longer period of time and artificially fed for about 3 months with marine-derived pellets
125 (Biomar, Nersac, France), before their release in the Gave de Pau River on June 15th of 2009.
126 In addition, six to ten fries from the two hatcheries were also collected in 2009 and their otoliths
127 examined for chemical profiles.

128 A second group of otoliths was also obtained from a one hundred thirty-nine-day
129 experiment conducted in 2011 at the Lapitxuri field-station (Martin, 2013; ECP, INRAE, 2018)
130 located on a tributary of the Nivelle River (France) ($1^{\circ}29'W$, $43^{\circ}17'N$; Figure S1). Briefly,
131 Atlantic salmon embryos (forty-eight-day-old) conceived by wild genitors from the Bidassoa
132 River were incubated for sixty-two days in a flow-through incubator before the fry were
133 transferred to a Lapitxuri experimental channel for seventy-seven additional days under natural
134 conditions (i.e. photoperiod, water chemistry, temperature, natural prey found in the channel).
135 Following hatching in the flow through incubator (day fifty-five), fry experienced a flood event
136 on day ninety. Larvae were thus exposed to a 40% increase in the Ba/Ca ratio for ~ three weeks
137 (days ninety to one hundred and eleven). The Ba/Ca ratio then constantly decreased until day
138 one hundred thirty-two to finally remain relatively stable during the last two months of the
139 experiment —without returning exactly to pre-flood values. The Sr/Ca ratio stayed constant
140 during the overall experiment. To identify the transition period between hatching and release
141 dates in the Lapitxuri experimental channel, two Alizarin Red S ($C_{14}H_7O_7SNa$, Sigma-Aldrich
142 Corporation, Saint Quentin Fallavier, France) fluoromarking immersions were carried out. The
143 first was performed shortly post-hatching on day sixty and the second on day one hundred and
144 ten, i.e. five to ten days after larvae exhausted their vitelline reserve but before fry were
145 transferred to the experimental channel. One hundred and sixty fish survived the experiment.
146 However, large variation in the fork lengths was then observed (mean L_F : 53.5 ± 6.1 mm; range:
147 39-71 mm; Figure S2). To account for the size effect, twenty-four otoliths of one hundred
148 eighty-seven-day-old salmon juveniles were selected for otolith chemistry in three different size
149 classes: eight with L_F between [35-45] mm, nine with L_F between [50-55] mm and seven with
150 L_F between [60-75] mm (Table 1, Figure S2).

151

152 **Water sampling and analysis**

153 Water samples were collected in 2010 every two weeks over a two month-period at the
154 Medous hatchery (n=5, mid-February to mid-April) and over a four month-period at the Sassis
155 hatchery (n=8, mid-February to mid-June). Water sampling was conducted every week at the
156 Lapitxuri field-station (n=18) from January 17, 2011 to June 1, 2011. Water chemistry was also
157 monitored in the Gave de Pau River every week in 2007 (n=31), every month in 2010 (March
158 to August, n=7) and on more scattered dates in 2009 and 2011 years (n=7). The 2007 dataset
159 covered the entire hydrological cycle and were part of a water pollution-monitoring program of
160 trace metals that received extra funding. Temperature (°C) and pH were also recorded at the
161 time of each water withdrawal in the Gave de Pau (2007, 2009, 2010, 2011). Water temperature
162 was measured daily every two hours at the Lapitxuri experimental station.

163 Water samples were filtered through a PVDF Whatmann 0.45 µm filters with syringes
164 into 125 ml acid-washed LDPE (low-density polyethylene) bottles and acidified (2%) using
165 ultrapure nitric acid HNO₃ (JT Baker, Ultrex II). All plastic materials used for sample collection
166 were acid-washed prior to use. Samples were immediately transferred to a cooler and
167 refrigerated upon return to the laboratory.

168 Filtered water samples were analysed for calcium (Ca), strontium (Sr) and barium (Ba)
169 concentrations using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry -
170 ACTIVA, Jobin Yvon) using external calibration (Martin et al., 2013a). Certified reference
171 materials (SLRS-5, NRCC, Canada – SRM1640A, NIST, USA) were analysed every ten
172 samples. Precision and accuracy were determined by analysing reference materials ($n = 48$) and
173 were all well within 3% of the targeted value except the precision for Ba/Ca that was slightly
174 higher (<10%).

175

176 **Otolith analyses**

177 Otoliths were processed using the protocol described in Martin et al. (2013a). Briefly,
178 one *sagitta* per fish was embedded in epoxy resin (Araldite 2020, Escil) with the primordial
179 surface downwards. Resin blocks were ground gradually with ultrapure water and sandpaper
180 (1200– 4000 grit) until the primordium was reached and polished with diamond-tipped
181 polishing powder. Finally, otoliths were carefully rinsed with ultrapure-water, and then air-
182 dried before being stored in individually labelled plastic vials.

183 Detection of Alizarin Red S marks on otoliths collected at the Lapitxuri experiment was
184 carried out using an Eclipse 90i compound microscope (Nikon, Champigny sur Marne, France),
185 equipped with a Nikon G-2A filter cube (excitation filter: 510–560 nm / dichromatic mirror:
186 565 nm / barrier filter: 590). Digital images of each structure were recorded using the Nikon
187 digital camera DXM1200C. Otoliths were carefully examined under transmitted light at 400×
188 and 1000× magnifications to check for hatch and yolk absorption marks. Distances from the
189 otolith core to the two Alizarin Red S marks were measured. The second otolith of some
190 Lapitxuri parr was prepared on a transverse plan for daily growth rate analysis. For this purpose,
191 otoliths were embedded in epoxy resin Araldite 2020 (Escil, France) and ground on the
192 transversal plane with abrasive papers (2 400 grit) to expose the daily growth increments until
193 satisfactory readability and polished with diamond solutions (0.25 µm; Escil; Chassieu, France).
194 Otolith photographs were taken and assembled into a large image using Photostitch software
195 (Canon; www.canonutilities-photostitch.en.lo4d.com). Using ImageJ (NIH, USA), fish growth
196 increments were counted along the profile from the core to the outer edge, and the distance
197 between each count was incremented to estimate daily growth rates. Counts were performed
198 twice by two independent readers.

199 Cleaned otoliths were analysed for Sr, Ba, and Ca concentrations using an Elan DRC 2
200 (Perkin Elmer, USA) inductively coupled plasma quadrupole mass spectrometer (ICP-MS)
201 coupled to a high repetition rate Infra-Red femto-second laser ablation system (Alfamet-

202 Novalase, France; Martin et al., 2013a). Strontium and Ba concentrations were then expressed
203 as molar ratios to calcium in $\text{mmol}\cdot\text{mol}^{-1}$ for Sr/Ca and $\mu\text{mol}\cdot\text{mol}^{-1}$ for Ba/Ca on the basis of
204 the stoichiometry of Ca carbonate ($9.73 \text{ mmol Ca}\cdot\text{g}^{-1}$ otolith) (Brown and Severin, 2009).

205

206 **Data treatment and statistical analysis**

207 Predicted Sr/Ca and Ba/Ca otolith profiles (water-based otolith composition) were
208 calculated by multiplying partition coefficients (D_{element}) with the water chemical composition.
209 Partition coefficients for Sr/Ca (D_{Sr}) and Ba/Ca (D_{Ba}) ratios have been shown to be influenced
210 by water concentration, temperature, diet and growth rates (DiMaria et al., 2010; Miller et al.,
211 2020). We calculated D_{Sr} and D_{Ba} from otolith and water data from thirteen rivers covering a
212 relatively broad spectrum of Sr/Ca and Ba/Ca ratios (Table 2; Martin et al., 2013a,b). Both D_{Sr}
213 and D_{Ba} were obtained using chemical ratios of the outer edge of otoliths (i.e., the last 100 μm
214 that represents about one to two months of life) and the average water ratios of the
215 corresponding summer months. We assumed the effects of temperature and growth across the
216 rivers are limited and that predicted otolith signals must be in agreement with the chemical
217 otolith ratio recorded during that summer period. Because no significant trend was found
218 between D_{Sr} and the Sr/Ca water composition (Figure S3), the average value of the thirteen
219 rivers was used, $D_{\text{Sr}} = 0.26 \pm 0.05$ (Table 2). Conversely, as the D_{Ba} decreases exponentially with
220 the water composition (Figure S3), three average values were defined (Table 2): one for the
221 lowest water ratios ($D_{\text{Ba}} = 0.026 \pm 0.008$ that was used for the Medous and Sassis hatcheries),
222 one for intermediate ratios ($D_{\text{Ba}} = 0.024 \pm 0.006$ that was used for the Gave de Pau River) and
223 finally one for the highest water ratios ($D_{\text{Ba}} = 0.017 \pm 0.005$ that was used for the Lapitxuri
224 experiment). The uncertainty on the partition coefficients ($\Delta D_{\text{element}}$) was determined from
225 standard deviation (SD) from the chemical composition of waters and otoliths according to the
226 following equation:

227

$$\Delta D_{Me} = [(\sigma_{Me}^{otolith} / \bar{X}_{Me}^{otolith}) + [(\sigma_{Me}^{water} / \bar{X}_{Me}^{water})] \times D_{Me} \quad (1)$$

229

230 where Me corresponds to the Sr/Ca or Ba/Ca ratios. Predicted average, minima and maxima for
231 Sr/Ca and Ba/Ca ratios were calculated and compared with the measured signals (Figures 1, 2,
232 3, 4 and 5). We made the assumption that deviation from the prediction was significant when
233 the measured signal was below the minima or above the maxima. Because the water database
234 from the Gave de Pau River was collected over several years, a certain degree of heterogeneity
235 can be observed, yet a general annual trend can also be detected. We thus decided to process
236 all data as an integrative and smoothed curve representative of a median annual hydrological
237 cycle. As such, a polynomial regression fitting was applied to the data to predict seasonal
238 changes in Sr/Ca and Ba/Ca water patterns into the Gave de Pau River (Figure S4).

239 For each individual, raw elemental values (Sr/Ca or Ba/Ca, mmol.mol⁻¹), water-based
240 prediction of otolith composition and temperature were reported to the number of days since
241 egg deposition (Lapitxuri field-experiment) or to a relative temporal scale corresponding to the
242 age of parr in months. Well-defined temporal anchor points, i.e. alizarin marks for the Lapitxuri
243 mesocosm experiment and breakpoints related to habitat changes of known calendar date
244 (hatcheries to Gave de Pau River), were used to delineate segments where a constant growth
245 rate was applied for a given individual. For age-1⁺ parr, the annual growth mark was used to
246 better constrain the otolith chemical signals as a function of time. The two known temporal
247 markers (two fluorescent marks: AM1 and AM2) at 60 and 110 days post egg deposition,
248 respectively, were used on otoliths of juveniles reared at the Lapitxuri field-station to transform
249 distances (µm) on otolith chemical profiles to days by assuming constant growth rate on each
250 interval. In the case of hatchery-reared individuals stocked in the Gave de Pau River, we used
251 otolith ⁸⁷Sr/⁸⁶Sr profiles (Martin et al., 2013a) to delimit the Gave de Pau habitat from that of

252 Sassis hatchery. Strontium isotopic signatures for this site (0.7092-0.7095) were indeed
253 significantly different from the one for the Gave de Pau River (0.710-0.7103) (Martin, 2013).
254 For the Medous hatchery, comparison of parr profiles to those of fry collected in this hatchery
255 allowed us to identify changes in habitat as both Sr/Ca and Ba/Ca ratios exhibit rapid and
256 significant changes following stocking. Assuming constant growth rate through time may not
257 be optimal and could lead to time deviation for some otolith chemical profiles compared to
258 others at least between the anchor points (Sturrock et al., 2015); however, we think it is a
259 reasonable assumption for our study.

260 The Fulton's index (for the entire fish life) and the otolith growth rate (OGR; for specific
261 periods of time) were determined. The Fulton's index (K-factor) was determined to estimate
262 the body condition using the following equation (Fulton, 1904):

$$263 \quad K = 100 * W/L^3 \quad (2)$$

264 Where W is the weight in g and L the length in cm.

265 Average OGR were calculated for age-0⁺ parr by dividing the distance between anchor
266 points by the number of associated days both for the Gave de Pau development period (from
267 the date of stocking until collection in the fall) and the Lapitxuri experiment (AM2-edge). OGR
268 of two specific periods during the experimentation in the Lapitxuri channel, i.e. the flood event
269 and the normal flow conditions during the last month, were determined by averaging daily
270 growth rates.

271 Individual raw elemental values (Sr/Ca and Ba/Ca, mmol.mol⁻¹ and μmol.mol⁻¹,
272 respectively) were modeled using generalized additive mixed-models (GAMM, Lin and Zhang,
273 1999) implemented using the *mgcv* package (version 1.8-24) in R 3.2.2 (Wood, 2017). Various
274 models were fitted for each life-history scenario, including varying fixed and random effects.
275 While the null model (m0) only contains an intercept, the saturated model (m1= full model)
276 contains the following explanatory variables: i) Water-based prediction of otolith composition

277 (mean-value, $\text{mmol}\cdot\text{mol}^{-1}$ for Sr/Ca and $\mu\text{mol}\cdot\text{mol}^{-1}$ for Ba/Ca), ii) water temperature ($^{\circ}\text{C}$), and
278 iii) individual Fulton's index. Other models were submodels of the saturated one and consisted
279 in all possible combinations of one, two or three variables (see Table S1 for further details on
280 models' formula). This approach allows to capture potential non-linear trends between the
281 elemental composition of otoliths and the explanatory variables. In these mixed-models, water-
282 based otolith composition (linear trend), water temperature (non-linear trend), and Fulton's
283 index (linear trend) are treated as a fixed effect. In contrast, 'individuals' were treated as random
284 effects (i.e. factor-smoother interaction between individuals and water-based prediction of
285 otolith composition within *mgcv*), allowing for the baseline value of elemental composition (i.e.
286 smoothness parameter) to vary for each fish. Given the presence of a random effects, the
287 restricted maximum likelihood (REML) estimator was preferred for fitting models (Reiss and
288 Ogden, 2009; Wood, 2011). Akaike information criterion (AIC) is a robust approach to compare
289 the different model structures, accommodating the effects of penalization and smoothing
290 parameter uncertainty (Wood et al. 2016). Here, Akaike weight was used for model selection.
291 Akaike weights represent the relative likelihood of a model. The Akaike weight is a value
292 between 0 and 1, with the sum of Akaike weights of all models in the candidate set being 1, and
293 can be considered as analogous to the probability that a given model is the best approximating
294 model. In all the datasets, full saturated models appeared among the most likely ones (Table
295 S2) and were therefore subsequently used. Full models are described as follow:
296 "Otolith_Composition = Water-based otolith composition + $f(\text{Water-based otolith composition})$
297 + $f(\text{Temperature})$ + Fulton's + $\zeta_{\text{individuals}}$ + ϵ_i ", where $f(x)$ is a smooth function of the covariate x ,
298 $\zeta_{\text{individuals}}$ is the random effect for fish [smoothness parameter of $f(\text{Water-based otolith}$
299 composition) allowed to vary for each fish] and ϵ_i is a Gaussian error term. Summary, including
300 fixed terms and approximate significance of smooth terms, of the full saturated model for each
301 scenario is reported in supplementary material (Table S3).

302 The fit of the full model was appraised by comparing the overall deviance explained
 303 by the full model with deviance explained by a null model (intercept only) as:

$$304 \frac{deviance_{(null\ model)} - deviance_{(full\ model)}}{deviance_{(null\ model)}} \quad (3)$$

305 Likewise, partial deviance explained by each variable in the full model was estimated by:

$$306 \frac{deviance_{(model\ without\ the\ explanatory\ variable)} - deviance_{(full\ model)}}{deviance_{(null\ model)}} \quad (4)$$

307 These calculations are used to determine the relative importance of each predictor (in terms of
 308 variance). Summed partial deviance explained for explanatory variables did not always add up
 309 to the total deviance explained due to concurvity (i.e. the fact that one smooth term can be
 310 approximated by some combination of the other smooth terms in the model. In other words,
 311 due to the overlap in the variance explained by different variables within the same model).

312

313 To test the role of individual OGR for explaining the large inter-individual variability
 314 of Sr/Ca and Ba/Ca ratios during specific periods (equilibrium stage, unexpected peaks, flood
 315 event), Pearson or Spearman correlation tests were conducted depending on the data normality
 316 according to the Shapiro-Wilk normality test. Statistical analyses were carried out using R
 317 software version 3.2.2.

318

319 RESULTS

320 Elemental otolith composition: Measured vs water-based predictions

321 Predicted Sr/Ca and Ba/Ca ratios are shown as average (bold), minima (dotted) and maxima
 322 (dotted) lines over the entire fish life for qualitative comparison with all the measured profiles
 323 of each individual parr according to their capture site (Gave de Pau River, Lapitxuri channel)
 324 and their hatchery of origin (Medous, Sassis). Overall, Sr/Ca and Ba/Ca ratios in otoliths usually
 325 effectively represent changes in water chemical composition (Fig. 1, 2, 3, 4 and 5). However,

326 the measured ratios in otoliths differed significantly from the predicted ratios for specific
327 periods of time (Sr/Ca: every winter; Ba/Ca: following the stocking or in response to the flood
328 event) and depending on the individual.

329

330 *Sr/Ca ratio*

331 A significant decrease in Sr/Ca_{otolith} occurred when parr experienced a steep decrease in the
332 water Sr/Ca ratio, i.e. when they were moved from the Sassis hatchery to the Gave de Pau River
333 (Fig. 1). Variability was observed among individuals for the time required for the Sr/Ca_{otolith} to
334 reach a new equilibrium, ranging from one month to two and a half months, resulting in
335 significant differences between the measured and predicted ratios when individuals were
336 transferred from their original habitat to another. Beside that episode, the predicted Sr/Ca ratios
337 generally matched the measured ratios.

338 For the individuals only subjected to slight seasonal fluctuations (Medous moved to the
339 Gave de Pau River), the measured Sr/Ca_{otolith} generally partially matched the predicted trend
340 from water chemistry (Fig. 2a,b). Surprisingly, fry reared at the Medous hatchery exhibited
341 maximal values for Sr/Ca_{otolith} during their early life in the Gave de Pau (age-0⁺, Fig.2a) but
342 also during their second winter (age-1⁺, Fig. 2b,c), while water chemical composition displayed
343 minimal ratio for the same time period (Fig. 2b; Figure S4). The measured Sr/Ca_{otolith} was
344 slightly higher than Sr/Ca ratio predicted from the water chemistry during the stay at the
345 Medous hatchery, probably because of the contribution of Sr-enriched diet. Furthermore,
346 although seasonal variation of Sr/Ca_{water} was only ~7%, seasonal changes in measured Sr/Ca
347 ratio in otoliths were ~23-37%.

348

349 *Ba/Ca ratio*

350 Following significant increases –three and six-fold increase for larvae from the Medous and
351 Sassis hatcheries, respectively– in water Ba/Ca ratio, all otolith profiles for age-0⁺ parr display
352 maxima values coinciding with the fish's release in the Gave de Pau River (Fig. 3a, b). These
353 maxima were relatively variable depending on the individual, and usually occurred within one
354 to two weeks after the stocking date (Fig. 3a, b) and were poorly predicted by the water-based
355 otolith composition. Unlike the Sr/Ca ratio, only one maximum in Ba/Ca_{otolith} was observed for
356 age-1⁺ parr again following the fish's release in the Gave de Pau River (Fig. 4). Measured
357 Ba/Ca_{otolith} signals reasonably matched the predictions throughout the remaining profile but
358 fine-scale variations did not perfectly match the fluctuations of the chemical composition of the
359 water (Fig. 4).

360 No otolith response to small (40%) and short period of time (3 weeks) increases of the water
361 Ba/Ca ratio at the Lapitxuri River, was detected immediately after changes in the water
362 chemistry (Fig. 5). An increase in Ba/Ca_{otolith} was generally detectable after day 110 suggesting
363 a ~20 days delayed response for the otolith to properly record a change in water signal.
364 Additionally, as in the Gave de Pau River, the Ba/Ca_{otolith} profiles displayed a wide variation
365 regardless of the size classes, some otolith transects being characterized by high Ba/Ca_{otolith}
366 maxima (up to ~7 $\mu\text{mol}\cdot\text{mol}^{-1}$) well over predicted values while others displayed no major peaks
367 but a rather gradual increase more in accordance with water-based predictions (Fig. 5).
368 Following the flood response, Ba/Ca_{otolith} gradually decreased to a relatively stable value (Fig.
369 5) but the dispersion among individuals remained still high (Table 3, CV = 15-17%). Finally,
370 predicted and measured mean values were in relatively good agreement during normal flow at
371 the end of the experiment for all size classes (Table 3).

372

373 **Contribution of covariables based on GAMMs**

374 Very good fits (>90% of deviance explained) were found for only two scenarios: (1) age-0⁺
375 with steep Sr/Ca decrease and (2) six-fold Ba/Ca increase. For the three other scenarios
376 involving age-0⁺ individuals, GAMMs only fit 66%, 72% and 80% of the deviance, for slight
377 Sr/Ca increase, three-fold and transient Ba/Ca increase, respectively (Table 4). The covariates
378 used could only explain less than half of the deviance in scenarios where fishes of age-1⁺ were
379 mainly subject to slight seasonal variations in Sr/Ca and Ba/Ca chemical ratios (Table 4), with
380 only 42% and 28% of deviance explained, respectively. GAMMs applied to our data clearly
381 highlight that interindividual variability (independently from individual condition index)
382 significantly contributed to the Sr/Ca and Ba/Ca variations in otoliths of salmon for all
383 scenarios — with contribution being at least as important as water-based prediction (Table 4).
384 Given that the mean value was used for the water-based otolith composition, the contribution
385 of ‘interindividual variability’ is therefore probably overestimated. Temperature seems a poor
386 predictor in explaining Sr/Ca and Ba/Ca variations in otoliths, yet this explanation cannot be
387 totally discarded because temperatures partially correlate with water composition in our
388 datasets.

389

390 **OGR vs interindividual variability**

391 *Sr/Ca ratio*

392 For age-0⁺ parr from the Gave de Pau, the delay to reach Sr/Ca_{otolith} equilibrium ratio during the
393 summer period presented a large range of individual responses and was not significantly
394 correlated to the average OGR during the stay in the Gave de Pau regardless of the hatchery of
395 origin ($r=-0.14$, $p\text{-value}= 0.52$, Figure S5). Sr/Ca_{otolith} ratios above the Sr/Ca ratio predicted
396 during the winter period (Fig. 2a,b) were not significantly correlated to the OGR ($r=-0.08$, $p\text{-}$
397 $value= 0.65$). At equilibrium, Sr/Ca_{otolith} was relatively constant between individuals (CV: 11%)

398 and unrelated to the OGR ($r=-0.20$, p -value= 0.33) and the somatic growth rate ($r=-0.31$, p -
399 value= 0.13).

400

401 *Ba/Ca ratio*

402 Among age-0⁺ parr, the lag to reach Ba/Ca_{otolith} equilibrium value correlates significantly with
403 the average OGR during their stay in the Gave de Pau River whether individuals were
404 previously reared in the Medous or Sassis hatcheries ($r=-0.61$, p -value= 0.0012, Figure S6).
405 However, this delay was not significantly linked to outlier's value ($r=-0.04$, p -value= 0.85).
406 Ultimately, Ba/Ca_{otolith} during the maxima or at equilibrium were not significantly correlated
407 with the average OGR ($r=-0.059$ with p -value = 0.78 and $r=-0.14$ with p -value = 0.51,
408 respectively) and somatic growth rate ($r=-0.36$ with p -value = 0.11 and $r=-0.14$ with p -value =
409 0.38, respectively).

410 Regarding the controlled experiment, the ratio of Ba/Ca_{otolith} during the flood episode correlated
411 significantly with the OGR average for individuals that stayed in the Lapitxuri channel ($r =$
412 0.74 , p -value = 0.001), but not significantly with the OGR recorded during the flood ($r = 0.48$,
413 p -value = 0.10). No significant relationship was found between the Ba/Ca_{otolith} equilibrium
414 value and the OGRs for the Lapitxuri experiment ($r = 0.32$, p -value = 0.24) or the normal flow
415 period ($r = 0.17$, p -value = 0.58). On average, individuals of the [50-55] and [60-75] size classes
416 were more prone to accumulate higher Ba/Ca in their otolith than the lowest size class [35-45]
417 (Table 4) during the flood episode (multiple comparison Dunn test: p -value = 0.0052 for [50-
418 55] vs [35-45] and p -value = 0.0109 for [60-75] vs [35-45]). Predicted Ba/Ca_{otolith} during the
419 flood episode better reflected individuals from the smallest size class [35-45] (Fig. 5, Table 3),
420 while individuals growing faster ([50-55] and [60-75] size classes) over-accumulated the Ba
421 quite significantly, well above predicted values (Fig. 5). Overall, a wide range of Ba/Ca_{otolith}
422 profiles was observed regardless of the size classes. On average, individuals of the [50-55] and

423 [60-75] size classes were more prone to accumulate higher Ba/Ca in their otolith than the lowest
424 size class [35-45] (Table 3) during the flood episode (multiple comparison Dunn test: *p-value*
425 = 0.0052 for [50-55] vs [35-45] and *p-value* = 0.0109 for [60-75] vs [35-45]).

426

427 **DISCUSSION**

428 Our study consisted in examining fish otolith response among freshwater systems exhibiting
429 contrasted or slightly variable water chemistry while allowing factors like temperature, diet and
430 physiology to vary. We acknowledge that our approach might be challenging for isolating
431 specific effects driven by temperature, food or growth rate. Modeling otolith variations (via
432 GAMM) with three explanatory variables treated as fixed effect (water-based prediction of
433 otolith composition, temperature, Fulton's index) and 'individual' treated as random effect,
434 allowed us to demonstrate that water chemistry alone cannot fully explain measured ratios in
435 otoliths, except in scenarios involving significant changes in water chemistry (Table 4).
436 Furthermore, individual variability partly explains the otolith variations in most scenarios, even
437 if its contribution may be overestimated by our approach (the use of only mean-value for the
438 water-based otolith composition). Additionally, the covariates included in the models did not
439 fully or poorly explain the observations in some scenario (30-80% of deviance explained),
440 especially when fish were exposed to slight seasonal variations in Sr/Ca and Ba/Ca water ratios
441 or limited increase in Ba/Ca water ratios (Table 4). Taken together our new results are partially
442 in agreement with previous studies indicating that Sr and Ba otolith composition of Atlantic
443 salmon roughly reflects changes in freshwater systems (Wells et al., 2003; Martin et al., 2013a;
444 Carlson et al., 2017). Indeed, although we collected evidence that water chemistry clearly drove
445 significant shift in otolith composition when there is a significant change in water chemistry,
446 we also found that additional factors (intrinsic, i.e. growth rate, metabolism, and/or extrinsic,
447 i.e. temperature) may lead to discrepancies between otolith elemental records and water-based

448 otolith prediction when habitat chemistry varies slightly. When facing identical/synchronous
449 environmental changes, the large inter-individual variation in chemical profiles may support
450 the combined influence of intrinsic factors (e.g., physiology) as previously suggested for marine
451 fish (Brown and Severin, 2009; Sturrock et al., 2014, 2015).

452

453 **Parameters influencing seasonal variations in Sr/Ca_{otolith}**

454 Seasonal variations in otolith Sr/Ca ratios were already examined for various fish species. They
455 are generally related to changing habitats especially for species known to perform seasonal
456 migrations between chemically distinct habitats such as fresh and marine waters (Brown and
457 Severin, 2009; Hüsey et al., 2020). In some circumstances, however, habitat change cannot be
458 invoked to explain variation in the otolith elemental composition and other factors must be
459 considered (Sturrock et al., 2015; Loewen et al., 2016; Hüsey et al., 2020). Our study
460 demonstrated that seasonal fluctuations in Sr/Ca were captured by otoliths for parr from the
461 Gave de Pau River. It is highly unlikely that these fluctuations depend exclusively on changes
462 of the water chemistry since fish were only exposed to very slight variations (~7%) whereas
463 otolith Sr/Ca ratios significantly exceeded the predicted ratio during low growing periods
464 (37±11% and 23±12%, respectively for the first and second winter). Seasonal variations in
465 Sr/Ca_{otolith} associated with the opaque (growth season) or translucent (winter period) zones of
466 otoliths were already reported and related to mixed influence of temperature and growth rate
467 (Sturrock et al., 2015; Hüsey et al., 2020). In this study, otolith Sr/Ca ratios for salmon parr
468 varied seasonally in the opposite direction to water temperature and water chemistry.

469 Our results collected from the Gave de Pau River showed that otolith Sr/Ca_{otolith} values
470 of Atlantic salmon parr decreased with higher water temperature values, in contradiction with
471 one of the few studies based on another freshwater species (*Perca flavescens*, Collingsworth
472 et al. 2010). Answering whether water temperature influences Sr incorporation in otoliths of

473 salmon parr remains a challenge and additional work is still required. Nonetheless, we found a
474 variation of about 0.1-0.15 mmol.mol⁻¹ between winters and summer periods, which is about
475 the same order of magnitude as for the variation produced by a 10°C temperature change on eel
476 Sr/Ca_{otolith} (0.2 mmol.mol⁻¹: Marohn et al., 2011). Temperature effects were also reported to be
477 consistently lower than environmental gradient experienced by the fish when changing habitat
478 (Hüssy et al., 2020). The 0.1-0.15 mmol.mol⁻¹ seasonal fluctuations we observed was low in
479 comparison to the overall range of Sr/Ca_{otolith} encountered among habitats in the Adour basin
480 (0.29 to 0.95 mmol.mol⁻¹, Table 2).

481 The results of our study support, in addition to the water chemistry, a mixed
482 physiological/temperature influence on Sr/Ca_{otolith} in freshwater systems as recently
483 underpinned for some marine species (Sturrock et al., 2014, 2015; Grammer et al, 2017; Hüssy
484 et al., 2020). Using a one year-long experiment involving monthly measurements of both
485 environmental and physiological parameters, Sturrock et al. (2014, 2015) showed that sex and
486 reproductive status of the European plaice (marine fish species) triggered specific variations in
487 the Sr/Ca composition of the otolith unrelated to the surrounding water. Further, Izzo et al.
488 (2018) have conducted a meta-analysis (85% from marine species) combining water
489 composition, otolith chemistry and some environmental parameters. They clearly emphasized
490 the influence of temperature and salinity, as well as the role of intrinsic factors (growth, diet)
491 that can act either independently or interactively. Mixed models combining field
492 biogeochemical tracers with fish growth and otolith chemistry also revealed that Sr/Ca ratio is
493 affected by physiological effects (growth, assimilation rates) variable through time (Grammer
494 et al., 2017) in the marine environment. Additionally, Barnes and Gillanders (2013) were able
495 to demonstrate that genetics had a significant interactive effect with environmental factors
496 (temperature and salinity) on both Sr/Ca and Ba/Ca ratios within otoliths.

497 While there is still no consensus, recent findings suggested that growth negatively
498 influences Sr/Ca composition in blood plasma and otolith for marine fish (Secor and Rooker,
499 2000; Marohn et al., 2011; Daverat et al., 2012; Stanley et al., 2015; Sturrock et al., 2014, 2015;
500 Miller et al., 2020). However, controlled laboratory experiments found no relationship between
501 growth rate and Sr/Ca_{otolith} (Hoff and Fuiman, 1995; DiMaria et al., 2010; Marohn et al., 2011).
502 Additionally, how food ration and composition would affect otolith Sr remains evasive
503 (Walther et al., 2010; Hoff and Fuiman, 1995; Gallahar and Kingsford, 1996). In salmon parr,
504 higher Sr otolith concentrations typically occur in translucent zones under transmitted light
505 (autumn-winter low growth period) while lower levels appear in opaque zones (growth season
506 –spring to mid-summer, Bacon et al., 2005; Strothotte et al., 2005), consistent with a negative
507 effect of growth. Growth is connected to temperature variations and involves multiple
508 physiological pathways, which result in the production and the transport of organic substances
509 in the blood and thus to the endolymph, subsequently influencing the transport and
510 incorporation of chemical elements into the otolith aragonite (Gronkjaer, 2016). In addition,
511 growth can act on the incorporation of elements into the otolith through kinetic dilution effects
512 related to crystal accretion rates, high calcification rates resulting in lower Sr/Ca incorporation
513 into otolith (Campana, 1999; Hamer et al., 2007; Hüsey et al., 2020). A fraction of Sr likely
514 binds to protein complexes in the blood and endolymph fluids (Funamoto and Mugiya, 1998;
515 Thomas et al., 2017). Although data are scarce, as high as 2/3 of the Sr was reported to be
516 protein-bound in the plasma of the goldfish *Carassius auratus* (Funamoto and Mugiya, 1998).
517 Furthermore, a positive relationship was demonstrated in the blood plasma between Sr
518 concentration and protein content for a marine fish (Sturrock et al., 2014), suggesting Sr
519 availability to the otolith can be strongly controlled by physiological variations. Strontium
520 availability in the blood plasma (Sturrock et al., 2014) and the endolymph fluid could thus be
521 lower during the growing season as higher concentrations of organic substances are expected

522 in these fluids (Hüssy and Mosegaard, 2004; Gronkjaer, 2016). We favor a model where otolith
523 Sr/Ca fluctuations recorded in salmon parr would result from interactive effects of both
524 temperature and physiology, whether through the control of available Sr by organic molecules
525 in the blood and the endolymph or kinetic effects on element incorporation during the growing
526 season.

527 Finally, time variability observed for individuals to reach a new equilibrium (from high
528 Sr/Ca ratio values to lower values) supports a significant influence of the fish's metabolism.
529 However, Sr/Ca otolith was not significantly related to both somatic growth rate and average
530 OGR in our study. Before their sequestration in otoliths, dissolved ions go through several
531 biochemical processes across various membranes that ultimately alter their original
532 bioavailability (Kalish 1989, 1991, Campana 1999, Payan et al. 2004, Melancon et al. 2009). A
533 multitude of physiological and biochemical mechanisms are probably involved in the
534 continuously forming otolith and can explain the wide range in individual responses for a given
535 freshwater system.

536

537 **Parameters influencing the variations in Ba/Ca_{otolith}**

538 When exposed to different water chemistry, we found the Ba incorporation in otolith of parr
539 was generally delayed by ~2-3 weeks. Our findings are similar to previous experimental studies
540 showing that there was a significant time lag (≥ 20 d) between variations in water chemical
541 composition and subsequent changes in otolith microchemistry (Elsdon and Gillanders, 2005;
542 Lowe et al., 2009; Macdonald and Crook, 2010; Yokouchi et al., 2011). Elemental
543 incorporation in otoliths is a multi-barrier process (Campana, 1999). Barium requires more time
544 than other elements before being precipitated onto the otolith surface (Elsdon and Gillanders,
545 2003) suggesting a strong biological control. Miller (2011) estimated that changes in otolith

546 composition of juveniles Chinook salmon were detected within two to three days of a change
547 in water chemistry but otolith compositions did not stabilize until fifteen days.

548 Laboratory controlled experiments were inconclusive regarding the influence of water
549 temperature on the Ba/Ca_{otolith} . Some studies reported small positive effects (Miller, 2009; Reis-
550 Santos et al., 2013; Stanley et al., 2015; Walsh and Gillanders, 2018), negative effects (DiMaria
551 et al., 2010; Sturrock et al., 2015) or no significant effects (Martin and Thorrold, 2005; Martin
552 and Wuenschel, 2006; Miller et al., 2020). The effects of food ration and growth rate were
553 tested and demonstrated an inverse response for Ba/Ca_{otolith} (Walther et al., 2010; Miller, 2011;
554 Sturrock et al., 2015), but no significant relationship was found between growth rate and
555 Ba/Ca_{otolith} for larval fish (Bath et al., 2000; Martin and Thorrold, 2005). Interactions between
556 temperature, diet and growth rate in the field make it difficult to separate their effects. Indeed,
557 increasing temperatures typically result in increased ration ingestion and growth rates.

558 A wide scattering was found in Ba/Ca_{otolith} following an increase of Ba/Ca_{water} for
559 individuals that experienced similar environmental conditions, thus ruling out an environmental
560 cause (water chemistry composition and temperature), but suggesting variable physiological
561 effects with a level-response specific to each individual. A similar variable response in otolith
562 Ba/Ca ratio was previously observed in wild salmon juveniles during the transition from the
563 yolk sac to the surrounding environment before the final equilibrium value was reached several
564 weeks later (Martin et al., 2013a). Peak values at this transition appeared unrelated to ambient
565 Ba/Ca ratios because they did not significantly change among fishes that experienced low / high
566 Ba/Ca_{water} ratios (Martin et al., 2013a). Other field-based studies that examined fish movements
567 from the sea (low Ba/Ca_{water}) into lower salinity environments (high Ba/Ca_{water}) reported
568 significant maxima in Ba/Ca_{otolith} (Hale and Swearer, 2008). A laboratory experiment also
569 reported euryhaline Australian bass *Macquaria novemaculeata* exhibiting a steep unpredictable
570 increase in Ba/Ca_{otolith} immediately after their transfer into a low salinity system (Macdonald

571 and Crook, 2010). Substantial and consistent two- to three-fold variations in metabolic rate that
572 were linked with variations in behaviour and critical life-history traits were demonstrated
573 among individuals of a same species (Norin et al., 2015; Norin and Gamperl, 2017). In case
574 where Ba/Ca_{otolith} is mainly controlled by metabolic rate, a broad response among individuals
575 exposed to unique environmental condition would be expected. As early life stages are subject
576 to intense physiological processes (de Pontual et al., 2003) with possible ontogenetic
577 asynchrony between individuals, it can explain the overall large variation in Ba/Ca ratio among
578 age-0⁺.

579 We propose that a sudden increase in water Ba concentration could trigger physiological
580 responses, inducing a progressive regulation of Ba incorporation into otolith following an
581 overdone response relatively variable depending on the individual physiology (Kalish, 1989;
582 Sadovy and Severin, 1994). Our study, as well as previous observations, isolate the following
583 pattern: short-time high and variable Ba uptake / progressive exponential decrease in the Ba
584 uptake / stabilization of the ratio which can take weeks to months.

585 Our controlled-experiment conducted at the Lapitxuri station showed that fast-growing
586 individuals accumulated more Ba than slower growing individuals during the flood event and
587 that Ba/Ca_{otolith} correlated significantly with the overall OGR during the stay in the channel (r
588 = 0.75, p -value = 0.001) but poorly with the specific OGR during the flood (r = 0.48, p -value
589 = 0.10). Surprisingly, no correlation was however found between the overall OGR over their
590 time in the Gave de Pau River and the Ba/Ca_{otolith} recorded during their early life (peak
591 response). The reasons remain unclear. The main difference between the two situations was the
592 magnitude of the change in the Ba/Ca_{water} ratio experienced by the fishes, +40% increase in the
593 mesocosm experiment (Lapitxuri) against +120% and +400%, respectively for Medous and
594 Sassis fry. Consequently, our data suggest that OGR cannot alone explain the high inter-
595 individual variance of this ratio in otoliths of freshwater fish as was previously reported for

596 marine species (Bath et al., 2000; Walther et al., 2010; Miller, 2011; Sturrock et al., 2015).
597 Other intrinsic factors would thus need to be considered. Future investigations using
598 experimental approaches should examine Ba transport in blood and endolymph, and its uptake
599 onto otolith as all these processes involves significant physiological regulation (Melancon et
600 al., 2009; Payan et al., 2002). However, a previous study found no evidence for significant
601 physiological control of Ba uptake into the blood of European plaice *Pleuronectes platessa* L.
602 in a marine environment (Sturrock et al., 2014). But, unlike our study, their fishes were not
603 exposed to a sudden and marked change in ambient Ba/Ca_{water} ratio.

604 Discreet seasonal fluctuations in Ba/Ca_{otolith} , hidden by the wide individual response
605 (Fig. 4), seems to vary inversely to both Ba/Ca_{water} ratio (Figure S4) and temperature (Fig. 2c).
606 We found no evidence that Ba concentration in otoliths increases with temperature, which is in
607 agreement with previous studies examining marine fishes (Bath et al., 2000; Fowler et al.,
608 1995). Relative effects of temperature on otolith of a freshwater fish (*Perca flavescens*) and an
609 estuarine species (*Acanthopagrus butcheri*) were however noticed (Collingsworth et al., 2010;
610 Elsdon and Gillanders, 2002) but were not significant.

611 Changes in physiological processes that affected the composition and abundance of
612 many organic molecules into the blood plasma over seasons may also control the amount of
613 bioavailable Ba (Kalish, 1991; Gronkjaer, 2016; Thomas et al., 2017; Hüseyin et al., 2020) and
614 thus the Ba/Ca_{otolith} . A study demonstrated that Ba concentration in blood plasma exhibited
615 limited temporal variability and was not related to changes in the protein content through time
616 in a marine fish, suggesting a reduction in biological fractionation for this element and thus
617 greatest potential link to environmental fluctuations (Sturrock et al., 2014). Barium was also
618 observed to be less protein-bound than Sr in otoliths (Izzo et al., 2016). Our results also support
619 a restricted influence of blood protein on Ba/Ca_{otolith} during periods of stable ambient water

620 chemistry, although discreet seasonal oscillations with slightly higher value in autumn-winter
621 and lower in spring-summer may be in favour of regulation by the latter.

622

623 **Implications for environmental reconstructions**

624 Robust reconstruction of fish origin and movements through distinct habitats requires several
625 fundamental assumptions: (1) otoliths record the original signal from the waters the fish lived
626 in without major alteration; (2) any individuals living in the same location or moving between
627 two habitats display consistent recordings (e.g. amplitude, time-lag) in their otoliths (Campana,
628 1999; Campana and Thorrold, 2001; GrønkJær, 2016; Walther, 2019). Although water
629 chemistry controls the baseline level for otolith elemental ratio for a given site, our data show
630 it is not always true. Deviations from the water chemistry are possible and potentially related
631 to physiological effects on Sr and Ba incorporation (Walther, 2019; Marino et al., 2020).

632 We found that temperature and/or growth rate could induce seasonal variations in $\text{Sr}/\text{Ca}_{\text{otolith}}$ by
633 an order of 0.1-0.2 $\text{mmol}\cdot\text{mol}^{-1}$. Compared to the range of variations generally observed within
634 freshwater systems, this alteration can be considered weak between some rivers, but may impair
635 the ability to distinguish among rivers at fine spatial resolution, especially those having close
636 $\text{Sr}/\text{Ca}_{\text{water}}$ signatures. Using a multi elemental approach (beyond Ba and Sr) would be more
637 efficient for discrimination among rivers.

638 We showed that otolith response to a steep increase in freshwater Ba/Ca ratio resulted
639 in a wide initial dispersal in the $\text{Ba}/\text{Ca}_{\text{otolith}}$ ratio, implying physiological driven factors could
640 largely outweigh the environmental signal in some individuals and thus should be considered
641 while assessing lifetime movements from high-resolution Ba chemical profile. Furthermore,
642 the time-lag to reach a new equilibrium when fish moved among habitats, highly differs
643 according to individuals. Consequently, brief incursions in habitats having Ba-enriched levels
644 might be thus challenging to assess for sure. Determining accurate habitat fingerprints with

645 enough confidence will thus require selecting a suitable portion of the otolith on each individual
646 in order to sample exclusively the environmental signal and remove noise associated with
647 physiological processes.

648

649 **Conclusions**

650 Our findings show that Sr/Ca and Ba/Ca ratios in freshwater systems control the baseline level
651 for their otolith elemental ratios at a given site. However, variations through time would still be
652 either controlled by extrinsic (temperature) and/or intrinsic factors (growth rate, metabolism)
653 and/or changes in water chemistry (i.e., flood events). Opposite Sr/Ca fluctuations between
654 otolith and water chemistry suggest that seasonal changes in physiology (growth, blood
655 complexing capacity) and temperature affect the otolith signal by adding noise to the global
656 background driven by the water chemistry. Otolith Ba/Ca ratio can also largely deviate from
657 the water-predicted ratios mostly at transitions following abrupt increases in the water ratio for
658 resident fish (flood event) or for fish changing habitats (movement between streams). Finally,
659 wider inter-individual variability in the Ba/Ca otolith response was observed compared to
660 Sr/Ca, either during periods of ambient water stability or environmental changes, reducing its
661 discriminating power in otolith freshwater application. Strontium and barium have been the two
662 pillars of the research analyzing otoliths, undoubtedly major breakthroughs were made by
663 measuring Sr/Ca and Ba/Ca ratios but they also display some limitations and the community
664 should incorporate additional elements, specifically for freshwater systems.

665

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676

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678

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681

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- 925

1 **Figures**

2

3 Fig. 1. $\text{Sr}/\text{Ca}_{\text{water}}$ temporal variability (black squares) and $\text{Sr}/\text{Ca}_{\text{otolith}}$ profiles of *Salmo salar* parr
4 of age-0⁺ (continuous thin black lines) caught in September 2009 (green) and that experienced
5 steep decrease in $\text{Sr}/\text{Ca}_{\text{water}}$. Predicted $\text{Sr}/\text{Ca}_{\text{otolith}}$ ratio (average: thick blue line; minima and
6 maxima: dotted blue lines) assumes that otolith variations through time rely exclusively on
7 changes in the ambient chemistry. Vertical lines delimit the successive habitats occupied by
8 fish since eggs deposition.

9

10 Fig. 2. Results for parr that did not experience significant change in $\text{Sr}/\text{Ca}_{\text{water}}$. Panel a (top):
11 $\text{Sr}/\text{Ca}_{\text{water}}$ temporal variability (black squares) and $\text{Sr}/\text{Ca}_{\text{otolith}}$ profiles of *Salmo salar* parr of
12 age-0⁺ (continuous thin lines: black=2006, green=2009 and orange=2010). Panel b
13 (intermediate): $\text{Sr}/\text{Ca}_{\text{otolith}}$ profiles of *Salmo salar* parr of age-1⁺ during their stay in the Gave
14 de Pau River and collected in September 2006 (each colour represents an individual). Both
15 panels: Predicted $\text{Sr}/\text{Ca}_{\text{otolith}}$ ratios (average: thick blue line; minima and maxima: dotted blue
16 lines) assumes that otolith variations through time rely exclusively on changes in the ambient
17 chemistry. Vertical lines in panel a) delimit the successive habitats occupied by age-0⁺ fish
18 collected in autumn. Vertical dotted line in panel b) delimits grow years 2005 and 2006. Panel
19 c) seasonal temperature (°C) variability.

20

21 Fig. 3. $\text{Ba}/\text{Ca}_{\text{water}}$ temporal variability (black squares) and $\text{Ba}/\text{Ca}_{\text{otolith}}$ profiles of *Salmo salar*
22 parr of age-0⁺ (continuous thin lines: black=2006, green=2009 and orange=2010) that were
23 exposed to significant increase in $\text{Ba}/\text{Ca}_{\text{water}}$ ratio following their stocking. Panel a (top) and b
24 (bottom) show data for parr reared in the Medous and Sassis hatcheries, respectively. Predicted
25 $\text{Ba}/\text{Ca}_{\text{otolith}}$ ratio (average: thick blue line; minima and maxima: dotted blue lines) assumes that

26 otolith variations through time rely exclusively on changes in the ambient chemistry. Vertical
27 lines delimit the successive habitats occupied by fish since eggs deposition.

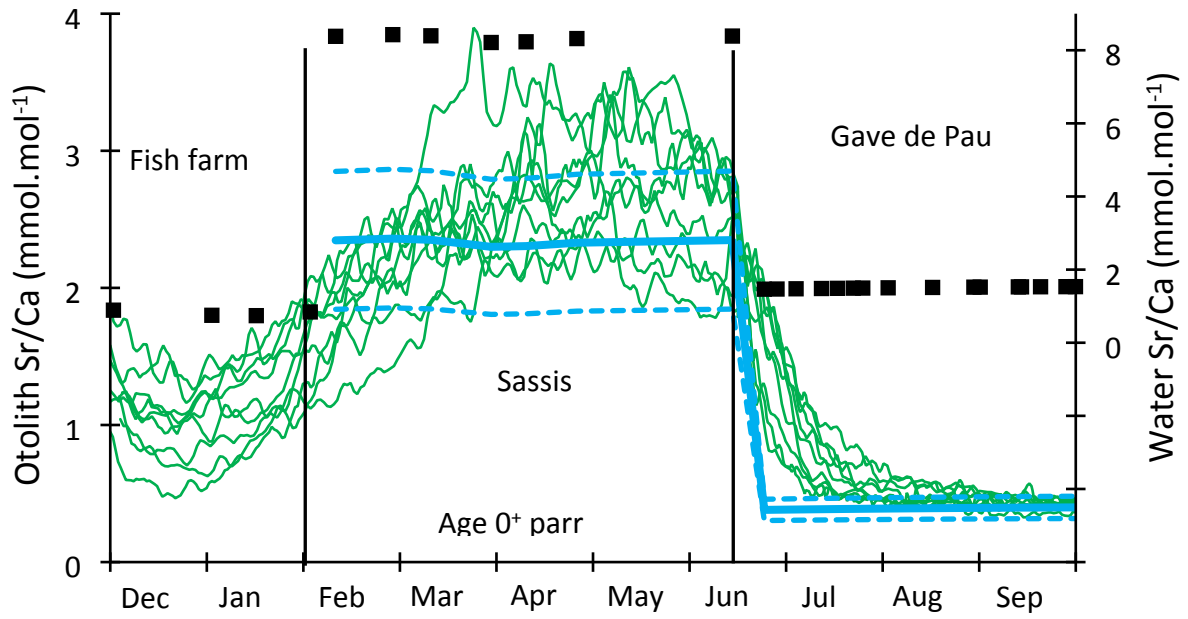
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29 Fig. 4. Results for age-1⁺ parr caught in 2006 that experience slight seasonal fluctuations in
30 Ba/Ca_{water} (Gave de Pau River). Each colour represents an individual as in figure 2b. Predicted
31 Ba/Ca_{otolith} ratios (average: thick blue line; minima and maxima: dotted blue lines) assuming
32 that otolith variations through time rely exclusively on changes in the ambient chemistry.
33 Vertical dotted line delimits year 2005 and 2006.

34

35 Fig. 5. Otolith response to a three weeks pulse in the Ba/Ca_{water} ratio during juvenile growth
36 under natural conditions in the Lapitxuri field-station. Green, orange and blue refer to [35-45],
37 [50-55] and [60-75] range sizes (mm), respectively. Predicted Ba/Ca_{otolith} ratio (average: thick
38 black line; minima and maxima: dotted black lines) assumes that otolith variations through time
39 rely exclusively on changes in the ambient chemistry. Vertical dotted line at day one hundred
40 and ten corresponds to the second alizarine redS marks (AM2). The shaded area corresponds to
41 the period of 40% increase of water Ba/Ca ratio under the effect of the flood event.

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44 Fig. 1.

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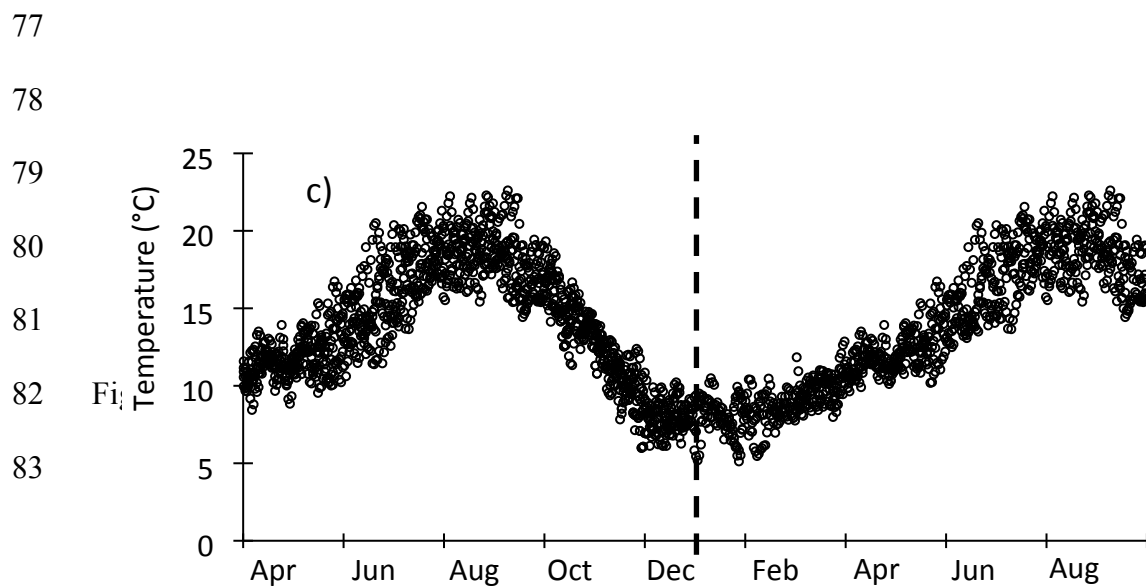
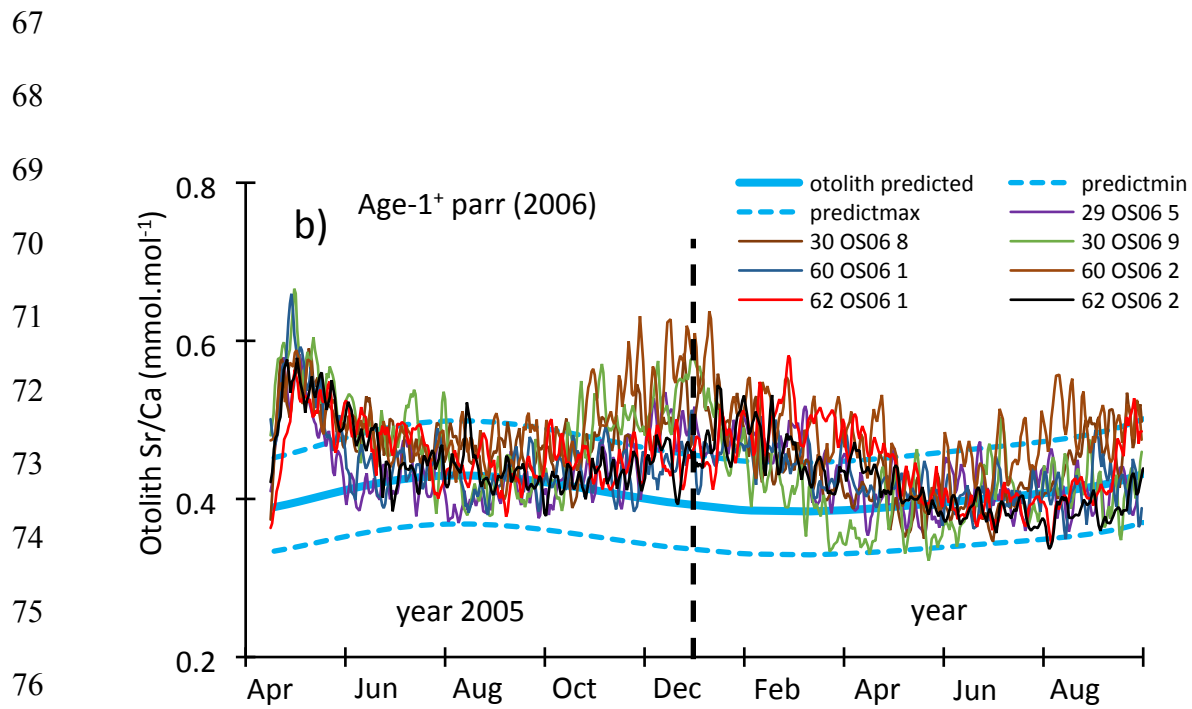
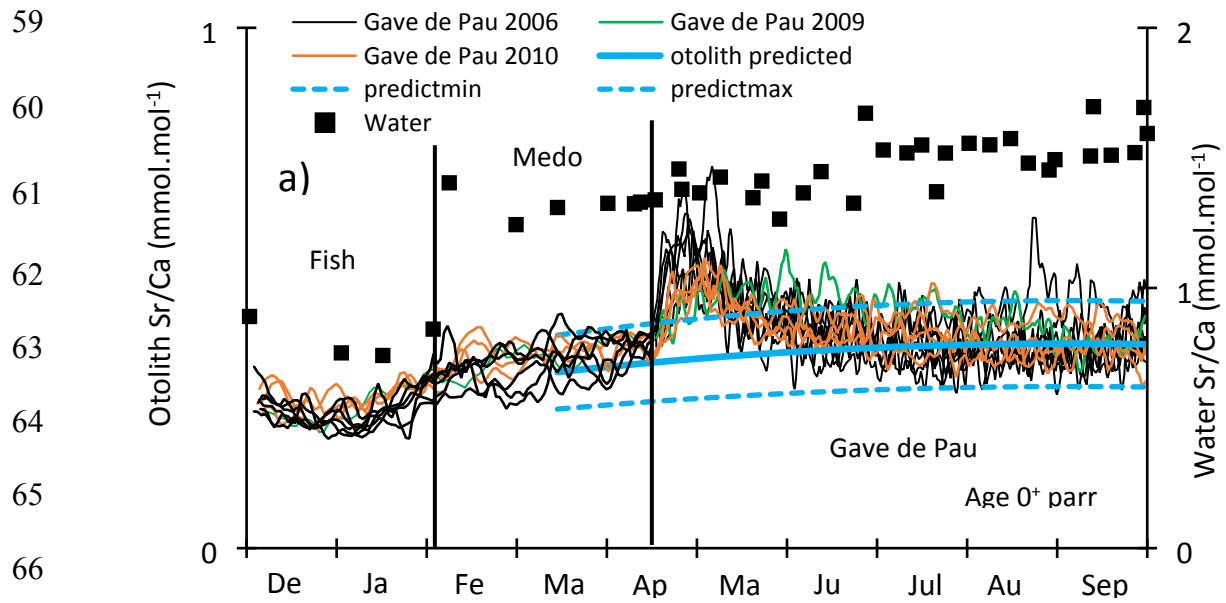
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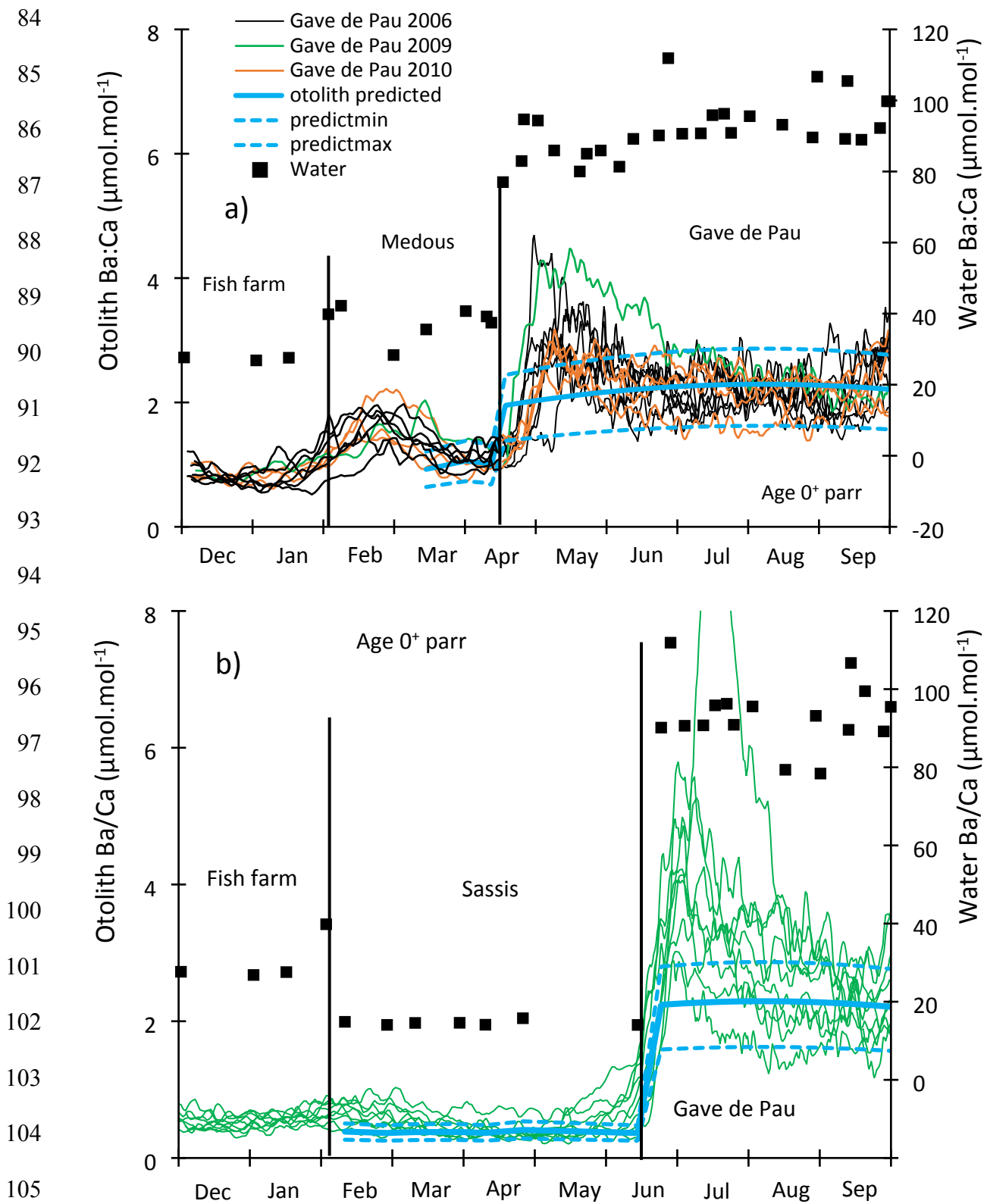
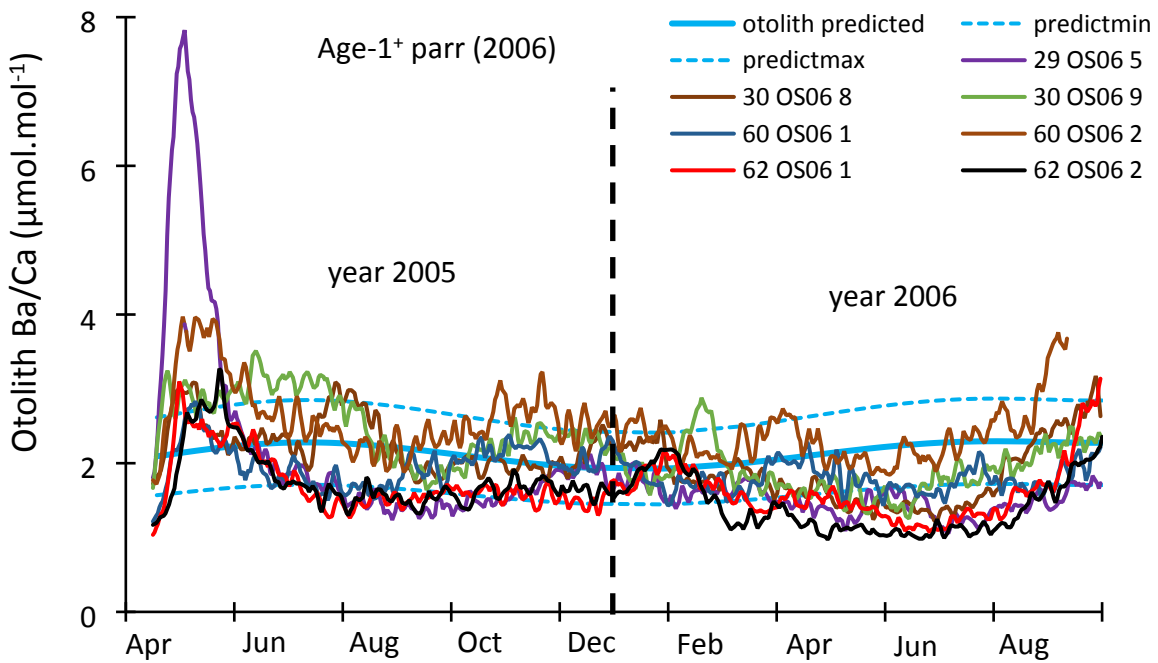


Fig. 3.

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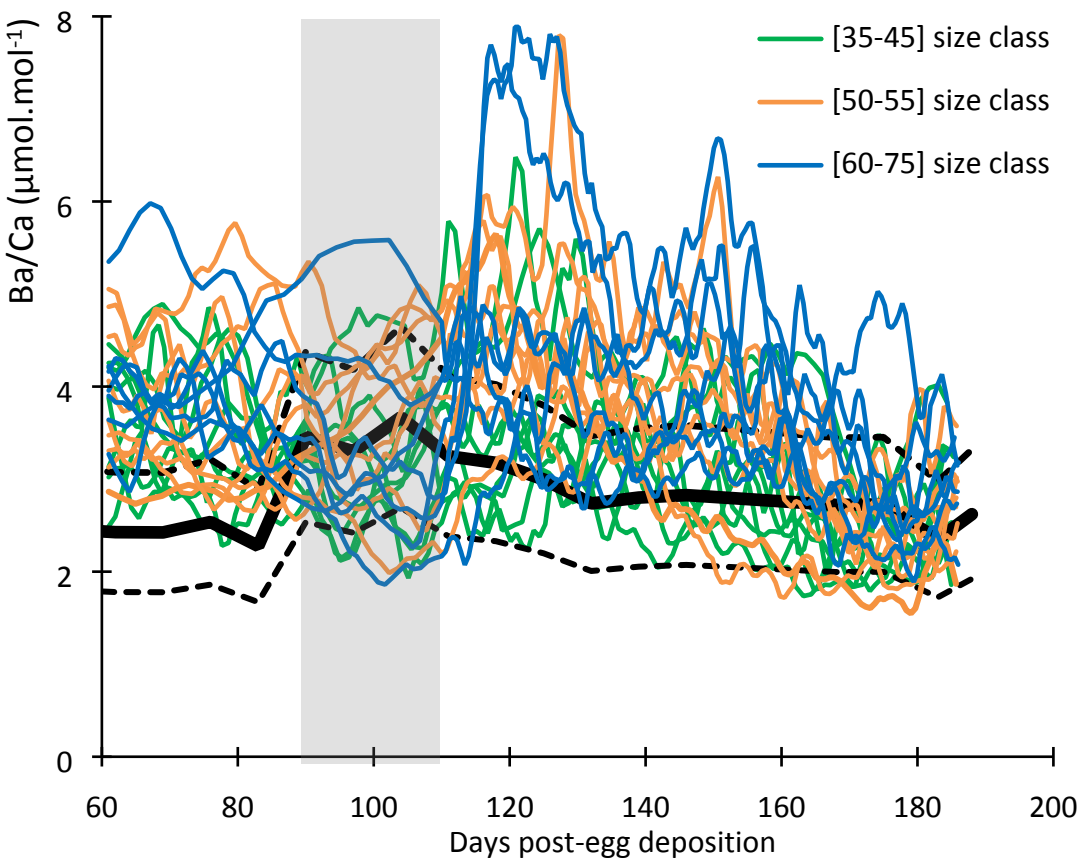
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110 Fig. 4.

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113 Fig. 5.

1 Table 1. Chemical scenarios studied and fish data (sampling year, number, age, site of collection) are presented for the Sr/Ca and Ba/Ca elemental
2 ratios. Aims of the work and statistical analyses used are also reported in the last two column.

3

4 Table 2. Average and standard deviation of Sr/Ca ($\text{mmol}\cdot\text{mol}^{-1}$) and Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) ratios in otoliths and waters from 13 rivers of the Adour
5 basin taken from a previous study (Martin et al., 2013a), from the Lapitxuri facility and from the Medous and Sassis hatcheries. For each river,
6 ratios correspond to the average of the last 100 μm of otolith accretion of n fish collected in September (Adour) and June (Lapitxuri) and n water
7 samples collected during the end of the summer period (August to September months) and May month (Lapitxuri). The average of D_{Sr} from all the
8 rivers as well as the three average D_{Ba} from three concentrations range, used in this study to predict otolith Sr/Ca and Ba/Ca ratios from the water
9 chemistry are also given.

10

11 Table 3. $\text{Ba}/\text{Ca}_{\text{otolith}}$ (mean, SD, CV) and otolith growth rate (OGR) for three time periods (overall stay in the Lapitxuri channel, flood event, normal
12 flow period at the end of the experiment) according to the three size classes investigated. Different letters A, B, a, b and c refer to significant
13 difference between size classes at the 0.05 confidence level. Statistical significance in linear relationships between $\text{Ba}/\text{Ca}_{\text{otolith}}$ and OGR are also
14 provided.

15

16 Table 4: Total deviance (%) explained by the full model and partial deviance explained by each explanatory variable for each case study using a
17 Generalized-Additive-Mixed-Models (GAMM). Water-based otolith composition (mean-value only, non-linear trend), water temperature (non-
18 linear trend) and individual Fulton's index (linear trend) were considered as variables treated as fixed effect and 'individuals' as variable treated as
19 random effect.

20

21

22 Table 1

Elemental ratio	Year of capture (n)	Fish Age	Treatment / Scenario	Aims of the work	Statistical tests
Sr/Ca	2009 (n=9)	0 ⁺	Sassis to Gave de Pau (mid-June): steep decrease in water Sr/Ca	i) otolith Sr/Ca and Ba/Ca ratios reliably reflect life-history scenarios?	Generalized-Additive-Mixed-Model (GAMM): □ <i>water-based prediction of otolith composition; water temperature and individual Fulton's index</i> as fixed effect □ 'individuals' as random effect
	2006 (n=8)	0 ⁺	Medous to Gave de Pau (mid-April): slight increase in water Sr/Ca		
	2009 (n=1)				
	2010 (n=4)				
2006 (n=7)	1 ⁺	Medous to Gave de Pau (mid-April): slight seasonal fluctuations in water Sr/Ca	ii) temperature, fish condition factor and individual variability explain/influence deviations between observed and water predicted ratios?		
2009 (n=9)	0 ⁺	Sassis to Gave de Pau (mid-June): six fold increase in water Ba/Ca			
2006 (n=8)	0 ⁺	Medous to Gave de Pau (mid-April) : three fold increase in water Ba/Ca			
2009 (n=1)					
2010 (n=4)					
Ba/Ca	2006 (n=7)	1 ⁺	Medous to Gave de Pau (mid-April) : three fold increase + slight seasonal fluctuations in water Ba/Ca	Pearson or Spearman correlation tests: Otolith growth rate (OGR) vs interindividual variability	
	2011	6 months	Lapitxuri experiment: transient increase in water Ba/Ca		
	[35-45 mm]: n=8				
	[50-55 mm]: n=9				
[60-75 mm]: n=7					

23

24 Table 2

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	Otolith Sr/Ca (mmol.mol ⁻¹)			Water Sr/Ca (mmol.mol ⁻¹)			D _{Sr}	ΔD _{Sr}	Otolith Ba/Ca (μmol.mol ⁻¹)			Water Ba/Ca (μmol.mol ⁻¹)			D _{Ba}	ΔD _{Ba}	
	<i>n</i>	<i>mean</i>	<i>SD</i>	<i>n</i>	<i>mean</i>	<i>SD</i>			<i>n</i>	<i>mean</i>	<i>SD</i>	<i>n</i>	<i>mean</i>	<i>SD</i>			
<i>Rivers</i>																	
Lourdios	12	0.71	0.04	4	2.76	0.65	0.26	0.08	12	0.91	0.16	4	39.4	6.6	0.023	0.008	
Verts	10	0.66	0.07	8	2.39	0.18	0.28	0.05	10	1.02	0.22	8	40.9	2.7	0.025	0.007	
Ossau	27	0.52	0.05	11	1.83	0.15	0.28	0.05	27	1.13	0.24	11	40.9	4.2	0.028	0.009	
Aspe	14	0.95	0.05	6	4.01	0.45	0.24	0.04	14	1.15	0.24	6	42.9	4.4	0.027	0.008	
Laurhibar	9	0.78	0.08	6	3.35	0.18	0.23	0.04	9	1.27	0.19	6	49.5	7.8	0.026	0.008	
Oloron	22	0.76	0.11	14	2.63	0.34	0.29	0.08	22	1.52	0.42	14	51.9	6.5	0.029	0.012	
Saison	13	0.75	0.06	12	2.64	0.28	0.28	0.05	13	1.70	0.35	12	60.3	5.5	0.028	0.008	
Ouzom	16	0.42	0.04	4	1.76	0.11	0.24	0.04	16	1.71	0.42	4	75.7	1.2	0.023	0.006	
Downstream																	
Pau	13	0.40	0.04	8	1.58	0.18	0.25	0.06	13	2.26	0.39	8	95.5	8.7	0.024	0.006	
Béhérobie	17	0.46	0.03	6	1.65	0.25	0.28	0.06	17	2.51	0.35	6	104.7	14.3	0.024	0.007	
Upstream Pau	4	0.41	0.03	7	1.69	0.08	0.24	0.03	4	2.58	0.24	7	132.3	10.5	0.019	0.003	
Arnéguy	17	0.29	0.04	5	1.15	0.17	0.25	0.07	17	4.34	0.93	5	253.4	52.7	0.017	0.007	
Lapitxuri	24	0.36	0.04	6	1.22	0.01	0.30	0.04	24	2.62	0.57	6	171	13	0.015	0.004	
							Average										
							D _{Sr}	0.26	0.05								
															Water Ba/Ca <50 μmol.mol ⁻¹	0.026	0.008
															Water Ba/Ca 80-120 μmol.mol ⁻¹	0.024	0.006
															Water Ba/Ca >120 μmol.mol ⁻¹	0.017	0.005
<i>Hatcheries</i>																	
Medous	10	0.39	0.05	4	1.27	0.16	-	-	10	1.19	0.30	4	36.7	6.3	-	-	
Sassis	9	2.60	0.44	7	8.34	0.08	-	-	9	0.44	0.14	7	14.6	0.6	-	-	

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27 Table 3

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Life period	Size class (mm)	Otolith Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$)				OGR ($\mu\text{m}\cdot\text{day}^{-1}$)				Ba/Ca vs OGR (α)	
		Mean	SD	CV (%)	predicted \S	Mean	SD	CV (%)			
AM2-edge (110-187)	[35-45]	3.08	0.54	A	18	α 1.62	0.13	A	8	$r = 0.66$	
AM2-edge (110-187)	[50-55]	3.52	0.52	B	15	2.99 \pm 0.45	α 1.95	0.14	B	7	$p = 0.006$
AM2-edge (110-187)	[60-75]	3.9	0.83	B	21		α 2.09	0.16	B	8	
Flood event (110-135)	[35-45]	3.52	0.54	a	15		\$1.54	0.16	a	10	
Flood event (110-135)	[50-55]	4.44	0.59	b	13	3.49 \pm 0.20	\$1.79	0.24	b	14	$p = 0.001$
Flood event (110-135)	[60-75]	4.64	0.97	b	21		\$1.81	0.24	b	13	
Normal flow (168-187)	[35-45]	2.48	0.43	c	17	2.66 \pm 0.20	\$1.85	0.23	c	12	$r = 0.32$
Normal flow (168-187)	[50-55]	2.46	0.43	c	17		\$1.90	0.22	c	12	$p = 0.24$
Normal flow (168-187)	[60-75]	2.73	0.42	c	15		\$1.96	0.33	c	17	

α otolith growth rate estimated as the distance between anchor points divided by the number of days between them (AM2 to edge)

\S mean daily growth rates over the defined period of time

\S otolith Ba:Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) predicted from water chemistry

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33 Table 4

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Elemental ratio	Treatment	Chemical scenarios	Fish Age	Total explained deviance (%)	Partial deviance (%)			
				Full model	Water-based otolith composition	T (°C)	Fulton's index	Interindividual variations
Sr/Ca	Sassis to Gave de Pau (mid-June)	Steep decrease (five fold)	0+	97.6	4.48	3.67	1.62 10 ⁻⁴	6.42
	Medous to Gave de Pau (mid-April)	Sligth increase (7%)	0+	66.1	10.93	15.17	4.94 10 ⁻⁶	20.89
		Sligth seasonal oscillations (7%)	1+	42	18.76	9.43	1.33 10 ⁻⁵	18.54
Ba/Ca	Sassis to Gave de Pau (mid-June)	Six fold increase	0+	92	24.34	4.34	4.71 10 ⁻⁵	23.2
	Medous to Gave de Pau (mid-April)	Three fold increase	0+	71.9	6.42	22.94	2.25 10 ⁻³	23.35
		Three fold increase + sligth seasonal oscillations (15%)	1+	28.5	8.88	4.77	1.75 10 ⁻⁴	21.09
		Lapitxuri experiment	Transient increase	6 months	79.9	23.4	2.96	1.56 10 ⁻⁴

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