Trans-Amazonian natal homing in giant catfish

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41   Running head: Trans-Amazonian natal homing in giant catfish

42   **Summary**

43   1.   Knowledge of fish migration is a prerequisite to sustainable fisheries management and

44   preservation, especially in large international river basins. In particular, understanding

45   whether a migratory lifestyle is compulsory or facultative, and whether adults home to their

46   natal geographic area is paramount to fully appraise disruptions of longitudinal connectivity

47   resulting from damming.

48   2.   In the Amazon, the large migratory catfishes of the *Brachyplatystoma* genus are apex

49   predators of considerable interest for fisheries. They are believed to use the entire length of

50   the basin to perform their life cycle, with hypothesized homing behaviours. Here we tested

51   these hypotheses, using the emblematic *B. rousseauxii* as a model species.

52   3.   We sampled adults close to major breeding areas in the Amazon basin (upper Madeira

53   and upper Amazonas) and assessed their lifetime movements by measuring variations in

54    $^{87}Sr/^{86}Sr$ along transverse sections of their otoliths (ear stones) using laser ablation

55   multicollector mass spectrometry (LA-MC-ICPMS).

56   4.   We demonstrate that larvae migrate downstream from the Andean piedmont to the

57   lower Amazon, where they grow over a protracted period before migrating upstream as

58   adults. Contrary to prevailing inferences, not all fish spend their nursery stages in the Amazon

59   estuary. By contrast, the passage in the lower or central Amazon seems an obligate part of the

60   life cycle. We further evidence that most adults home to their natal geographic area within the

61   Madeira sub-basin. Such long-distance natal homing is exceptional in purely freshwater

62   fishes.

63   5.   *Synthesis and applications.* By using otolith microchemistry, we were able to

64   demonstrate a seemingly compulsory basin-wide migratory life cycle of large Amazonian

65   catfishes. This makes them the organisms performing the longest migrations (>8000 km) in

66   fresh waters. This exceptional life history is already jeopardized by two dams recently built in

67   the Madeira River, which block a major migration route and access to a substantial part of

68   their spawning grounds. Major impacts can be anticipated from the current and forthcoming

69   hydroelectric development in the Amazon basin, not only on the populations and fisheries of

70   this apex predator, but also on Amazonian food webs through trophic cascades.

71

74

Introduction

76
77   Inland waters and their biodiversity provide a wealth of goods and services, but are

78   increasingly threatened by anthropogenic activities worldwide (Nilsson *et al.* 2005;

79   Hoeinghaus *et al.* 2009; Vörösmarty *et al.* 2010; Winemiller *et al.* 2016). Although relatively

80   preserved until recently in comparison with other large river systems, the Amazon basin,

81   which holds the most diverse freshwater fish fauna on Earth (Pimm *et al.* 2014), is no longer

82   an exception (Castello *et al.* 2013). Fisheries, one of the most important ecosystem services of

83   Amazonian fresh waters, largely rely on migratory Characiformes and Siluriformes (Lucas &

84   Baras 2001; Carolsfeld *et al.* 2003). The utmost importance of migratory animals in

85   ecosystem functioning and resilience has been stressed in recent studies, emphasizing their

86   major roles in coupling resident communities and shaping food web dynamics along their

87   migration routes (Wilcove & Wikelski 2008; Bauer & Hoye 2014). In-depth knowledge of the

88   movement patterns of these migratory species is required to fully appraise their structuring

89   importance and to implement relevant management policies. Such crucial information is

90   lacking for most migratory Amazonian fishes and in particular for some of the most

91   ecologically and economically important species, the *Brachyplatystoma* spp. (Pimelodidae).

92   These large (up to 3 m) catfishes are apex consumers (Barthem & Goulding 1997). They

93   support the largest fisheries of the Amazon basin and are often overexploited (Petrere Jr *et al.*

94   2004 ; García Vasquez *et al.* 2009 ; Agudelo *et al.* 2013). Based on strong circumstantial

95   evidence, these catfishes – at least *B. rousseauxii* (Castelnau 1855) – were hypothesized to

96   undertake the longest migrations ever described in fresh waters, from the spawning areas in

97   the Andean piedmont to the nursery in the Amazon estuary and back (Barthem & Goulding

98   1997): small juveniles are caught in the lower Amazon and in the freshwater part of the

99   estuary, body size in fisheries landings increases with distance from the estuary, while ripe

100   adults and newly hatched larvae are exclusively observed in white water tributaries of the

101   Andean piedmont in Bolivia, Colombia, Ecuador and Peru (Barthem & Goulding 1997;

102   García Vasquez *et al.* 2009 ; Cañas & Pine 2011; Agudelo *et al.* 2013). Recently, using

103   otolith microchemistry Hegg, Giarrizzo & Kennedy (2015) provided evidence that the

104   Amazon estuary might not be the sole nursery area for this species, as was thought originally

105   (Barthem & Goulding 1997). The hypothesis of a single panmictic population of *B.*

106   *rousseauxii* at the scale of the Amazon basin (Batista & Alves-Gomes 2006) was recently

107   refuted by the existence of several reproductive units, with at least one exclusively found in

108   the Madeira sub-basin (Carvajal-Vallejos *et al.* 2014). This genetic structure can be explained

109   either by a resident lifestyle, with individuals spending their entire life in the Madeira or by a

110   natal homing behaviour. Natal homing, the return of migratory individuals to reproduce in the

111   geographic area where they originated is widespread among terrestrial and aquatic animals

112   (Papi 1992). Some of the most fascinating examples of this adaptive strategy are found in

113   diadromous and marine fishes (Lucas & Baras 2001), such as salmons (Scheer 1939), eels

114   (Tesch & White 2008) and tunas (Rooker *et al.* 2008). Evidence of long-range natal homing is

115   scarce, however, for purely freshwater fishes (Lucas & Baras 2001). This behaviour has been

116   hypothesized in *B. rousseauxii* (Batista & Alves-Gomes 2006 ; Carvajal-Vallejos *et al.* 2014)

117   but never demonstrated.

118   Elucidating the migratory patterns of this large apex predator roaming the rivers of six

119   Amazonian countries with different fisheries policies is paramount for designing adequate

120   conservation and management strategies, especially in view of the current and proposed

121   hydroelectric development throughout the Amazon basin (Finer & Jenkins 2012; Castello *et*

122   *al.* 2013). In particular, two large dams (Santo Antonio and Jirau) have been erected since

123   2011 in the Brazilian part of the Madeira River, near Porto Velho (Fearnside 2014, Fig. 1).

124   The impacts of these and other forthcoming dams on *B. rousseauxii* largely depend on their

125   migration patterns: do these fish actually make basin-wide migrations? Do they make a single

126   or multiple runs? Do they exhibit homing behaviour?

127   Here, in order to address these questions, we sampled adults of *B. rousseauxii* close to

128   some of their main breeding areas in the Upper Amazonas and Upper Madeira (Fig. 1a),

129   before the construction of the Madeira dams. We measured ^{87}Sr/^{86}Sr ratios in transects along

130   transverse sections of their otoliths and we compared these observed profiles with those

131   expected from different scenarios of space utilization and migration (Fig. 2).

132

133   **Material and methods**

134

135   STUDY AREA

136   In Amazonia, the term Amazon is generally restricted to the river stretch between the estuary

137   and the confluence with the Negro River, near Manaus (Fig. 1). Further west, the river is

138   named Solimões until the tri-frontier between Peru, Colombia and Brazil near Tabatinga.

139   Further upstream, up to the confluence between the rivers Ucayali and Marañon (upstream of

140   Iquitos, Peru), it is known as Amazonas.

141

142   STUDY DESIGN

143   Most studies of fish migration rely on mark–recapture or biotelemetry (Lucas & Baras 2001).

144   The implementation of these methods, however, is unrealistic for the study of

145   *Brachyplatystoma* spp. migrations, given the small size of their migratory larvae (Cella-

146   Ribeiro *et al.* 2015) and the sheer dimensions of the Amazonian basin. The screening of fish

147   hard body parts for environmental chemicals (such as strontium or barium) or isotopes that

148   naturally substitute for calcium can be used as a proxy to indicate the fish's habitat at a

149   particular age or size (Campana 1999; Kennedy *et al.* 2000; see Appendix S1 in Supporting

150   Information for details). Methodologies using laser ablation (LA) combined with

151   multicollector (MC) and inductively coupled plasma mass spectrometry (ICPMS) for the

152   measurement of Sr isotopes and their ratio ($^{87}Sr/^{86}Sr$) along otolith transects enable the fine

153   reconstruction of habitat use during part or the entire fish's life (Outridge *et al.* 2002; Barnett-

154   Johnson *et al.* 2005).

155   Otoliths are made of relatively inert aragonitic calcium carbonate laid down on an

156   organic matrix, and contrary to other hard body parts, they are not subjected to any resorption

157   process. In contrast to the situation of many other chemicals, strontium (Sr) isotopic ratios in

158   otoliths are unaffected by confounding effects of biotic processes (physiological or dietary

159   dynamics) and abiotic factors (such as temperature) (Kennedy *et al.* 1997; Kennedy *et al.*

160   2000; Walther & Limburg 2012). The diversity of bedrocks with contrasting strontium

161   isotopic ratios ($^{87}Sr/^{86}Sr$) across the Amazonian Basin, together with the rather limited

162   variability of these ratios across years and seasons (Palmer & Edmond 1992; Gaillardet *et al.*

163   1997; Queiroz *et al.* 2009; Pouilly *et al.* 2014; Santos *et al.* 2015) provide heuristic

164   opportunities for identifying fish geographical origin or reconstructing their migration

165   patterns (Pouilly *et al.* 2014; Garcez *et al.* 2015; Hegg, Giarrizzo & Kennedy 2015), at least at

166   the scale of large sub-basins. A finer discrimination between tributaries is rendered more

167   difficult or impossible by the similarity of their underlying watershed geology.

168   Here we use the classification of Hegg, Giarrizzo & Kennedy (2015), slightly

169   modified in the light of additional data of strontium isotopic ratios in Amazonian waters

170   (Palmer & Edmond 1992). We distinguish three main riverine regions (Fig. 1): (i) the Western

171   Andean tributaries (hereafter, WAT), the Amazon River mainstem and its floodplain

172   tributaries; (ii) the Madeira sub-basin and lower Negro River; and (iii) tributaries draining the

173   Brazilian and Guyanese granitic shields, referred to hereafter as "granitic shield tributaries"

174   (GST). There is some overlap between the Sr isotopic signatures of Madeira waters and those

175   of the lower course of some GST, especially the Tapajos and Lower Negro River (Fig. 1,

176   Table S1). Decades of intensive fisheries and scientific sampling, however, never yielded any

177   ripe adult or larva of *B. rousseauxii* in GST (Barthem & Goulding 1997), in contrast to their

178   routine occurrence in Andean white water tributaries (Barthem & Goulding 1997; García

179   Vasquez *et al.* 2009; Cañas & Pine 2011; Agudelo *et al.* 2013). Therefore, otolith core

180   signatures comprised between 0.715 and 0.726 (range of available ^{87}Sr/^{86}Sr signatures in the

181   Madeira waters, Fig. 1, Table S1) will be considered as evidence of hatching within the

182   Madeira sub-basin. Likewise, otolith core signatures < 0.712 will be assigned to fish hatched

183   in WAT waters.

184

185   FISH SAMPLING

186   Fish were sampled between 2005 and 2009, before the construction of the Santo Antonio and

187   Jirau hydroelectric dams on the Madeira River. Eighteen fish (95–120 cm standard length,

188   SL) were obtained directly on the fishing grounds of the Bolivian upper Madeira, near Puerto

189   Villaroel on the Mamoré River, and near Rurrenabaque on the Beni River. Nineteen other fish

190   (89–141 cm SL) were purchased in Iquitos market, Peru, which lands fish caught within ~

191   300 km, including the Amazonas, and the lower parts of the Ucayali, Napo and Marañon river

192   systems. For each specimen, SL was measured (nearest cm) and otoliths (lapilli) were

193   extracted, rinsed in clear water, dried and stored for later examination.

194

195   OTOLITH ANALYSIS

196   Every collected otolith was processed at the MARBEC laboratory, Montpellier (France),

197   mounted in Araldite epoxy resin and a transverse section was made with a low-speed Isomet

198   saw (Buehler, Düsseldorf, Germany) to obtain a dorso-ventral slice including the otolith core.

199   The section was then ground and polished using polishing papers and ultra-pure water until

200   seeing the core on one side.

201   Analyses of Sr isotopes in fish otoliths were carried out in the laboratory LCABIE-IPREM

202   (Université de Pau et des Pays de l'Adour, France), using a Nu Plasma high resolution MC-

203   ICPMS (Nu instruments, Wrexham, UK) coupled to an Alfamet femtosecond (fs) laser

204   ablation system (Nexeya SA, Canejan, France). The laser ablated a transect from the core

205   (hatching of the fish) to the edge (death of the fish) of the otolith (i.e. perpendicular to the

206   growth marks) at a speed of 5 μm second^{-1}. The laser was operated at 500 Hz with a pulse

207   energy of 8 μJ to limit the in-depth ablation to no more than 30 μm while the scanner was

208   doing a permanent 200-μm wide back and forth movement at a speed of 1 mm second^{-1}. In

209   order to prevent blast effect on the nucleus, the ablation was started 200 μm before the core.

210   Twice a day, we analysed an in-laboratory $^{87}Sr/^{86}Sr$-certified otolith powder pellet (NIES22,

211   certified Reference Material produced by the National Institute for Environmental Studies,

212   Japan) to check the reliability of $^{87}Sr/^{86}Sr$ measurements (Martin *et al.* 2013). In all cases fs-

213   LA-MC-ICPMS $^{87}Sr/^{86}Sr$ values (mean ± 2 SD = 0.709289 ± 0.000379, *n* = 38) corresponded

214   well with the in-laboratory-certified values (mean ± 2 SD = 0.709240 ± 0.000021, *n* = 54).

215   Further details about analytical processes are given in Appendix S1.

216

217 ESTIMATION OF THE BODY LENGTH OF DOWNSTREAM AND UPSTREAM

218 MIGRANTS IN THE MADEIRA

219 On the basis of environmental signatures, the limit of ^{87}Sr/^{86}Sr between the Madeira and

220 Amazon waters was set at 0.715 (Fig. 1). We considered that *B. rousseauxii* exited the

221 Madeira when their otolith Sr isotopic ratios were consistently lower than 0.715. Similarly,

222 we assumed that fish had left the Amazon and re-entered the Madeira when their otolith Sr

223 isotopic ratios became anew consistently higher than 0.715. For both events, the distances

224 from the otolith's core were noted (nearest 25 μm). In order to back-calculate the fish SL at

225 these moments, we used a relationship between the otolith dimension along the transverse

226 radius and the fish SL, produced from 351 *B. rousseauxii* caught between 2009 and 2012 by

227 the Laboratory of Ichthyology and Fisheries of the Universidade Federal do Rondônia (UNIR)

228 in the Brazilian stretch of the Madeira River. The relationship is linear in small fish (40–50

229 cm SL) then becomes curvilinear in larger individuals (Fig. S1), as the otoliths of older fish

230 are thicker. Henceforth, two distinct equations were used for fish ≤ and > 45 cm SL.

231
232 SIZE OF *B. ROUSSEAUXII* AT FIRST SEXUAL MATURITY

233 The size at first sexual maturity (L_m) is defined as the SL at which 50% of the fish are

234 observed at an advanced maturation stage during the breeding season (Núñez & Duponchelle

235 2009) (i.e. when oocytes are visible to the naked eye, which means vitellogenesis has started).

236 L_m is estimated by fitting the fraction of mature individuals per 5-cm SL intervals to a logistic

237 function (García Vasquez *et al.* 2009; Agudelo *et al.* 2013), where the contribution of each

238 size class to the model is statistically weighted by the total number of individuals in the same

239 size class:

240 $$M = 100(1 + e^{(-a(L_i - L_m))})^{-1}$$

241 where *M* (%) is the proportion of mature individuals by size class, L_i is the central value of

242 size class *i*, and *a* and L_m are the coefficients of the model. Here, the model was adjusted using

243   152 female *B. rousseauxii* (37–125 cm SL) caught in the Brazilian part of the Madeira sub-

244   basin and for which we histologically determined the stage of gonad maturation. The values

245   of coefficients a and L_m in the model (Fig. S2) were fitted using a non-linear regression with

246   the quasi-Newton adjustment and were 0.3402 ($\pm$ 0.0141 SE) and 73.33 ($\pm$ 0.14 SE) cm SL,

247   respectively ($P < 0.0001$ in both cases).

248

249   **Results**

250   As for all migratory fishes, several scenarios can be hypothesized for *B. rousseauxii*,

251   including resident behaviour in the hatching tributary or sub-basin, long-range migration from

252   the hatching tributary to the Amazon mainstem followed by homing or straying, and multiple

253   runs between the Amazon mainstem and the same or different sub-basins. The corresponding

254   theoretical $^{87}Sr/^{86}Sr$ patterns in the otoliths of fish hatched in the Madeira and WAT are

255   distinct (Fig. 2). Several patterns can be univocal, whereas others may refer to several

256   scenarios. In particular, on the sole basis of Sr isotopic signatures, it is uncertain whether fish

257   ascending the Madeira before their capture did so during their very first upstream run or if

258   they migrated to WAT in the meanwhile (Fig. 2b, 2e). Likewise, it is almost impossible to

259   distinguish between fish spending all their life in WAT and those migrating once or several

260   times between these places and the middle or lower Amazon River (Fig. 2g, 2h, 2j). It is also

261   difficult to discriminate between fish venturing in the Madeira sub-basin and those entering

262   GST, because of the overlap between the Sr isotopic signatures of these rivers (Fig. 1b). This

263   confusion would only arise for intermediate runs, and exclusively in situations where fish in

264   GST would remain in waters with signatures < 0.726, thereby resulting in otolith profiles

265   resembling those illustrated in Fig. 2d or 2k. Intermediate runs in more radiogenic GST would

266   produce univocal profiles, similar to those illustrated in Fig. 2f or 2l. Regarding the final

267   upstream run, the interpretation is straightforwardly deduced from the capture location.

269 INTERPRETATION OF MIGRATION PATTERNS

270   Twelve of the 18 fish sampled in the Madeira sub-basin could be ranked as natal

271 homers (Fig. 3a, Fig. 2b). Their core Sr isotopic signatures (0.716–0.722) were typical of

272 Madeira waters, they moved downstream into waters with Amazon and WAT signatures,

273 grew in these waters, then homed to the Madeira sub-basin, where they remained until their

274 capture. As explained above, it is uncertain whether these fish ventured into WAT before

275 returning to the Madeira (Fig. 2b, 2e). By contrast, it is certain that they did not make any

276 intermediate run in the Madeira or in any GST, as these migratory patterns would have

277 involved a passage in the Amazon on the way back to the Madeira, which would have

278 produced Sr isotopic profiles close to those in Fig. 2d and 2f, respectively. Likewise, there is

279 no ambiguity for the final upstream run, as these fish were captured in the upper Madeira.

280 Based on the relationship between otolith radius and body length in *B. rousseauxii* (Fig. S1),

281 these fish would have exited the Madeira at 3.7 ± 1.6 cm SL (mean ± S.D.) and returned at

282 86.0 ± 5.7 cm SL, slightly above the mean size at first sexual maturity (73.3 ± 4.7 cm SL; Fig.

283 S2).

284   Ten other fish, all collected in Iquitos, exhibited no single Sr isotopic signal > 0.713 in

285 their otoliths (Fig. 3b). Therefore, it can be assumed that these fish never entered the Madeira

286 or GST, and they remained in the Amazon or WAT for their entire lives. By contrast, it is

287 uncertain whether these profiles correspond to resident or migratory behaviours, with one or

288 several runs between the Amazon and WAT (scenarios in Fig. 2g, 2h and 2j, respectively).

289 Based on existing knowledge, however, it is likely that these fish hatched in WAT, travelled

290 down to the Amazon at a small body size and subsequently returned to WAT. In contrast to

291 the clear-cut situation for fish homing to the Madeira, no information can be obtained here

292   regarding the size of fish during their (presumed) downstream and upstream runs, except that

293   all these fish had passed the size at first sexual maturity when they were captured.

294   Five other fish also hatched in waters with WAT or Amazon signatures and collected

295   later as adults in Iquitos, showed slightly less typical patterns (Fig. 3c). Four of them (Iq3,

296   Iq6, Iq8 and Iq11) repeatedly moved back and forth in waters with Sr isotopic ratios

297   intermediate between those of the Amazon and Madeira. The fifth fish (Iq12) showed a

298   signature profile matching the scenario of multiple runs in several Amazonian sub-basins

299   (Fig. 2l). This fish, which hatched and lived in waters with WAT and Amazon signatures,

300   thereafter migrated in highly radiogenic waters ($^{87}Sr/^{86}Sr > 0.730$), which are found

301   exclusively in some GST (probably the Negro or Tapajos rivers, see Fig. 1 and Table S1). It

302   eventually migrated back to the WAT, where it was caught.

303   For the remaining 10 fish, the edge signatures departed from the core signatures (Fig.

304   3d). For two of the six fish caught in the Upper Madeira (Ma10 and Be8), the core signatures

305   were typical of WAT, matching the straying scenario illustrated in Fig. 2c. The core

306   signatures of the remaining eight fish (0.7125–0.7147) fell in between the ranges of Madeira

307   and WAT.

308

Discussion

310   The migratory patterns of most fish were relatively univocal and indicated long-range

311   migration and natal homing behaviour (Fig. 3a). By contrast, the isotopic profiles of several

312   fish illustrated in Fig. 3c and 3d were less straightforward and require further discussion. Four

313   fish (Iq3, Iq6, Iq8 and Iq11) apparently moved back and forth in waters with Sr isotopic ratios

314   intermediate between those of the Amazon and Madeira (Fig. 3c). These ratios could

315   correspond to sojourns in non-sampled tributaries or refer to fish movements in confluence

316   areas between waters with highly contrasting signatures (Bouchez *et al.* 2010), as occur in

317 several places in the Amazon River (e.g. confluences with more radiogenic tributaries, such as

318 the Negro, Madeira and Tapajos rivers; Fig. 1). White water tributaries with high sediment

319 loads have a much higher and lasting impact than clear or black water tributaries when mixing

320 with waters of contrasting signatures (Bouchez *et al.* 2010). Hence, it is likely that most

321 profiles depicted in Fig. 3c and 3d correspond to movements within the confluence area of the

322 Amazon and Madeira or similar situations. Eight fish from Fig. 3d had core signatures

323 (0.7125–0.7147) falling in between the ranges of Madeira and WAT. These atypical

324 signatures might correspond to tributaries for which there is currently no information on Sr

325 isotopic ratios, and in this particular case, all eight fish would be strayers. Alternatively, it is

326 possible that these atypical core signatures originated from fish hatched in the Madeira sub-

327 basin, but moving downstream much faster than others, as can typically happen under higher

328 flows. Several authors have indeed demonstrated that *B. rousseauxii* reproduces during

329 contrasting hydrological periods in the main rivers of the Upper Madeira (Cañas & Pine 2011;

330 Cella-Ribeiro *et al.* 2015). On the basis of hydrological data (HYBAm) upon river discharge,

331 flow and width in the Madeira River, it can be reasonably assumed that fish hatching and

332 drifting during high flows could be flushed down into the Amazon at least three times faster

333 than during periods of low flows. While taking into account the methodological limitations

334 pertaining to the depth of laser ablation for otolith screening, such faster movements would

335 inevitably lead to some degree of integration of the Amazon signature into the core

336 signatures, and thus to lower Sr isotopic ratios than expected for fish hatched in Madeira

337 waters. If this hypothesis were valid, then four of the eight fish illustrated in Fig. 3d and that

338 were caught in Iquitos (Iq1, Iq4, Iq7 and Iq19) would be strayers from the Madeira (as

339 illustrated in Fig. 2i) and the remaining four fish caught in the Madeira (Ma7, Be1, Be2 and

340 Be5) would be natal homers.

341   Our results tend to indicate the absence of multiple upstream and downstream runs of

342   *B. rousseauxii* between the Madeira and the Amazon (Fig. 2d), which had been formerly

343   hypothesized (Barthem & Goulding 1997). The species fine-scale movement patterns cannot

344   be detailed yet, given the limited variability of ^{87}Sr/^{86}Sr ratios along the Amazon and WAT

345   mainstems and it is uncertain whether fish hatched in the Madeira and WAT share the same

346   nurseries.

347   There is strong evidence, however, that not all *B. rousseauxii* migrate downstream to

348   the Amazon estuary, as recently suggested by Hegg, Giarrizzo & Kennedy (2015) on the basis

349   of fish caught near Manaus. Indeed, fish hatched in the Madeira or WAT and growing

350   thereafter in the estuary must travel in the meanwhile through the lower Amazon (between the

351   mouth of the Madeira and Santarem, near the estuary), where the contributions of the Madeira

352   River and highly radiogenic GSTs tend to increase significantly the Sr isotopic ratio in the

353   Amazon mainstem to values ranging from 0.7103 to 0.7123 (averaging 0.7115 in Obidos and

354   Santarem, downstream of sampling points 27; Fig. 1, Table S1). Three fish hatched in WAT

355   and later caught in Iquitos (Iq 2, Iq 9 and Iq 10) had no single otolith ^{87}Sr/^{86}Sr value falling

356   within this range, thereby indicating that these fish never entered the Amazon estuary at any

357   life stage. Two other fish hatched in WAT, one caught in Iquitos (Iq 5) and the other one

358   caught in the Upper Madeira (Ma 10), exhibited such Sr isotopic ratios in their otoliths, but

359   only at relatively large body sizes (50 and 47 cm SL, respectively), thereby indicating that

360   they did not move into the estuary during their early juvenile stages. Our results suggest that

361   the nurseries of these five fish were somewhere upstream of the Obidos-Santarem area. No

362   such evidence of an absence of movement into the estuary can be deduced for fish hatched in

363   the Madeira, as these fish always inevitably cross waters with Sr isotopic ratios between

364   0.7103 and 0.7123 on their way down to the Amazon. Whatever the exact place of the

365   nurseries and their connections with the spawning areas of *B. rousseauxii*, a protracted period

366   in the lower or central Amazon River at the juvenile stage seems an obligate part of the life

367   cycle of *B. rousseauxii*, thereby refuting the hypothesis of an alternative resident lifestyle

368   (Carvajal-Vallejos *et al.* 2014). This certainly applies to fish hatched in the Madeira, for

369   which evidence is provided here, and presumably to those hatched in the WAT.

370   Our results provide the very first demonstration of natal homing of *B. rousseauxii* in

371   the Amazon basin, at least for the Madeira sub-basin. This is the longest homing migration

372   ever documented in potamodromous fishes. Once *B. rousseauxii* have homed to the Madeira,

373   at about the size of first sexual maturity, they never return to the Amazon. The data base of

374   water isotopic signatures in the Amazon basin is currently too limited to test whether *B.*

375   *rousseauxii* home precisely to their hatching tributary, although the pelagic nature of their

376   semi-buoyant eggs may suggest that salmon-like precision in homing is unlikely (Papi 1992;

377   Lucas & Baras 2001). Homing is seemingly the predominant behaviour, at least in the

378   Madeira River, but some fish do stray between sub-basins (Fig. 3d). The relatively low

379   number of otolith samples analysed in the present study precludes a reliable estimation of the

380   proportions of different behaviours. The occurrence of both homers and strayers here is

381   nevertheless consistent with molecular analyses indicating at least three reproductive units of

382   *B. rousseauxii* in the Amazon basin, one of which is restricted to the Madeira basin (Carvajal-

383   Vallejos *et al.* 2014).

384   The movement patterns highlighted here somehow differ from those recently

385   published for the same species by Hegg, Giarrizzo & Kennedy (2015), who did not evidence

386   fish originating from or moving into the Madeira. These differences presumably owe to at

387   least three factors. Their sampling localities were different, lying in the estuary (Belem) or

388   central Amazon (Manaus), and their fish were caught before their upstream spawning runs.

389   Finally, in contrast to the present study, where fish were collected at all seasons over several

390   years, all fish studied by Hegg, Giarrizzo & Kennedy (2015) were caught within less than a

391 month. The latter context is more likely to generate a sampling bias that could account for the

392 absence of fish hatched in the Madeira in their study. Alternatively it cannot be excluded that

393 fish hatched in the Madeira effectively contribute to the estuarine stock to a lower extent than

394 those hatched in WAT, especially if their main nursery areas were somewhere else. This brief

395 comparison emphasizes the need for further studies to elucidate the migrations of *B.*

396 *rousseauxii* in the Amazonian basin, in particular to understand the contributions of different

397 sub-basins and tributaries to recruitment and fisheries. In the study by Hegg, Giarrizzo &

398 Kennedy (2015) and in the present study, sample size was limited to a few tens of fish, but in

399 both cases the method used generated large numbers of samples over the life of individual

400 fish. These observations may be auto-correlated, but they nevertheless provide unprecedented

401 information on the life of this ecologically and economically important species.

402

403 In synthesis, the present study demonstrates basin-wide migrations of *B. rousseauxii*,

404 with a downstream migration into the Amazon at a small (<3–4 cm SL) body size, a

405 protracted growth period in the lower or central Amazon at the juvenile stage and a return

406 upstream run as adults. On the basis of fish captured in the Madeira, this long-range migratory

407 lifestyle is seemingly compulsory, with no single specimen exhibiting otolith strontium

408 isotopic profiles characteristic of a resident behaviour. These findings have profound

409 consequences for the management of this ecologically and commercially important species.

410 The now demonstrated existence of transboundary movements in *B. rousseauxii* further

411 advocates for the need of an international fisheries management coordination of this resource

412 across the Amazon basin (Fabré & Barthem 2005). Beyond current issues of overexploitation

413 and complex fisheries management, *B. rousseauxii*, in view of its compulsory basin-wide

414 migrations, might be imperilled to a greater extent than other migratory fishes by disruptions

415 of longitudinal connectivity. Until recently the Amazon basin had been moderately affected

416   by damming (Nilsson *et al.* 2005; Vörösmarty *et al.* 2010), with no single dam below 400 m

417   a.s.l. (Finer & Jenkins 2012) that could have interfered with the migratory life cycle of *B.*

418   *rousseauxii*. Since 2011, two large dams were erected in the lower Madeira River. Their fish

419   passage facilities have been found ineffective for accommodating the upstream runs of large

420   catfishes, including *B. rousseauxii* (Fearnside 2014). As *B. rousseauxii* spawns exclusively in

421   the white water tributaries of the Andean piedmont (WAT and upper Madeira), it can be

422   assumed that the access of *B. rousseauxii* to a substantial part of its possible spawning

423   grounds in the Amazon basin is already strongly compromised. This calls for urgent

424   modifications of the fish passages of both Jirau and Santo Antonio dams in order to restore

425   the connectivity between the upper and lower portions of the Madeira. Our results further

426   urge the need to preserve river connectivity in the still undammed parts of the Amazonian

427   lowlands and midlands, while tens of other dams are already planned in these regions (Finer

428   & Jenkins 2012; Castello *et al.* 2013 ; Fearnside 2014).

429   The impacts of multiple obstacles on the migration routes of *B. rousseauxii* might just

430   be as dramatic as those suffered in temperate regions by anadromous salmonids, which also

431   undertake basin-wide migrations (Lucas & Baras, 2001), or even worse, for two reasons.

432   First, *B. rousseauxii* makes its downstream migrations at a much younger age and smaller

433   body size than salmonids (Celia-Ribeiro *et al.* 2015; present study). Smaller fish have a lower

434   probability to escape predation or adverse physico-chemical conditions in lentic reservoirs

435   upstream of dams, because of their limited swimming capacities, and fish-attracting or

436   deterring devices aiming to facilitate a safe downstream passage are certainly less efficient

437   than for larger fish (Lucas & Baras 2001; Pelicice, Pompeu & Agostinho 2015). Second, *B.*

438   *rousseauxii* is an apex consumer, with key ecological functions (Barthem & Goulding 1997),

439   and its decline could thus have profound top–down effects on the ecosystem through trophic

440   cascades (Estes *et al.* 2011). To our knowledge, it is most infrequent that large piscine

441 predators exhibit long-range migrations in continental ecosystems. As a consequence,

442 damming in the Amazon basin could have more severe impacts on ichtyofauna and food webs

443 than in any other large river system. These issues add to a long list of concerns regarding the

444 sustainability of large-scale hydroelectric development (Nilsson *et al.* 2005; Vörösmarty *et al.*

445 2010; Ansar *et al.* 2014), and may require re-evaluating the actual pros and cons of large

446 hydropower plants in the Amazon basin (Finer & Jenkins 2012; Stickler *et al.* 2013; Kahn,

447 Freitas & Petrere Jr 2014; Winemiller *et al.* 2016), which hosts the world's richest

448 biodiversity.

449

457

458 **Data accessibility**

459 Source data of fish 87Sr/86Sr profiles are available in Supporting Information Table S3.

460

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589

590   **Figure Legends**

591

592 **Figure 1. (a) Map of the reference water ^{87}Sr/^{86}Sr values in the Amazon basin.** Yellow

593 squares refer to some of the cities mentioned in the text and yellow stars to fish sampling

594 localities. Black dots refer to water sampling stations, with the following colour code: blue for

595 the Western Andean tributaries, the Amazon and floodplain tributaries, red for the Madeira

596 and Lower Negro and purple for the granitic shield tributaries with black or clear waters.

597 Coloured circles with a white ring correspond to multi-year samples from SO-HYBAm

598 sampling stations (Table S1). The main Andean white water tributaries are highlighted in dark

599 blue. The two transverse black bars on the Madeira River upstream of Porto Velho represent

600 two large hydroelectric dams erected since 2011 (Santo Antônio and Jirau). **(b) Mean**

601 **isotopic water signatures (± SD) along the Amazon basin** following a west-to-east gradient

602 from the Andes to the Amazon mouth. Individual water data are provided in Table S1.

603

604 **Figure 2**. **Isotopic Sr profile patterns corresponding to alternative migration scenarios**

605 **for *Brachyplatystoma rousseauxii* in the Amazon basin.** The light red and blue bands

606 correspond to the ranges of water signatures in the Madeira sub-basin, and in the Amazon and

607 Western Andean tributaries, respectively (see Fig. 1). Rectangles on the horizontal axis of

608 each graph refer to the inferred downstream (closed symbols) and upstream movements

609 (hatched symbols), in the granitic shield tributaries (GST, purple), Madeira (red) or Western

610 Andean tributaries (WAT, blue). The bold black line is the corresponding Sr isotopic ratio in

611 the fish otolith. For the sake of simplicity, only situations for homers were illustrated for

612 scenarios involving multiple runs (d, e, f, j, k, l).

613

614 **Figure 3. Laser ablation-MC-ICPMS transects of $^{87/86}$Sr along otolith transverse sections**

615 **(from the core to the edge) of *Brachyplatystoma rousseauxii*.** Each curve corresponds to

616   one individual caught either in the upper Madeira (Mamoré, Ma and Beni, Be rivers) or in the

617   upper Amazon (fish landed in Iquitos, Iq; see Fig. 1). Four migratory patterns were identified

618   among transects (a, b, c, d, see text). The dashed line (Fig. 3a) represents the size at first

619   sexual maturity of *B. rousseauxii* in the Madeira basin (Fig. S2).

620

621

622

Supporting Information

624 Additional Supporting Information may be found in the online version of this article:
625

626 **Appendix S1 -** Detailed methods.

627

628 **Table S1.** Detailed water ^{87}Sr/^{86}Sr data.

629

630 **Table S2.** Laser ablation and multicollector settings.

631

632 **Figure S1**. Relationship between the radius of the otolith transverse section and the standard

633 length of *B. rousseauxii* from the Madeira basin.

634

635 **Figure S2**. Percentage of mature individuals as a function of standard body length in female

636 *B. rousseauxii* from the Madeira River basin.

637

638 **Table S3**. Otolith transect data. Table of raw otolith transect data for each sample analysed
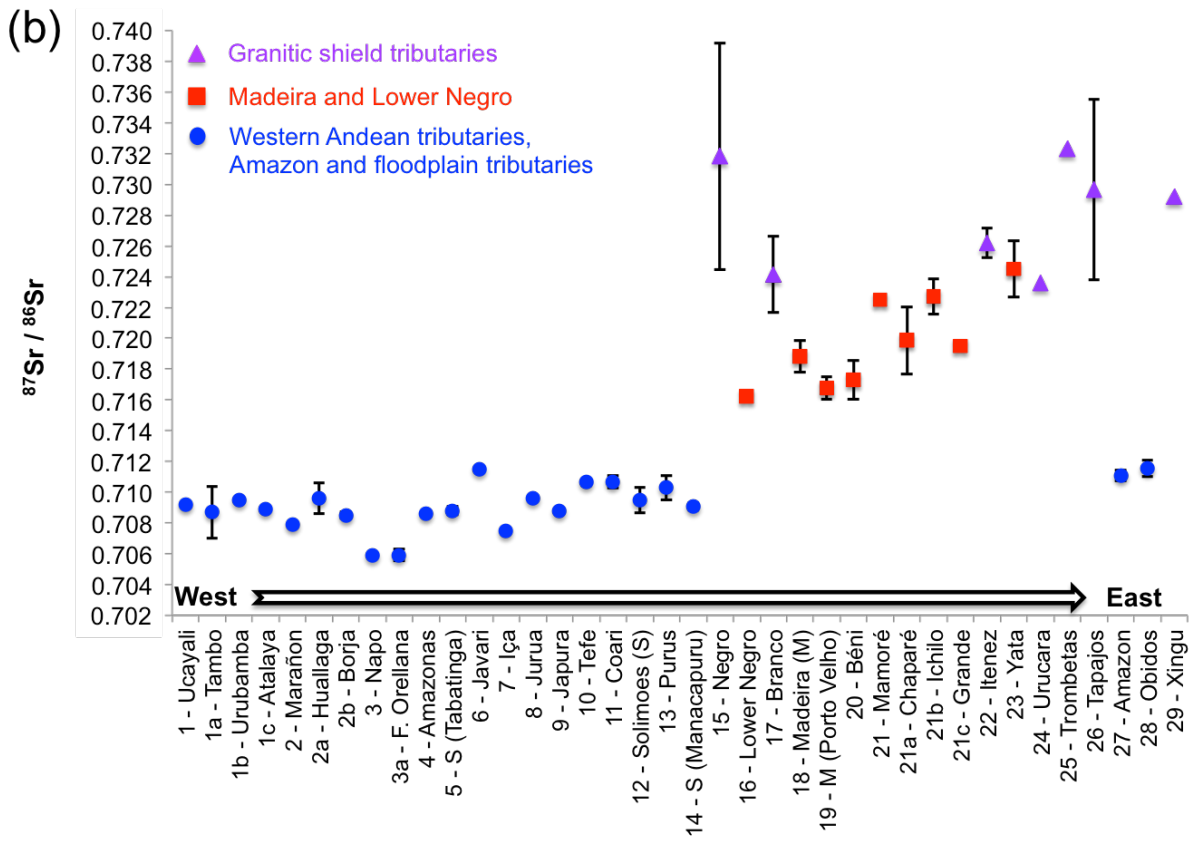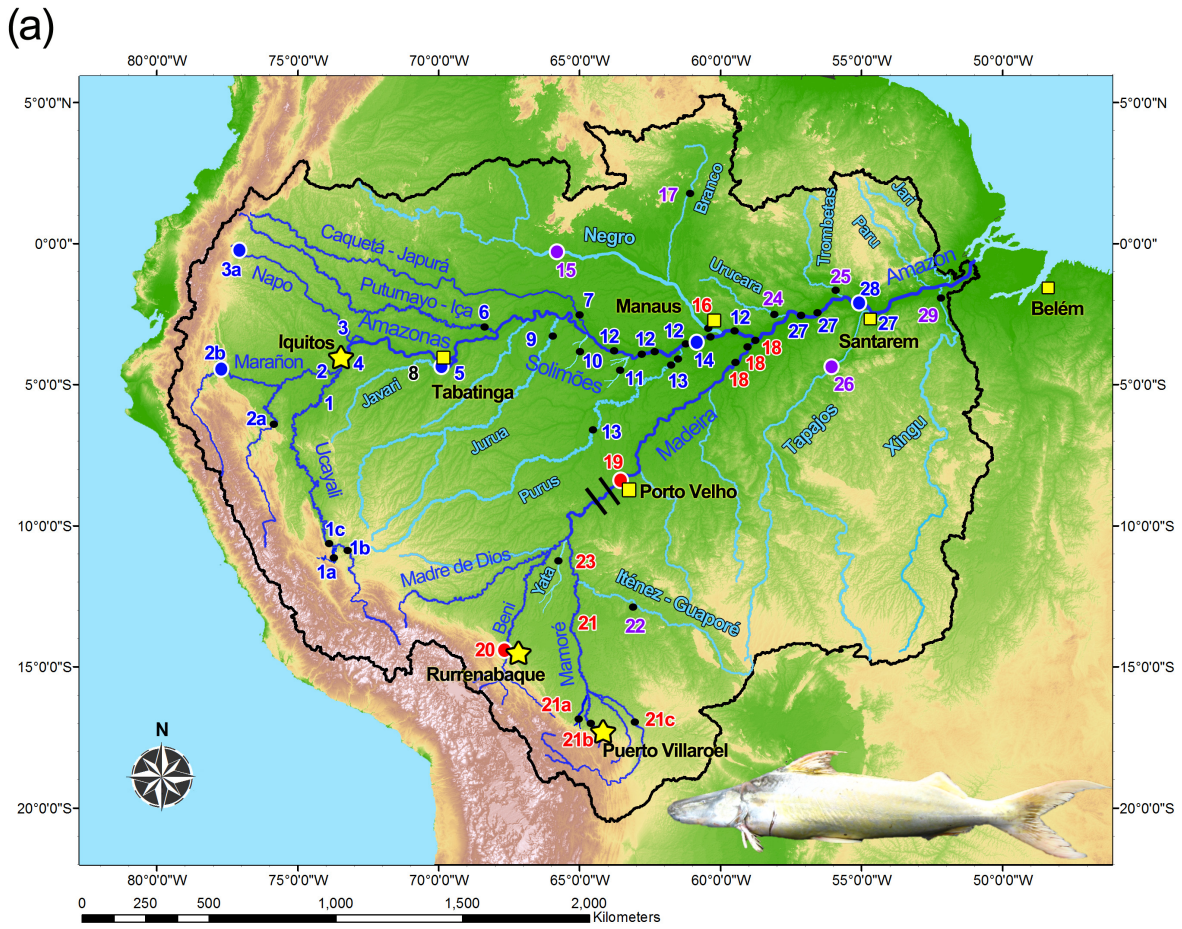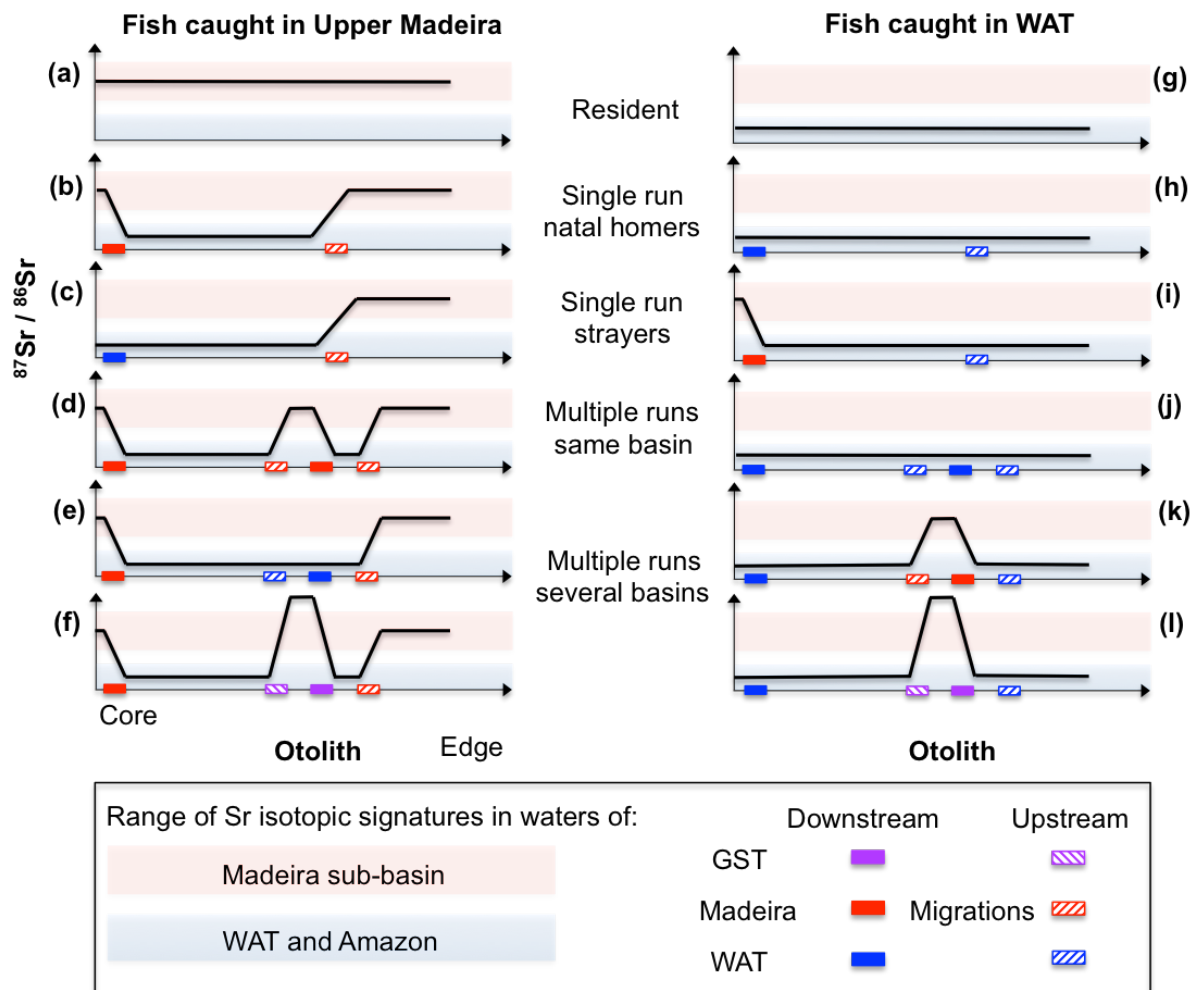
639 (XLSX).

(a)

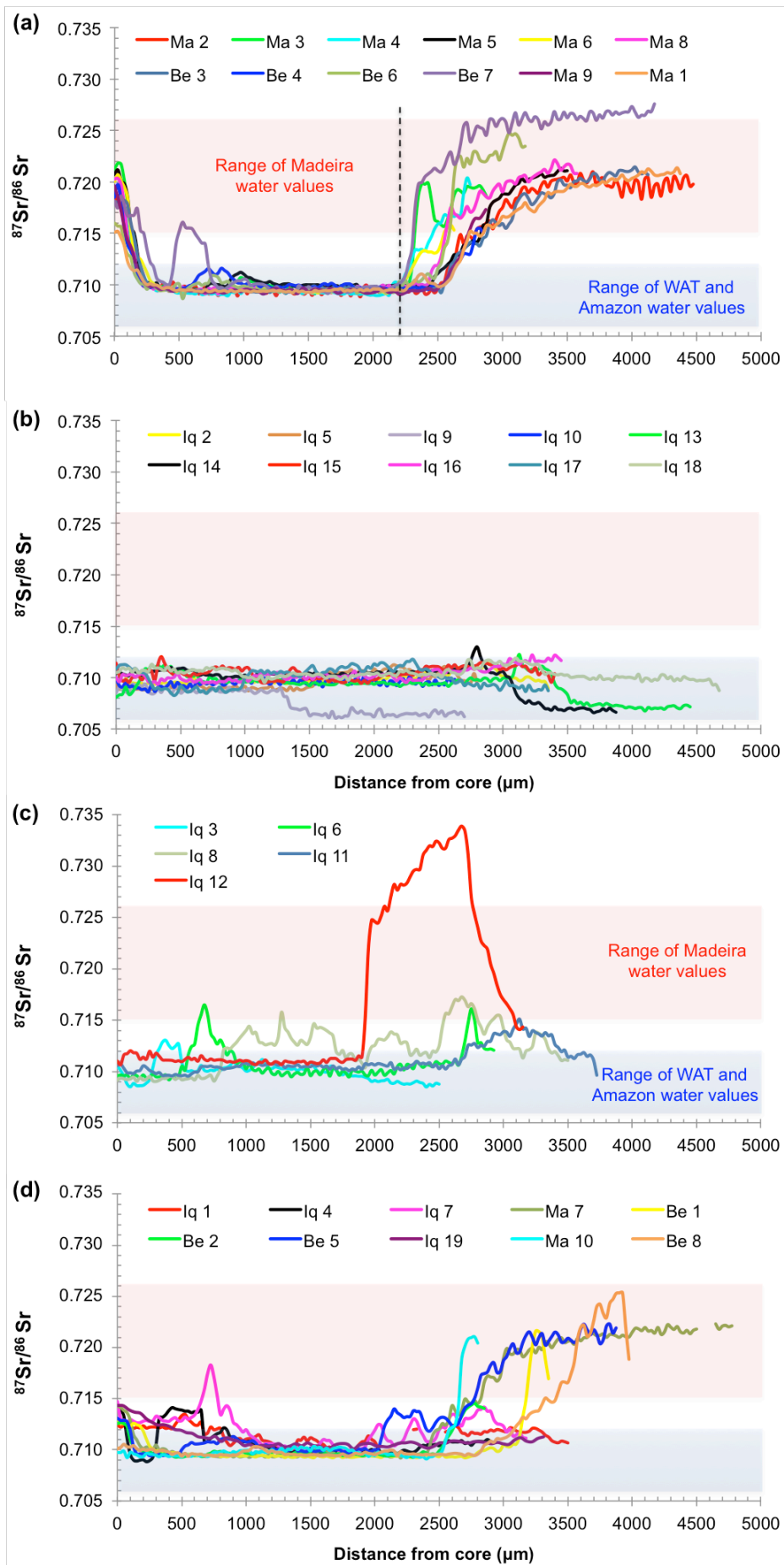(b)

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2
3   Fig. 1.

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Fig. 2.

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15   Fig. 3
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1   **Supporting Information for Duponchelle, F. et al. "Trans-**

2   **Amazonian natal homing in giant catfish"**

3

4   **Appendix S1: detailed methods**

5   **Reconstructing fish migrations using microchemistry of hard body parts.** The bones,

6   cartilages, scales and otoliths (ear bones) of a fish grow continuously during its life, forming

7   seasonal growth rings. The screening of these body hard parts for chemical elements or

8   isotopes gives the opportunity to reconstruct habitat conditions during the fish's life

9   (Campana 1999). As fish grow, natural chemical elements such as strontium, barium, lead or

10   zinc, and their isotopes, readily substitute for calcium in their hard body parts, generally in

11   proportion to their ambient dissolved concentrations or occurrence in food. Their quantitative

12   analysis in fish hard body parts can thus be used as a proxy of the fish's habitat at a particular

13   age or size. Otoliths are made of relatively inert aragonitic calcium carbonate laid down on an

14   organic matrix. In contrast to other hard body parts, they are not affected by any resorption

15   process and are thus considered as reliable internal data loggers registering ambient

16   environmental conditions (Panfili *et al*. 2002). Trace element concentrations or elemental

17   ratios, which vary widely between marine and fresh waters, have been extensively used for

18   studying the migrations of diadromous fishes (Tsukamoto *et al*. 2008 ; Campana & Thorrold

19   2001 ; Walther & Limburg 2012). However, their use in purely freshwater fishes has proved

20   more challenging, owing to reduced chemical gradients across a river basin and possible

21   confounding effects of biotic processes (physiological or dietary dynamics) or abiotic factors

22   (temperature) on their incorporation into fish otoliths. By contrast, strontium (Sr) isotopic

23   ratios in otoliths are unaffected by the latter potential biases; they depend almost exclusively

24   on the age and bedrock geological composition of watersheds, and thus, they barely vary

25   across years and represent accurate habitat signatures (Walther & Limburg 2012; Kennedy *et*

26 *al*. 1997 ; Kennedy *et al*. 2000). Recently, methodologies using laser ablation (LA) combined

27 with multicollector (MC) and inductively coupled plasma mass spectrometry (ICPMS) for the

28 measurement of Sr isotopes and their ratio ($^{87}Sr/^{86}Sr$) along otolith transects have opened new

29 horizons in fish science, as they enable the fine reconstruction of habitat use during part or the

30 entire fish's life (Outridge *et al*. 2002; Barnett-Johnson *et al*. 2005). The utility of Sr isotopic

31 ratios and LA-MC-ICPMS for retracing the movements and habitat use of Amazonian fish

32 has recently been demonstrated (Pouilly *et al*. 2014; Santos *et al*. 2015; Hegg, Giarrizzo &

33 Kennedy 2015).

34

35 **Sr isotopic analyses in fish otoliths.** Analyses of Sr isotopes in fish otoliths were carried out

36 in the Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE) of the

37 Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux (IPREM) at the

38 Université de Pau et des Pays de l'Adour, France. All measurements were carried out on a Nu

39 Plasma HR-MC-ICPMS (Nu instruments, Wrexham, UK) coupled to an Alfamet femtosecond

40 (fs) laser ablation system (Nexeya SA, Canejan, France). This laser is fitted with a diode-

41 pumped Yb:KGW crystal laser source (HP1, Amplitude Systèmes, Pessac, France) delivering

42 360-fs pulses at 1030 nm. The laser source operates within a wide range of repetition rates (1-

43 100,000 Hz) and low energy (from <25 µJ pulse^{-1} at 100,000 Hz to <1,000 µJ pulse^{-1} below

44 1,000 Hz), which represents a new approach in analytical applications where high energy and

45 low repetition rate are commonly used. The laser beam is focused with a 25-mm lens

46 providing a 15-µm diameter spot size, and it can be rapidly moved (up to 0.5 m/s) with high

47 repositioning precision owing to 2D galvanometric scanning module fitted to the optical line.

48 The optical field covered by the laser beam is 25 mm^2. Further details of a similar laser

49 ablation system (operating in the IR region) are described elsewhere (Claverie *et al*. 2009;

50 Tabouret *et al*. 2010). Here the laser was operated at 500 Hz with a pulse energy of 8 µJ to

51 limit the in-depth ablation while the scanner was doing a permanent 200-μm wide back and

52 forth movement at a speed of 1 mm/s then resulting in an apparent 15 x 200 μm laser ablation

53 sample, the long axis of which was parallel to the otolith's growth marks (which are

54 considered as pseudo linear at this scale). This "sharp blade" virtual shaping allows

55 improving MC-ICPMS signal intensity while keeping high spatial resolution. Combined to

56 this back and forth movement, the sample was continuously moved from the core to the edge

57 of the otolith (i.e. perpendicular to the growth marks) at a speed of 5 $\mu m.s^{-1}$. In order to

58 prevent blast effect on the nucleus, the ablation was started 200 μm before the core. The

59 ablation depth never exceeded 30 μm.

60 The LA-MC-ICPMS coupling was carried out using a 2-m long antistatic tube of 6 mm

61 external diameter and 4 mm internal diameter (Legris SA, Rennes, France) into the ICP torch,

62 using helium as carrier gas. A two-inlet torch was used to mix the laser-generated aerosol

63 together with a liquid aerosol (2% HNO_3 solution in ultra pure water [18.2 MOhm] nebulised

64 by means of a pneumatic 200 $\mu L.min^{-1}$ self-aspirating micro-concentric nebuliser combined

65 with a mini-cyclonic Cinnabar spray chamber) before introduction into the plasma (Barats *et*

66 *al.* 2007). This dual-flow introduction system enables easy optimization of the MC-ICPMS by

67 nebulising a suitable solution for tuning. Measuring conditions were adjusted for maximum

68 sensitivity, stability, and plasma robustness (see Table S2). The contribution of ^{87}Rb to ^{87}Sr

69 intensity was deduced from the ^{85}Rb signal (Barnett-Johnson *et al*. 2010). The ^{83}Kr intensity

70 was monitored to control any potential interference of ^{84}Kr and ^{86}Kr on ^{84}Sr and ^{88}Sr,

71 respectively. The ^{86}Sr/^{88}Sr invariant ratio was used to correct the ^{87}Sr/^{86}Sr ratio from

72 instrumental mass bias using an exponential law (Walther *et al.* 2008). At the beginning and

73 the end of each half-day session, an in-lab ^{87}Sr/^{86}Sr certified otolith powder pellet (NIES22,

74 certified Reference Material produced by the National Institute for Environmental Studies,

75 Japan) was analysed to check the reliability of ^{87}Sr/^{86}Sr measurements (Martin *et al*. 2013). In

76   all cases fs-LA-MC-ICPMS ^{87}Sr/^{86}Sr values (mean ± 2 SD = 0.709289 ± 0.000379, *n* = 38)

77   were in very good agreement with the in-lab certified values (mean ± 2 SD = 0.709240 ±

78   0.000021, *n* = 54).

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