



HAL
open science

Unsupervised estimation of the age at sea departure of Atlantic salmon using magnesium, manganese, and zinc otolith chemistry

Anthony Fontaine, Matthias Vignon, H el ene Tabouret, Christophe P echeyran,
Gilles Bareille

► To cite this version:

Anthony Fontaine, Matthias Vignon, H el ene Tabouret, Christophe P echeyran, Gilles Bareille. Unsupervised estimation of the age at sea departure of Atlantic salmon using magnesium, manganese, and zinc otolith chemistry. *Journal of Fish Biology*, 2024, 10.1111/jfb.15829 . hal-04621470

HAL Id: hal-04621470

<https://univ-pau.hal.science/hal-04621470v1>

Submitted on 24 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche fran ais ou  trangers, des laboratoires publics ou priv es.

1 **UNSUPERVISED ESTIMATION OF THE AGE AT SEA DEPARTURE OF**
2 **ATLANTIC SALMON USING MAGNESIUM, MANGANESE, AND ZINC**
3 **OTOLITH CHEMISTRY**

4 A. Fontaine¹, M. Vignon², H. Tabouret¹, C. Pécheyrant¹, G. Bareille¹

5 ¹*University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France*

6 ²*University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France*

7

8 **Corresponding author:**

9 Anthony Fontaine

10 anthony.fontaine@univ-pau.fr

11 **ORCID ID:** <https://orcid.org/0000-0001-5779-5789>

12

13 **Funding information:**

14 This project was founded by the *Agence de l'Eau Adour-Garonne (AEAG)*, the *Office français*
15 *de la Biodiversité (OFB)*, the *Région Nouvelle-Aquitaine (RNA)*, the *Université de Pau et des*
16 *Pays de l'Adour (UPPA-E2S)*, and the North Atlantic Salmon Conservation Organization
17 (NASCO) – European Community.

18

ABSTRACT

19 Investigating the age at sea departure of returning Atlantic salmon is critical to better understand
20 the role of emigration timing in survival. Among the methods available, the use of otolith
21 chemistry can be challenging in anadromous fish, as the elements frequently used (*i.e.*
22 magnesium, manganese, and zinc) can jointly reflect individual metabolism and environmental
23 conditions. Here, we present a new unsupervised and robust method for successfully estimating
24 the outmigration age of Atlantic salmon in the Bay of Biscay, based on otolith zinc
25 concentrations.

26

27 **Keywords:** *Salmo salar*, Migration, Functional clustering, Freshwater

28 Age determination from scale and otolith annuli observations has been applied for more than
29 120 years in fisheries science, as it is crucial information to manage stock structure and
30 understand population dynamics (Campana and Thorrold, 2001). In recent years, researchers
31 have tried to combine chemical profiles from otolith analyses with traditional visual reading to
32 estimate fish age, especially for taxa that do not allow for clear determination of annuli
33 (Heimbrand et al., 2020) or with long-lived species. As some elements such as magnesium
34 (Mg), manganese (Mn), phosphorus (P) or zinc (Zn) are essential for fish and may vary with
35 individual metabolism (Hüssy et al., 2021b), their otolith concentrations are likely to be related
36 with physiological mechanisms such as ontogeny, growth, reproduction, metamorphosis or even
37 diet (Sturrock et al., 2012), thus reflecting seasonal patterns that match with annual opaque and
38 translucent zones in otoliths (Hüssy et al., 2016; Heimbrand et al., 2020).

39 To date, contrasted results have been pointed out in the literature concerning the
40 cause(s) of Mg, Mn, and Zn variations in otolith (Hüssy et al., 2021b). First applied to track
41 pollution exposure, the use of Zn otolith concentration has more recently emerged as a potential
42 tool to estimate fish age with marine (Sturrock et al., 2015a; Heimbrand et al., 2020) and
43 migratory species (Brûlé, 2022; Mainguy et al., 2023), as its accumulation would mainly come
44 from diet. Scientific literature exploring the uptake mechanisms of Mg and Mn in otolith is also
45 very limited. Magnesium incorporation is reported to be mostly related to individual growth rate
46 and, therefore possibly associated with foraging strategies and metabolic activity (Hüssy et al.,
47 2021b). Hypotheses about the main sources of Mn in otolith are even more inconsistent since
48 some studies have demonstrated a positive effect of growth (Martin and Thorrold, 2005) while
49 others did not report any effect (Sturrock et al., 2015a). Nevertheless, for both Mn and Mg, a
50 significant effect of diet does not appear to be a valid assumption, in contrast to Zn sources
51 (Hüssy et al., 2021b). Finally, a decrease in elemental concentrations over time seems to apply
52 commonly for Mg, Mn, and Zn in otoliths according to ontogenic changes in somatic growth

53 patterns. From the literature, reported as “chemical age-tracers”, Mg, Mn, and Zn otolith
54 concentrations are expected to vary seasonally, with peaks occasionally observed for all these
55 elements in the spring-summer period (*i.e.* otolith opaque zone) reflecting high metabolic activity
56 and minima in the winter period (*i.e.* otolith translucent zone), when foraging and growth are
57 very low (Martin and Thorrold, 2005; Hüsey et al., 2016; Mainguy et al., 2023).

58 To the best of the author's knowledge, every recent study that explored Mg, Mn, and Zn
59 as tracers to estimate fish age has combined their chemical results with optical aging to
60 corroborate the occurrence of elemental variation with otolith zones (opaque and translucent).
61 However, this work is time-consuming and requires expertise to adequately prepare and read
62 the otoliths (Hüsey et al., 2016). In this study, we used a functional clustering method to test the
63 usefulness of Mg, Mn, and Zn variation to assess in an unsupervised manner the age at sea-
64 departure (most likely hypothesized to be 1+ or 2+ in our study sites, based on previous studies
65 and reports from regional management associations) for mature returning Atlantic salmon
66 (*Salmo salar*). Combined with life history reconstruction, also inferred from otolith chemistry,
67 joint acquisition of age can greatly improve our knowledge about time-dependent migratory
68 patterns, yearly river-spawning contribution, and stocking-programs efficiency for salmon
69 populations (Martin et al., 2013; Sturrock et al., 2015b).

70 *Study area and sample collection*

71 As the incorporation of Mg, Mn, and Zn in the otolith matrix seems to be simultaneously
72 influenced by intrinsic and extrinsic factors (Hüsey et al., 2021b), their chemical profiles should
73 theoretically vary between distinct populations that experience contrasted habitat (*e.g.* river
74 length, temperature and discharge, food availability, density-dependence). To account for this
75 natural variability, we selected otoliths from adult Atlantic salmon from three French localities
76 (Adour, Gironde, and Bretagne), previously aged by traditional scale-reading method (double-
77 blind, unpublished data). In these regions, smolts commonly migrate to marine waters either

78 after one year (defined as "1+" hereafter) or two successive years (expressed as "2+" hereafter)
79 in freshwater, although the proportion of 1+ and 2+ is also expected to vary spatially and
80 temporally between locations. In total, 377 returning adults collected in year 2015 in Bretagne
81 (1+ n=11, 2+ n=12) and from years 2010 to 2016 in the Gironde (1+ n=62, 2+ n=16) and Adour
82 (1+ n=236, 2+ n=40) were used in this paper (Table S1).

83 *Ethical statement*

84 All the otoliths analyzed in this study were sampled from monitoring and carcass surveys or
85 from amateur fishermen. Each individual was collected according to animal welfare laws,
86 guidelines and policies as approved by sampling permits or the number of individual tag.

87 *Otolith preparation and chemical analyses*

88 Detailed otolith preparation and parameters of chemical analyses can be found in Martin et al.,
89 2013. Briefly, sagittal otoliths were extracted from salmon heads, gently cleaned, and
90 embedded in epoxy resin (Araldite 2020, Escil) before being ground with distilled water and
91 sandpaper (Martin et al., 2013). Prepared otoliths in resin block were then analyzed for ^{24}Mg ,
92 ^{55}Mn and ^{65}Zn concentrations concurrently with strontium (^{86}Sr) and barium (^{138}Ba) using a
93 femtosecond-laser ablation system (fs-LA, 1030nm, Alfamet-Novalase, France) coupled to an
94 Inductively Coupled Plasma Mass Spectrometer (Agilent 8900 triple Quadrupole ICP-MS, USA).
95 Calcium (^{43}Ca) was used as an internal standard and concentrations of measured elements
96 were determined using three-point calibration curved based on certified glass standards NIST
97 610, 612, and 614 (NIST, USA). Accuracy was checked using otolith reference materials FEBS
98 1 (NRCC, Canada) and NIES 22 (NIES, Japan). Ultimately, Mg, Mn, and Zn concentrations are
99 expressed as a ratio of Ca concentration (Mg/Ca, Mn/Ca, and Zn/Ca respectively, in $\mu\text{g}\cdot\text{g}^{-1}$),
100 based on the concentration of Ca in otolith (0.383g of Ca per g of otolith).

101 *Modelling*

102 Profiles of elemental ratios were individually investigated to identify the part of the transect that
103 corresponds to the freshwater residence, from fish birth (*i.e.* primordia, visible with a
104 manganese peak (Ruttenberg et al., 2005) and systematically located 200 μ m after the ablation
105 starting point) to the departure at sea (*i.e.* outmigration, based on the significant increase in
106 Sr/Ca and concurrent decrease in Ba/Ca ratios along the otolith transect). To compare all
107 individuals, we standardized the distance obtained from each of them between the nucleus and
108 the outmigration by calibrating every profile between 0 and 1. As our method requires an
109 identical number of values per individual along transects, we used a generalized additive model
110 (GAM) to resample 150 equally spaced values per profile (*i.e.* the mean average number of data
111 points in our transects). The GAM was performed using the *mgcv* package (version 1.8-40, in R
112 version 4.2.1) with an equal number of splines per individual ($k=10$) and we used the GCV
113 criterion as the method for estimating the smoothing parameters. We then used an univariate
114 functional clustering approach based on an Expectation-Maximization (EM) algorithm following
115 Bouveyron and Jacques, 2011 (Table 1). Multivariate approaches were also tested but did not
116 outperform the results obtained via univariate analyses (Table 1). The method is an adaptation
117 from the traditional high dimensional data clustering (HDDC) method to functional data that was
118 achieved using the *funHDDC* package (version 2.3.1). We used this unsupervised (naïve)
119 approach to cluster each profile within two groups (most likely hypothesized to be 1+ or 2+ in
120 our study sites, based on the data from the *Centre national d'interprétation des captures de*
121 *salmonidés migrants* (CNICS), <https://professionnels.ofb.fr/>). We arbitrarily fixed the number
122 of groups equal to two since long-term surveys of salmon in our localities have revealed that a
123 hegemonic proportion of individuals remain either one or two years in freshwater before
124 migrating. For robustness purpose we also tested for three groups but the likelihood did not vary
125 between the two and three-cluster approaches (Table S2). The posterior probability for each

126 profile allowed classifying them into two groups (assumed to be either 1+ or 2+), with the
127 maximum probability of attribution always higher than 0.9, and we used the percentage of
128 correctly classified individuals as a performance index. Finally, we performed a cross-validation
129 approach using Out-of-Bag (OOB) sampling (training set: 75%, test set: 25%, number of
130 iterations: 1000) to evaluate the performance of the naïve approach for each metal.

131 Although Mg and Mn have been successfully used in a few studies to reconstruct the
132 “metabolically active” (Limburg et al., 2018; Hüssy et al., 2021a) period of fish, our results do not
133 indicate similar trends (**Error! Reference source not found.**). Considering the wide range of
134 observed raw values, which can be 100 to 500 times higher between salmons, especially for
135 Mn, the biggest challenge is to depict the expected patterns (1+ and 2+) of Mg and Mn profiles
136 from the very massive inter-individual variability. Clearly, in our study, the model does not
137 distinguish between 1+ and 2+ fish as almost all individuals are clustered in the 1+ group (Table
138 1). In both Mg and Mn profiles, elevated concentrations (Figure 1) are clearly identified in the
139 core region, which is consistent with previous studies (Ruttenberg et al., 2005). Yet, combined
140 with a steady decrease of Mg and Mn otolith concentration with fish growth (Hüssy et al.,
141 2021b), the annual fluctuation of both elements is probably masked in the broad spectrum of
142 inter-individual variability. Additionally, as Mg otolith concentration significantly differed between
143 high and low body conditions in cods (Limburg and Casini, 2018), it may reinforce the observed
144 variability within our sampling if such diversity is also occurring in Atlantic salmon. Our results
145 diverge from previous studies on marine (Limburg et al., 2018) fish species for both metals, but
146 also with northern Canadian populations of Atlantic salmon for Mg (Brûlé, 2022). Presumably, it
147 emphasizes the importance of environmental conditions, and more specifically the effect of
148 contrasted seasons in the Arctic area compared to our temperate zones, on elemental
149 availability (because of a decline in food availability) and reduced metabolic activity. In addition,
150 a confounding effect between localities would be a plausible hypothesis to explain the failure to

151 use Mg and Mn profiles. The mixing of three different geographic areas could mask a potential
152 site-specific signal, preventing the detection of a distinct pattern between 1+ and 2+ fish. It could
153 also explain why the multivariate analysis does not improve, even slightly, the performance
154 obtained with the univariate method. However, analyses computed separately for each locality
155 lead to the same inconclusive results.

156 In contrast, when looking at Zn variations, the visual pattern for 1+ and 2+ fish is much
157 more apparent than for Mg and Mn, although the signal varies by an order of magnitude 10
158 (from 50-60 $\mu\text{g}\cdot\text{g}^{-1}$ up to almost 600 $\mu\text{g}\cdot\text{g}^{-1}$, Figure 1). This is also in line with the literature as Zn
159 otolith concentration has been reported to highly vary among Baltic cod *Gadus morhua*
160 populations (Hüssy et al., 2016). Significant differences were also observed between males and
161 females of European plaice *Pleuronectes platessa* L.(Sturrock et al., 2015a), demonstrating the
162 importance of individual physiological processes in the uptake mechanism. Here, from the raw
163 Zn data (Figure 1), our model successfully reclassified 1+ and 2+ fish with a performance of
164 83.1% and 91.0% respectively (Table 1). Thus, the functional clustering method produced fairly
165 reliable results for this metal as we collected fish from various environments, with particular
166 sharp differences in water temperature, annual discharge, and food availability. In the Adour
167 and Gironde systems, juveniles inhabit rivers under strong influences of snow, with drastic
168 changes between spring (high river flow due to snowmelt and heavy rain associated with cold
169 temperatures) and summer period (low flow combined with high temperatures). In the Bretagne
170 region, seasonal changes are much less severe. Therefore, in theory, the timing and the
171 intensity of Zn incorporation in otolith would resultingly differ among locations, since the
172 synchrony of biotic and abiotic conditions is a prerequisite to the use of a chemical age-tracer
173 (Hüssy et al., 2016). Nevertheless, the good performance from the functional clustering
174 highlights the robustness of natural Zn signals (1+ vs 2+) across spatial and temporal scales in
175 the Bay of Biscay. Our results mirror previous patterns highlighted in studies on pelagic (Hüssy

176 et al., 2016; Heimbrand et al., 2020) and coastal (Sturrock et al., 2015a) marine fish, in addition
177 to diadromous (Brûlé, 2022; Mainguy et al., 2023) and freshwater fish species.

178 Hence, in our study and in light of current knowledge, the use of Zn to assess fish age
179 appears to be the most reliable tool across distinct environments (*i.e.* marine, estuarine, and
180 freshwater systems) and species. Although the reliability of Mg and Mn has been demonstrated
181 in other studies (Limburg and Casini, 2018; Hüsey et al., 2021a), we did not identify such trends
182 with Atlantic salmon from the Bay of Biscay. Their complex life cycle and the associated multiple
183 shifts in their foraging strategies and diet may be responsible for the lack of annual elemental
184 pattern in this study, in addition to the mix of three spatially separate populations. Future
185 research could examine the variability of P in otolith as it has been proposed as a promising
186 element to estimate fish age (Hüsey et al., 2021b).

187

ACKNOWLEDGEMENTS

188 The authors would like to thank the regional water agency (*Agence de l'Eau Adour-*
189 *Garonne*), the *Office français de la biodiversité* (OFB), the European Community (North Atlantic
190 Salmon Conservation Organization - NASCO) and the *Centre National de Recherche*
191 *Scientifique* (CNRS) for funding this study. We are also very grateful to every organization and
192 person that have helped in sample collection: MIGRADOUR (<https://www.migradour.com/>),
193 MIGADO (<http://www.migado.fr/>), *Bretagne Grands Migrateurs* (BGM, [https://www.observatoire-](https://www.observatoire-poissons-migrateurs-bretagne.fr/)
194 [poissons-migrateurs-bretagne.fr/](https://www.observatoire-poissons-migrateurs-bretagne.fr/)), the *Fédérations de pêche* 29, 64, and 65, *Maison Barthouil*,
195 Jean-Marc Casteigt, Patrick Balesta, and recreational anglers. Finally, many thanks to
196 Alexandre Holub, Jordan Lacoste, Fanny Claverie and Gaëlle Barbotin for their precious help in
197 otolith preparation and analyses.

198

199

AUTHOR CONTRIBUTIONS

200 **AF:** Formal analysis, Methodology, Validation, Investigation, Writing – Original Draft, Writing –
201 Review & Editing, Visualization

202 **MV:** Methodology, Validation, Writing – Review & Editing, Visualization

203 **HT:** Formal analysis, Investigation, Writing – Review & Editing

204 **CP:** Resources

205 **GB:** Conceptualization, Validation, Writing – Review & Editing, Supervision, Project
206 administration, Funding acquisition

REFERENCES

- 208 Bouveyron, C., Jacques, J., 2011. Model-based clustering of time series in group-specific functional
209 subspaces. *Adv. Data Anal. Classif.* 5, 281–300. <https://doi.org/10.1007/s11634-011-0095-6>
- 210 Brûlé, C., 2022. Occurrence de saumons atlantique (*Salmo salar*) estuariens dans la région de la baie
211 d'Ungava (Nunavik, QC) confirmée par la chimie des otolithes et la morphologie des écailles.
212 Université du Québec à Chicoutimi.
- 213 Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive
214 understanding of fish populations? *Can. J. Fish. Aquat. Sci.* 58, 30–38.
215 <https://doi.org/10.1139/f00-177>
- 216 Heimbrand, Y., Limburg, K.E., Hüsey, K., Casini, M., Sjöberg, R., Palmén Bratt, A., Levinsky, S.,
217 Karpushevskaya, A., Radtke, K., Öhlund, J., 2020. Seeking the true time: Exploring otolith
218 chemistry as an age-determination tool. *J. Fish Biol.* 97, 552–565.
219 <https://doi.org/10.1111/jfb.14422>
- 220 Hüsey, K., Gröger, J., Heidemann, F., Hinrichsen, H.-H., Marohn, L., 2016. Slave to the rhythm:
221 seasonal signals in otolith microchemistry reveal age of eastern Baltic cod (*Gadus morhua*).
222 *ICES J. Mar. Sci.* 73, 1019–1032. <https://doi.org/10.1093/icesjms/fsv247>
- 223 Hüsey, K., Krüger-Johnsen, M., Thomsen, T.B., Heredia, B.D., Næraa, T., Limburg, K.E., Heimbrand,
224 Y., McQueen, K., Haase, S., Krumme, U., Casini, M., Mion, M., Radtke, K., 2021a. It's
225 elemental, my dear Watson: validating seasonal patterns in otolith chemical chronologies.
226 *Can. J. Fish. Aquat. Sci.* 78, 551–566. <https://doi.org/10.1139/cjfas-2020-0388>
- 227 Hüsey, K., Limburg, K.E., de Pontual, H., Thomas, O.R.B., Cook, P.K., Heimbrand, Y., Blass, M.,
228 Sturrock, A.M., 2021b. Trace element patterns in otoliths: The role of biomineralization. *Rev.*
229 *Fish. Sci. Aquac.* 29, 445–477. <https://doi.org/10.1080/23308249.2020.1760204>
- 230 Limburg, K.E., Casini, M., 2018. Effect of marine hypoxia on baltic sea cod *Gadus morhua*: Evidence
231 from otolith chemical proxies. *Front. Mar. Sci.* 5, 482.
232 <https://doi.org/10.3389/fmars.2018.00482>
- 233 Limburg, K.E., Wuenschel, M.J., Hüsey, K., Heimbrand, Y., Samson, M., 2018. Making the otolith
234 magnesium chemical calendar-clock tick: Plausible mechanism and empirical evidence. *Rev.*
235 *Fish. Sci. Aquac.* 26, 479–493. <https://doi.org/10.1080/23308249.2018.1458817>
- 236 Mainguy, J., Arsenault, A., Tran, L., Martyniuk, M.A.C., Paquet, C., Moore, J., Power, M., 2023.
237 Otolith-inferred patterns of marine migration frequency in Nunavik Arctic charr. *J. Fish Biol.*
238 *jfb.15491*. <https://doi.org/10.1111/jfb.15491>
- 239 Martin, G., Thorrold, S., 2005. Temperature and salinity effects on magnesium, manganese, and
240 barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. *Mar.*
241 *Ecol. Prog. Ser.* 293, 223–232. <https://doi.org/10.3354/meps293223>
- 242 Martin, J., Bareille, G., Berail, S., Pécheyran, C., Gueraud, F., Lange, F., Daverat, F., Bru, N., Beall,
243 E., Barracou, D., Donard, O., 2013. Persistence of a southern Atlantic salmon population:
244 diversity of natal origins from otolith elemental and Sr isotopic signatures. *Can. J. Fish. Aquat.*
245 *Sci.* 70, 182–197. <https://doi.org/10.1139/cjfas-2012-0284>
- 246 Ruttenberg, B., Hamilton, S., Hickford, M., Paradis, G., Sheehy, M., Standish, J., Ben-Tzvi, O.,
247 Warner, R., 2005. Elevated levels of trace elements in cores of otoliths and their potential for
248 use as natural tags. *Mar. Ecol. Prog. Ser.* 297, 273–281. <https://doi.org/10.3354/meps297273>

- 249 Sturrock, A.M., Hunter, E., Milton, J.A., EIMF, Johnson, R.C., Waring, C.P., Trueman, C.N., 2015a.
250 Quantifying physiological influences on otolith microchemistry. *Methods Ecol. Evol.* 6, 806–
251 816. <https://doi.org/10.1111/2041-210X.12381>
- 252 Sturrock, A.M., Trueman, C.N., Darnaude, A.M., Hunter, E., 2012. Can otolith elemental chemistry
253 retrospectively track migrations in fully marine fishes? *J. Fish Biol.* 81, 766–795.
254 <https://doi.org/10.1111/j.1095-8649.2012.03372.x>
- 255 Sturrock, A.M., Wikert, J.D., Heyne, T., Mesick, C., Hubbard, A.E., Hinkelman, T.M., Weber, P.K.,
256 Whitman, G.E., Glessner, J.J., Johnson, R.C., 2015b. Reconstructing the migratory behavior
257 and long-term survivorship of juvenile Chinook salmon under contrasting hydrologic regimes.
258 *PLOS ONE* 10, e0122380. <https://doi.org/10.1371/journal.pone.0122380>
- 259

1 **UNSUPERVISED ESTIMATION OF THE AGE AT SEA DEPARTURE OF**
2 **ATLANTIC SALMON USING MAGNESIUM, MANGANESE, AND ZINC**
3 **OTOLITH CHEMISTRY**

4 A. Fontaine¹, M. Vignon², H. Tabouret¹, C. Pécheyrant¹, G. Bareille¹

5 ¹*University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France*

6 ²*University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France*

7

8 **Corresponding author:**

9 Anthony Fontaine

10 anthony.fontaine@univ-pau.fr

11 **ORCID ID:** <https://orcid.org/0000-0001-5779-5789>

12

TABLE CAPTION

13 Table 1: Proportions of correct reattribution and model convergence for each element under
14 study (Mg, Mn, and Zn), obtained from the functional clustering analysis with univariate and
15 multivariate approaches. The results originate from the cross-validation analyses (randomly
16 selected in-bag fraction: 0.75, number of iterations: 1000) we conducted on each metal and
17 each possible combination from the initial sampling (n=309 for 1+ fish, n=68 for 2+ individuals,
18 n=377 for total sample number). The parameters used to perform the analyses were set as
19 follows: $N=150$, $k=10$, $n^{basis}=5$, $n^{order}=4$, $itermax=10000$ and $eps=10^{-5}$ with N being the number
20 of datapoints per profile, k the number of basis to form a spline, n^{basis} the number of β splines
21 used, n^{order} define a polynomial of degree 4, $itermax$ the maximal number of iteration in the
22 *funHDDC* algorithm and eps the threshold of the convergence criterion.

TABLE

24 Table 1

Element(s)	Certified origin	Average proportion of fish correctly assigned \pm SD (%)	Proportion of model convergence (%)
<i>Univariate</i>			
Mg	1+	94.2 \pm 4.0	43.2
	2+	3.6 \pm 5.2	
Mn	1+	78.9 \pm 7.2	89.3
	2+	5.3 \pm 6.5	
Zn	1+	83.1 \pm 5.2	99.8
	2+	91.0 \pm 6.4	
<i>Multivariate</i>			
Mg-Mn	1+	79.2 \pm 6.9	89.8
	2+	5.3 \pm 6.2	
Mg-Zn	1+	83.0 \pm 5.0	99.9
	2+	91.0 \pm 6.4	
Mn-Zn	1+	82.6 \pm 5.4	100
	2+	91.3 \pm 6.3	
Mg-Mn-Zn	1+	82.8 \pm 5.3	99.9
	2+	91.5 \pm 6.3	

25 Abbreviations: Mg, Magnesium; Mn, Manganese; SD, Standard Deviation; Zn, Zinc

1 **UNSUPERVISED ESTIMATION OF THE AGE AT SEA DEPARTURE OF**
2 **ATLANTIC SALMON USING MAGNESIUM, MANGANESE, AND ZINC**
3 **OTOLITH CHEMISTRY**

4 A. Fontaine¹, M. Vignon², H. Tabouret¹, C. Pécheyran¹, G. Bareille¹

5 ¹*University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France*

6 ²*University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France*

7

8 **Corresponding author:**

9 Anthony Fontaine

10 anthony.fontaine@univ-pau.fr

11 **ORCID ID:** <https://orcid.org/0000-0001-5779-5789>

12

FIGURE CAPTION

13 Figure 1: Raw data distribution of Mg/Ca, Mn/Ca, and Zn/Ca profiles from Atlantic salmon
14 (*Salmo salar*) sagittal otoliths. The left boxes display chemical profiles that were classified as 1+
15 river-aged individuals while 2+ are shown in the right boxes. The standardized distance [0-1]
16 was obtained using the otolith length between the core and the departure for marine migration.

1 **UNSUPERVISED ESTIMATION OF THE AGE AT SEA DEPARTURE OF**
2 **ATLANTIC SALMON USING MAGNESIUM, MANGANESE, AND ZINC**
3 **OTOLITH CHEMISTRY**

4 A. Fontaine¹, M. Vignon², H. Tabouret¹, C. Pécheyran¹, G. Bareille¹

5 ¹*University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France*

6 ²*University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France*

7

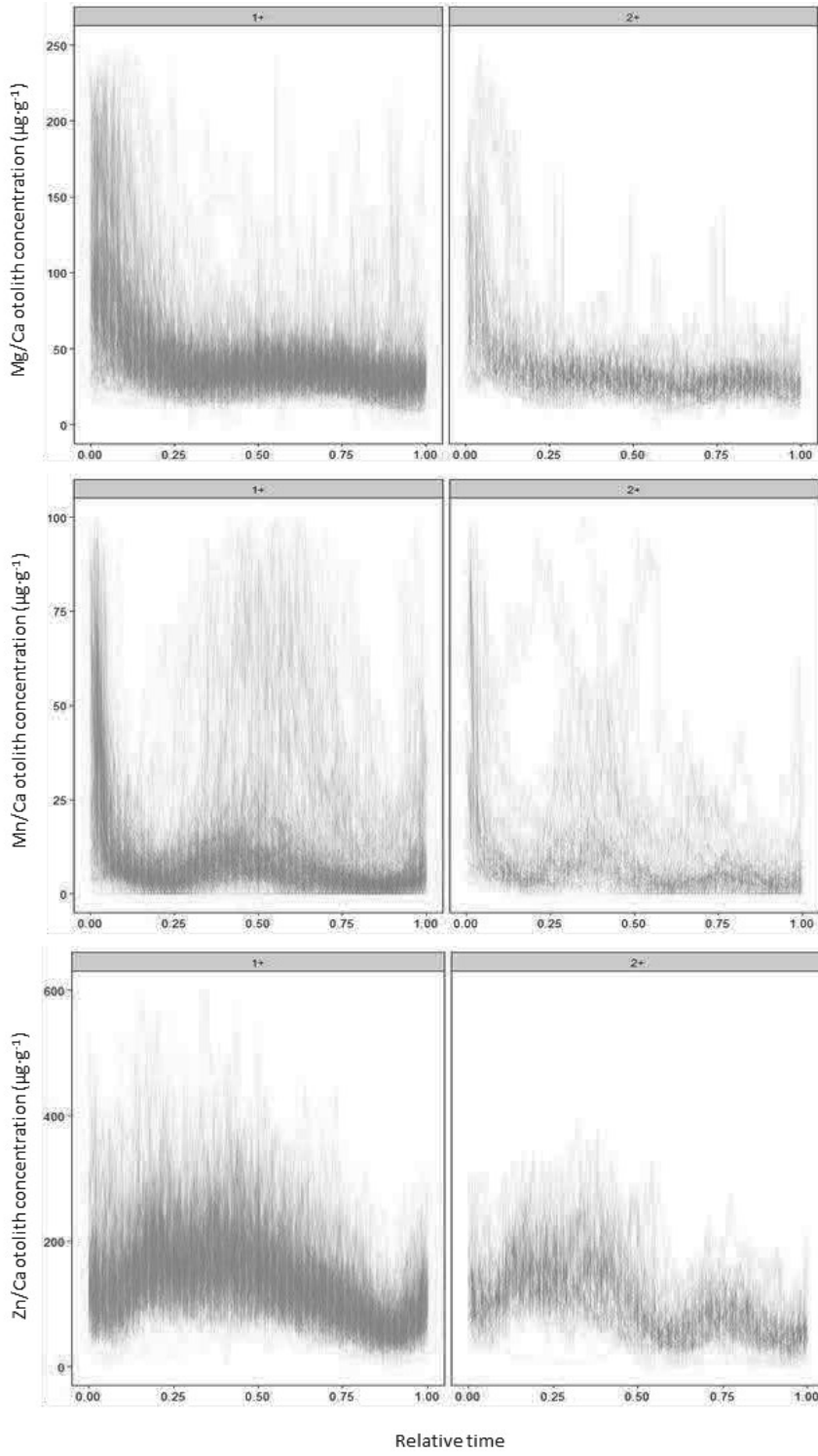
8 **Corresponding author:**

9 Anthony Fontaine

10 anthony.fontaine@univ-pau.fr

11 **ORCID ID:** <https://orcid.org/0000-0001-5779-5789>

FIGURE



1 **UNSUPERVISED ESTIMATION OF THE AGE AT SEA DEPARTURE OF**
2 **ATLANTIC SALMON USING MAGNESIUM, MANGANESE, AND ZINC**
3 **OTOLITH CHEMISTRY**

4 A. Fontaine¹, M. Vignon², H. Tabouret¹, C. Pécheyran¹, G. Bareille¹

5 ¹*University of Pau and the Adour Region, E2S UPPA, CNRS, IPREM, MIRA, Pau, France*

6 ²*University of Pau and the Adour Region, E2S UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France*

7

8 **Corresponding author:**

9 Anthony Fontaine

10 anthony.fontaine@univ-pau.fr

11 **ORCID ID:** <https://orcid.org/0000-0001-5779-5789>

12

SUPPORTING INFORMATION

13 Table S1: Detailed sampling information for 1+ and 2+ individuals by year of capture in the three
 14 localities under study: Adour, Bretagne, and Gironde.

Year of collection	Number of individuals captured in the Adour		Number of individuals captured in the Bretagne		Number of individuals captured in the Gironde		Total
	1+	2+	1+	2+	1+	2+	
2010					2		2
2011					7		7
2012	19	5			14	6	44
2013	70	7			9	4	90
2014	79	11			15	3	108
2015	60	11			7	3	81
2016	8	6	11	12	8		45
Total	236	40	11	12	62	16	

15

16 Table S2: Loglikelihood and proportion of convergence for each possible model tested in this
 17 study.

Element(s)	Number of groups (k)	Loglikelihood \pm SD ($\times 10^3$)	Proportion of model convergence (%)
<i>Univariate</i>			
Mg	2	-32.7 ± 1.5	43.2
	3	-33.1 ± 2.8	2.8
Mn	2	-26.1 ± 1.6	89.3
	3	-25.3 ± 2.5	3.7
Zn	2	-40.5 ± 3.2	99.8
	3	-41.4 ± 8.2	38.4
<i>Multivariate</i>			
Mg-Mn	2	-26.1 ± 1.6	89.8
	3	-24.8 ± 2.0	4.3
Mg-Zn	2	-40.4 ± 3.1	99.9
	3	-42.0 ± 8.3	35.9
Mn-Zn	2	-40.5 ± 3.1	100
	3	-42.2 ± 8.8	34.5
Mg-Mn-Zn	2	-40.5 ± 3.0	99.9
	3	-41.8 ± 8.1	35.2

18 Abbreviations: Mg, Magnesium; Mn, Manganese; SD, Standard Deviation; Zn, Zinc