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1 **Phenotypic—Morphological Variations** And Demographic Responses Of The
2 Mediterranean Pond Turtle *Mauremys Leprosa* To Heterogeneous Aquatic Habitats

3

4 **Author names and affiliations**

5 Anne-Sophie Le Gal ^{a,b,c}, Jean-Yves Georges ^c, Christine Sotin ^{a,b}, Bruno Charrière ^{a,b}, Olivier
6 Verneau ^{a,b,d}

7 ^a Université de Perpignan Via Domitia, Centre de Formation et de Recherche sur les
8 Environnements Méditerranéens, UMR 5110, 52 Avenue Paul Alduy, F-66860 Perpignan
9 cedex, France

10 ^b CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens, UMR
11 5110, 52 Avenue Paul Alduy, F-66860 Perpignan cedex, France

12 ^c Université de Strasbourg, CNRS, IPHC UMR 7178, 23 rue du loëss, 67000 Strasbourg, France

13 ^d Unit for Environmental Sciences and Management, North-West University, Potchefstroom
14 campus, Private Bag X6001, 20520 Potchefstroom, South Africa

15

16 **Corresponding author**

17 Anne-Sophie Le Gal

18 Université de Perpignan Via Domitia, Centre de Formation et de Recherche sur les
19 Environnements Méditerranéens, UMR 5110, 52 Avenue Paul Alduy, F-66860 Perpignan
20 cedex, France

21 asophielegal@gmail.com

22

23 **Highlights**

24 Phenotypes and population structure of *Mauremys leprosa* are influenced by water quality

25 Fast-growing turtles, as well as largest and heaviest males, are associated with the most polluted
26 habitats

27 Highest proportions of ~~immature~~juveniles are associated with the least polluted habitats

28 Eutrophication affects turtle phenotype and may adversely affect reproductive success

29 **Abstract**

30 Human activities affect terrestrial and aquatic habitats leading to changes at both individual and
31 population levels in wild animal species. In this study, we investigated the phenotype and
32 demographics of the Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) in
33 contrasted environments of Southern France: two peri-urban rivers receiving effluents from
34 wastewater treatment plants (WWTP), and another one without sewage treatment plant. Our
35 findings revealed the presence of pesticides and pharmaceuticals in the three rivers of
36 investigation, the highest diversities and concentrations of pollutants being found in the river
37 subsections impacted by WWTP effluents. Principal component analysis and hierarchical
38 clustering identified three levels of habitat quality, with different pollutant concentrations,
39 thermal conditions, nutrient, and organic matter levels. The highest turtle densities, growth
40 rates, and body sizes were estimated in the most disturbed habitats, suggesting potential adult
41 benefits derived from ~~bad~~ harsh environmental conditions induced by pollution and
42 eutrophication. Conversely, juveniles were the most abundant in the ~~habitats of best quality~~ least
43 polluted habitats ~~(16-45% of the captures)~~ to the habitats of worst quality ~~(7-9% of the~~
44 ~~captures)~~, suggesting adverse effects of ~~degraded habitats~~ pollution on juvenile survival or adult
45 reproduction. This study suggests that turtles living in polluted habitats may benefit from
46 enhanced growth and body size, at the expense of reproductive success.

47

48 **Keywords**

49 Freshwater turtles; Wastewater treatment plant; Water pollution; Population structure

50 **Abbreviations**

51	AGO: Agouille de la Mar River	56	OSR: Operational sex ratio
52	AMPA: Aminomethylphosphonic acid	57	PCA: Principal component analysis
53	BAI: Baillaury River	58	SCL: Straight carapace length
54	BM: Body mass	59	SMI: Scaled mass index
55	FOS: Fosseille River	60	WWTP: <u>W</u> wastewater treatment plant

61

62 **1. Introduction**

63 Sauropsid reptiles have evolved over 300 million years, resulting in the emergence of several
64 morphs adapted to aquatic and terrestrial life (Reisz, 2005). They are unique among vertebrates,
65 encompassing various biological and behavioral characteristics, that include ectothermy,
66 delayed sexual maturity, migratory patterns, and cryptic behavior specially during early
67 developmental stages (Hopkins, 2000). They possess physical defenses such as scales and shells
68 that operate as protective armor against predation and desiccation, contributing to their
69 particularly long lifespan even in harsh conditions (Reinke et al., 2022). ~~Because variations in~~
70 ~~external temperature can alter metabolic rate and development, r~~They reptiles need to require
71 periods of thermoregulation-thermoregulate to reach their physiological optimum -(Pettersen,
72 2020). In extreme environmental ~~conditions~~, however, they-they can slow down metabolism
73 by entering into hibernation, which limits energy expenditure and enhances survival (Holden et
74 al., 2021). Nevertheless, major and rapid environmental changes following anthropization are
75 responsible for their decline worldwide (~~Doherty et al., 2020;~~ Gibbons et al., 2000; Todd et al.,
76 2010; Weir et al., 2010; Doherty et al., 2020). Accordingly, environmental pollutions can affect
77 animal survival (Trakimas and Sidaravičius, 2008; Owen-Jones et al., 20152016; Trakimas and

78 ~~Sidaravičius, 2008~~) and population stability (~~Boissinot et al., 2015~~; Gibbons et al., 2000; Irwin
79 and Irwin, 2006; Boissinot et al., 2015).

80 Reptiles can be exposed to pollutants such as pesticides, metals, and polychlorinated
81 biphenyls (PCBs) through ingestion, inhalation, dermal absorption, but also during maternal
82 transfer to the eggs (Freitas et al., 2020). In early life stages, micropollutants can interfere with
83 embryonic development and hatchling viability (Woodward et al., 1993; Willemsen and Hailey,
84 2001), ~~which may likewise alter sex ratio in species with temperature-dependent sex~~
85 ~~determination~~ (Barraza et al., 2021; Thompson et al., 2018). Pesticides which are widely used
86 for pest control in agriculture, can have adverse effects (~~Mingo et al., 2016~~; Weir et al., 2014;
87 Wagner et al., 2015; ~~Weir et al., 2014~~ Mingo et al., 2016). It was reported that organochlorines
88 (e.g., PCBs), triazines (e.g., atrazine, simazine), and phenylureas (e.g., diuron) can interfere
89 with sex hormones, damaging morphology and functionality of reproductive organs, with
90 implications for fertility (~~Crain and Guillette, 1998~~; Guillette et al., 1994; Crain and Guillette,
91 1998). Even though the lethal dose of a pollutant is not reached, chronic exposure can also harm
92 animals at low concentrations (Faburé et al., 2022). However, many questions still remain about
93 effects of chronic exposure to low concentrations of pollutants on animal population dynamics.

94 Among reptiles, aquatic turtles have the particularity of exploiting both aquatic and
95 terrestrial habitats. Because they are long-lived animals and prone to bioaccumulate pollutants,
96 they have been considered as relevant biological bio-probes indicators to monitor pollutants
97 exposure (Golden and Rattner, 2003; Hopkins et al., 2013; Slimani et al., 2018). When present
98 in food, water, and/or sediment, contaminants can impact turtles through dermal exposure
99 and/or ingestion (Leusch et al., 2021). Pollutants can affect metabolic profile (Meng et al.,
100 2023), sexual development (Matsumoto et al., 2014), growth rate (Holliday and Holliday,
101 2012), and increase incidence of juvenile deformities and mortalities (Adams et al., 2016).
102 Deleterious effects, such as lethal deformities in embryos (Bell et al., 2006), but also skin

103 lesions and physiological stress (Leusch et al., 2021) can occur as a result of chronic exposure
104 to a cocktail of pollutants. Furthermore, changes in the thermal regime in combination with
105 maternally-derived pollutants can unbalance sex ratio in clutches (Thompson et al., 20148).
106 However, despite the numerous studies that were conducted on the effects of environmental
107 pollution on turtle species, too few to date have focused on the same species occurring in
108 contrasted environments with varying degrees of pollution and eutrophication.

109 The Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) is a freshwater
110 turtle whose range extends from Northwest Africa (Western Libya, Tunisia, Algeria, and
111 Morocco) to Ssouthwest Europe (Spain, Portugal, and Southern France) (Bertolero and Busack,
112 2017). This species occurs mostly in permanent wetlands, living in stagnant water or slow-
113 flowing rivers (Keller, 1997; Díaz-Paniagua et al., 2015; ~~Keller, 1997~~). It is ~~also~~ adapted to
114 watercourses aquatic enclaves experiencing sharp seasonal changes and, consequently, large
115 fluctuations in water quantity and quality (Díaz-Paniagua et al., 2015). It is omnivorous, feeding
116 mainly on plants and macro-invertebrates yet with some scavenger behavior (Díaz-Paniagua et
117 al., 2015). Because it is a long-lived animal with a high somatic conversion rate of food, it can
118 bioaccumulate pollutants over a long period of time with various responses in contrasted
119 environments (Martínez-López et al., 2017; Slimani et al., 2018).

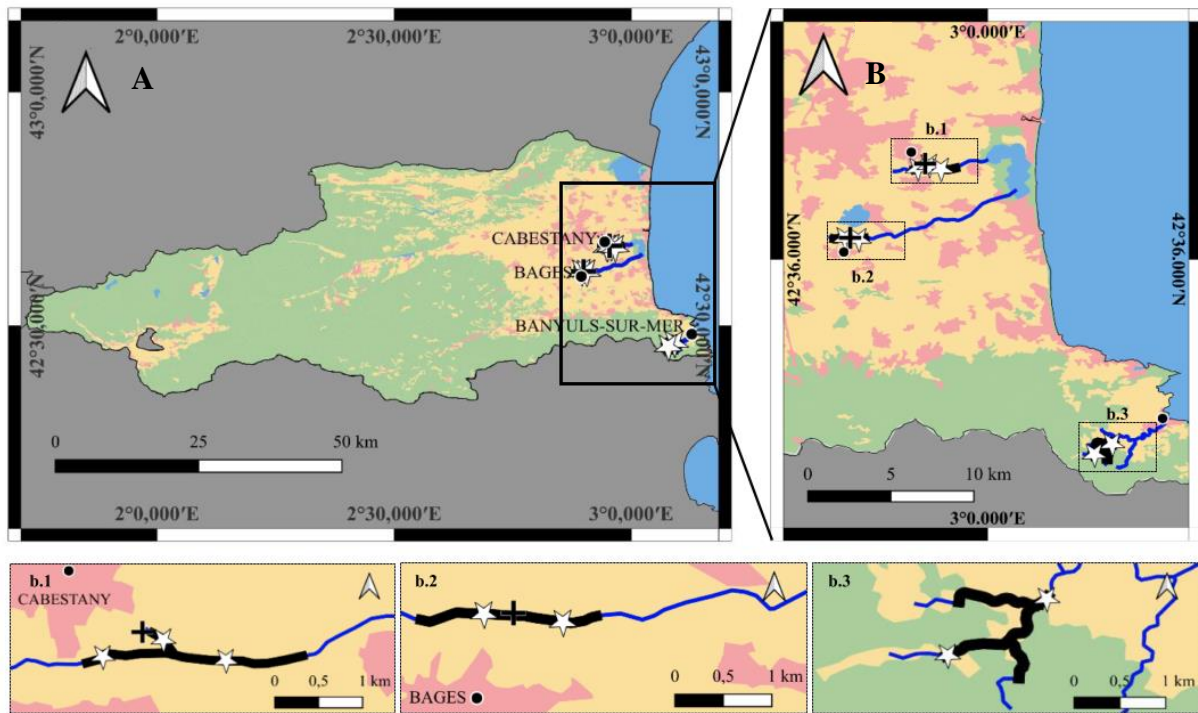
120 *Mauremys leprosa* ~~It~~ can ~~indeed~~ tolerate eutrophic waters (da Silva, 1995; Franch,
121 2003), and has even been reported in highly polluted habitats (Naimi et al., 2012~~El Hassani et~~
122 ~~al., 2019~~; Héritier et al., 2017; El Hassani et al., 2019~~Naimi et al., 2012~~). El Hassani et al.
123 (2019) showed that M. leprosa turtles, in Northwestern Morocco, could cope with habitat
124 pollution without exhibiting physiological disorders. On the opposite, Héritier et al. (2017)
125 showed that turtles living in aquatic habitats impacted from wastewater treatment plant
126 (hereafter referred as WWTP) effluents, in Southern France, exhibited high oxidative stress.
127 Despite a significant difference in pesticide pollution upstream and downstream of the WWTP

128 relarguing effluents on the river, over a nearly ten years study was revealed that turtles were
129 concentrated preferentially downstream of the WWTP, which was unexpected (Le Gal et al.,
130 2023)~~Le Gal et al. (2023) revealed over a nearly ten years study that turtles were concentrated~~
131 ~~preferentially downstream of the WWTP, which was unexpected.~~ Yet, additional comparative
132 studies are necessary to better understand the relationship between habitat alteration and the
133 demographics of *M. leprosa*.

134 The objectives of our study were to determine how *M. leprosa* responds to habitat alteration,
135 including pollution and eutrophication. Whether high degrees of habitat degradation can
136 promote large turtles and dense populations, a male-biased sex ratio was observed in the ~~worst~~
137 most polluted habitats (El Hassani et al., 2019; Le Gal et al., 2023), suggesting detrimental
138 effects on reproduction. In order to investigate the relationship between habitat alteration,
139 ~~structure of turtle populations~~morphological and demographic variations, and phenotypes, we
140 ~~studied~~ three populations of *M. leprosa* occurring along a latitudinal gradient, ~~which was~~
141 ~~characterized by three hydrologically disconnected and contrasted rivers~~ were studied. Our aims
142 were to assess the population structure and dynamics, and to correlate demographic parameters
143 to the quality of habitats.

144 **2. Materials and methods**

145 **2.1. Habitats of investigation** Study sites



146

147 **Figure 1.** (A) Region of Pyrénées-Orientales in Southern France, with aquatic areas in blue,
148 pristine areas in green, bare soil in yellow, cropland in orange and urbanized areas in red. (B)
149 The studied rivers are in navy blue, and their configuration is shown at the bottom of the main
150 map, with the river sections in black, the wastewater treatment plants represented by black
151 crosses, and water sampling points by white stars: (b.1) Fosseille River, (b.2) Aiguille de la
152 Mar River, and (b.3) Baillaury River.

153

154 **2.1.1. The Baillaury environment**

155 ~~The climate of the Albera Massif is characterized by mild winters and hot, dry summers. The~~
156 ~~thermal regime is marked by often large daily temperature variations (1 to 12 °C), but by annual~~
157 ~~variations of small amplitude.~~ The Baillaury River ~~and its tributaries~~ flows over 8.5 kilometers
158 ~~long through the Albera Massif,~~ from its source ~~located in the Puig del Torn (altitude, 600~~

159 meters high, to the Mediterranean Sea, at Banyuls-sur-Mer (Fig.1). ~~The Baillaury River~~
160 ~~watershed, with a surface area of 32 km², is traditionally cultivated with vineyards and is almost~~
161 ~~free of urban and industrial activities. The river~~It part of investigation consisted of three
162 ~~intereconnected tributaries (Rec de les Vinyes, Rec de Pouada, and Rec de les Abelles) flowing~~
163 ~~from an altitude of 120 to 60 meters, with a cumulative length of 1.62 kilometers, a width of~~
164 ~~0.5 to five meters, and a maximum depth of 0.5 meters. It~~ is characterized by a slow flow during
165 the dry season, when it partially dries up, and by a water regime increasing considerably during
166 seasonal rainfall events. The river section of investigation flows from an altitude of 120 to 60
167 meters, is 1.62 kilometers long, 0.5 to five meters wide, and 0.5 meters deep. Its watershed,
168 which is almost free of urban activities, is traditionally cultivated with vineyards. The edge
169 vegetation is mainly composed of the giant reed *Arundo donax* (Linnaeus, 1753), ~~the chaste~~
170 ~~tree~~ *Vitex agnus-castus* (Linnaeus, 1753), ~~the Mediterranean hackberry~~ *Celtis australis*
171 ~~(Linnaeus, 1753)~~, the European ash *Fraxinus excelsior* (Linnaeus, 1753), the cork oak *Quercus*
172 *suber* (Linnaeus, 1753), scrubland, and maquis. *Mauremys leprosa* is the only freshwater turtle
173 species present ~~in this river~~here, and other ~~most~~ frequently encountered aquatic species are
174 mostly native, among which the Mediterranean barb *Barbus meridionalis* (Risso, 1827), ~~the~~
175 ~~Catalan chub~~ *Squalius laietanus* (Doadrio, Kottelat and de Sostoa, 2007), the European eel
176 *Anguilla anguilla* (Linnaeus, 1758), the viperine water snake *Natrix maura* (Linnaeus, 1758),
177 the Perez's frog *Pelophylax perezi* (López-Seoane, 1885), and the spiny toad *Bufo spinosus*
178 (Daudin, 1803), ~~and the alien painted frog~~ *Discoglossus pictus* (Oth, 1837) (A. S. Le Gal,
179 ~~personal observations).~~

180

181 2.1.2. The Fosseille environment

182 ~~The Mediterranean climate in the plain of Roussillon is characterized by mild winters,~~
183 ~~consistently dry summers and moderate springs and autumns. In 2021, the weather in Cabestany~~

184 ~~and Bages cities (Fig.1) was characterized by large daily temperature variations (1 to 14 °C),~~
185 ~~and small annual temperature variations.~~ The Fosseille River is a lentic river, flowings in the
186 ~~plain of Roussillon over eight kilometers long, from its source located in tthe peri-urban peri-~~
187 ~~urban area~~ south of Perpignan to the Saint Nazaire lagoon, which is connected to the
188 Mediterranean Sea (Fig.1). ~~The Fosseille River watershed, with a surface area of 33 km², is~~
189 ~~highly anthropized by urban, industrial, and agricultural activities such as vine growing. The~~
190 river section of investigation flows from an altitude of 16 to two meters, is 2.6 kilometers long,
191 one to four meters wide, and two meters deep. The Iriver has been repurposed for agriculture,
192 ~~and~~ receives effluents from the WWTP of Cabestany, which contributes to maintain the
193 continuous water flow of the river year-round. ~~The river part of investigation flows from an~~
194 ~~altitude of 16 to two meters and has a cumulative length of 2.6 kilometers, a width of one to~~
195 ~~four meters, and a maximum depth of two meters. It consists of three subsections according to~~
196 the position of the WWTP: the upstream, outfall, and downstream river subsections (Fig.1). Its
197 watershed is highly anthropized by urban and agricultural activities such as vine growing. It
198 ~~was divided into two river sections, the second one starting at the connecting point between the~~
199 ~~Fosseille River and the WWTP outfall, which is 0.5 kilometer long. The first river section is~~
200 ~~located upstream of the WWTP, flows over 0.6 kilometer, and is hydrologically connected to~~
201 ~~the second river section only during rain events (Fig.1). The second river section is located~~
202 ~~downstream of the WWTP and flows over 1.5 kilometer long (Fig.1). Both river sections are~~
203 ~~lentic, with slow flow except during extreme rainfall events.~~ The riparian zone is homogeneous
204 ~~all along the investigated river,~~ with sparse border vegetation dominated by *A. donax*, the
205 common reed *Phragmites australis* (Cavanilles) Trinius ex Steudel, 1840, the lakeshore bulrush
206 *Schoenoplectus lacustris* (Linnaeus) Palla, 1888, and the yellow flag *Iris pseudacorus*
207 (Linnaeus, 1753). The frequently encountered aquatic species were *M. leprosa*, the European
208 roach *Rutilus rutilus* (Linnaeus, 1758), *Squalius* spp., *A. anguilla*, and *N. maura*. ~~Several a~~ Alien

209 species were ~~also also~~ encountered, ~~among which~~among which the red-eared slider *Trachemys*
210 *scripta elegans* (Wied, 1838), ~~and~~ the red swamp crayfish *Procambarus clarkii* (Girard, 1852),
211 ~~the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), and the nutria *Myocastor coypus*~~
212 ~~(Molina, 1782).~~

214 2.1.3. The Agouille de la Mar environment

215 ~~Similar to the Fosseille River, the Agouille de la Mar River is exposed to the Mediterranean~~
216 ~~climate. The Agouille de la Mar River is a lentic river, flows flowing in the Roussillon plain~~
217 ~~over 13 kilometers long, from its source located in the vicinity of Bages (altitude 25 m) to the~~
218 ~~Saint Nazaire lagoon (Fig.1). Its watershed, with a surface area of 62 km², is very anthropized~~
219 ~~like the Fosseille River watershed, because of urban, industrial, and agricultural activities. The~~
220 ~~Agouille de la Mar River has been repurposed for agriculture, and four WWTPs are~~
221 ~~hydrologically connected, which contribute to maintain the continuous water flow year round.~~
222 ~~The The river of investigation river part of investigation flows has at an altitude of about 15~~
223 ~~meters, is a length of 1.9 kilometers long, a width of three to eight meters wide, and a maximum~~
224 ~~depth of two meters deep. Two subsections were defined according to the position of the Bages~~
225 ~~WWTP, located: the upstream and the downstream subsections upstream and downstream of~~
226 ~~the Bages WWTP, which is the first wastewater processing company connected to the river.~~
227 ~~The upstream section is one kilometer long and the downstream section, 0.9 kilometer long~~
228 ~~(Fig.1). Its watershed is highly anthropized as the Fosseille River, because of urban and~~
229 ~~agricultural activities. Both investigated sections are lentic, with slow flow except during~~
230 ~~intense rainfall events.~~ The riparian zone is homogeneous all along the study section, with
231 sparse border vegetation dominated by *A. donax*, *P. australis*, *S. lacustris*, and *I. pseudacorus*.
232 The encountered aquatic species are the same as for the Fosseille River and consist of native
233 species, including *M. leprosa*, and alien species such as *T. s. elegans* and *P. clarkii*.

234

235 2.2. Habitat quality

236 2.2.1. Environmental parameters of investigation

237 A total of 14 water samples were collected to assess water quality of the three rivers, among
238 which seven were collected on 2021/05/07, and seven on 2021/09/27 (Fig.1). On the Baillaury
239 River, point sampling was done on the upper (BAI₁: N 42°27'25" E 3°4'57") and lower (BAI₂:
240 N 42°27'45" E 3°5'26") reaches. On the Fosseille River, point sampling was done on the
241 upstream (FOS₁: N 42°40'2" E 2°56'53"), outfall (FOS₂: N 42°40'11" E 2°57'26") and
242 downstream (FOS₃: N 42°40'1" E 2°58'1") subsections. Finally, on the Aguille de la Mar
243 River, point sampling was conducted on the upstream (AGO₁: N 42°36'57.5" E 2°53'41") and
244 downstream (AGO₂: N 42°36'53.6" E 2°54'24") sub-sections. Environmental parameters, such
245 as temperature (T, in °C), pH, dissolved oxygen (DO, in mg.L⁻¹), and conductivity (σ, in μS.cm⁻¹),
246 were measured *in natura* with a HQ40D Portable Meter (Hach).

247 Water samples were filtered on 0.45 μm (47 mm diameter) MF-Millipore membrane
248 filters to quantify suspended particulate matter (SPM, in mg.L⁻¹) at the [Centre de Formation et](#)
249 [de Recherche sur les Environnements Méditerranéens](#) CEFREM, and on 0.7 μm (47 mm
250 diameter) Whatman GF/F glass fiber filters (precombusted at 500 °C for four hours) to quantify
251 particulate organic matter, i.e., particulate organic carbon (POC), nitrogen (PON), and
252 phosphorus (POP) (in mg.L⁻¹), at the Mediterranean Institute of Oceanography (MIO ;
253 Marseille, France). Glass fiber filters were dried at 60 °C and ~~were~~ processed by the analytical
254 platform for the chemistry of marine environments at the MIO, with a SEAL analytical chain,
255 using the wet-oxidation technic (Raimbault et al., 1999). The detection limits were 120 ng.L⁻¹,
256 1 ng.L⁻¹, and 2 ng.L⁻¹ for POC, PON, and POP, respectively.

257

258 Nutrients, including nitrite (NO_2^- , in mg.L^{-1}), ammonium (NH_4^+ , in mg.L^{-1}), nitrate
259 (NO_3^- , in mg.L^{-1}), and orthophosphate (PO_4^{3-} , in mg.L^{-1}), as well as pesticides (N = 477
260 molecules ; in $\mu\text{g.L}^{-1}$) and pharmaceuticals (N = 23 molecules ; in $\mu\text{g.L}^{-1}$), were quantified from
261 the water samples by the Centre d'Analyses Méditerranée Pyrénées (Perpignan, France). The
262 analytical methods used to quantify the nutrients, with their detection limits, were the flow
263 analysis and spectrometric detection for NO_2^- and NH_4^+ (0.01 mg.L^{-1}), the liquid
264 chromatography assay for NO_3^- (0.2 mg.L^{-1}), and the spectrophotometry for PO_4^{3-} (0.02 mg.L^{-1}).
265 The liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and the gas
266 chromatography-mass spectrometry (GC-MS/MS), were used to quantify pesticides. ~~The LC-~~
267 ~~MS/MS~~ was also used to quantify pharmaceuticals. The detection limits for contaminants
268 depended on the molecules and varied from $0.005 \mu\text{g.L}^{-1}$ to $0.1 \mu\text{g.L}^{-1}$. Methods used for
269 nutrients and contaminants quantification were in compliance with the LAB GTA 05
270 accreditation program.

271

272 2.2.2. *Statistical analyses of water quality*

273 Concentrations of contaminants were compared between samples collected along the same
274 river, using Kruskal-Wallis rank sum and Dunn post hoc tests. Comparisons were done between
275 BAI_1 and BAI_2 of the Baillaury River, between FOS_1 and FOS_2 , FOS_1 and FOS_3 , and FOS_2 and
276 FOS_3 of the Fosseille River, and between AGO_1 and AGO_2 of the Aguille de la Mar River, for
277 both periods of sampling. The water quality parameters were also compared between samples
278 collected from the three rivers. A principal component analysis (PCA) was performed on the
279 water quality dataset ~~(N = 7 sampling points x 2 periods = 14 water samples)~~, ~~composed~~
280 ~~by including~~ the values of the physicochemical parameters, organic matter, nutrients, and
281 contaminants. ~~To select the relevant number of dimensions needed to interpretate the graphs,~~
282 ~~the relationships between the variables were first tested. Then, to characterize and compare the~~

283 ~~water quality of the distinct river sections, the variable graph and the individual graph were~~
284 ~~analyzed together. Finally, t.~~ To distinguish the different levels of habitat quality among the
285 seven ~~sampling points~~river subsections sampled in May and September, a hierarchical
286 clustering (HC) was used on the 14 water samples. Statistical tests were done on RStudio 3.6.2
287 (RStudio Team, 2020) with significance levels set at 5%. The Factoshiny R package (Lê et al.,
288 2008) was used for PCA and HC analyses, and the ggplot2 R package for data visualization
289 (Wickham, 2016).

290

291 *2.3. Turtles monitoring*

292 *2.3.1. Animals handling*

293 Three sessions of three days of capture-mark-recapture were conducted during the peak activity
294 of turtles, in May, June, and July 2021 in the three rivers of investigation. Baited crayfish traps
295 (60 x 30 cm and 80 x 35 cm), equipped with empty water bottles to avoid drowning of animals,
296 were deployed every 50 meters along each river. The yearly sampling effort was 450 traps.days
297 in the Baillaury River, 450 traps.days in the Fosseille River, and 288 traps.days in the Agouille
298 de la Mar River. Turtles were identified by a unique code following indentations on the
299 marginal scutes (Cagle, 1939). Sex of each turtle was determined from apparent secondary
300 sexual characters (MAL for males, and FEM for females), namely the concavity of the plastron
301 and the distance between the cloaca opening and the plastron notch (Pérez et al., 1979; ~~Meek,~~
302 ~~1987~~; Busack and Ernst, 1980; Meek, 1987; Fritz, 2003). Because these traits are only apparent
303 in turtles over 80 mm of size in the populations of interest, turtles smaller than 80 mm were
304 considered as ~~immature~~juveniles (IMMJUV). The body mass (BM) and the straight carapace
305 length (SCL) were measured with a ~~balance~~scale (precision = 0.1 g) and a Vernier caliper
306 (precision = 1 mm). After measurements, turtles were immediately released at the place of
307 capture. To estimate growth rates, unpublished data acquired following turtles monitoring in

308 the Baillaury River (2012-2021), Agouille de la Mar River (2014-2021), and Fosseille River
309 (2013-2021), as well as data retrieved from Le Gal et al. (2023) were also used. A ministerial
310 authorization was granted to the handlers of turtles by the DREAL Occitanie, and experiments
311 were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and
312 associated guidelines, EU Directive 2010/63/EU for animal experiments.

313

314 2.3.2. *Population structure*

315 Population size, density, and operational sex ratio (OSR), were estimated from the data gathered
316 in 2021. Because turtle population stability mainly depends on the adults (Crouse et al., 1987;
317 Galbraith and Brooks, 1987; Frazer, 1992; Heppell, 1998; Blamires and Spencer, 2013), the
318 population structure was estimated after excluding ~~immature~~juveniles from the dataset. Prior to
319 modeling, population closure was tested with the CloseTest software (Stanley and Burnham,
320 1999), trap-dependence was tested on the CAPTURE program implemented in MARK 6.2
321 (White and Burnham, 1999), and transience was analyzed using a goodness-of-fit test (Pradel
322 et al., 2005) performed on U-Care 2.3.2 (Pradel et al., 2010). The population size was then
323 estimated from CAPTURE (White and Burnham, 1999). Finally, the population density (in
324 adults.km⁻¹), and the OSR (MAL/(MAL+FEM)) were inferred from the raw data captures.

325 Differences in BM and SCL along the river subsections were assessed using Kruskal-
326 Wallis rank sum comparison tests, followed by Dunn post-hoc tests. ~~Size classes. Then,~~ body
327 condition, and growth rate were studied, ~~with,~~ only the first annual capture being considered to
328 account for pseudo replication. For body condition, the scaled mass index (SMI) was calculated
329 following the procedure developed by Peig and Green (2009). The SMI is a hypothetical mass
330 that allows for consideration of the relationship between body mass and body length within the
331 group of interest, and the impact of individual variability and measurement errors on BM and

332 ~~SCL. The differences in SCL and BM along the river sections were assessed using Kruskal-~~
333 ~~Wallis rank sum comparison tests, followed by Dunn's post hoc tests. The scaled mass index~~
334 ~~(SMI)The that corrects the BM for SCL was calculated using the procedure of Peig and Green~~
335 ~~(2009). The growth rate (GR) was calculated following the following equation designed by us:~~
336 $GR_i = (SCL_{2021} - SCL_y) / [(2021 - y) * SCL_y]$, with i the turtle, and y the first year of capture.
337 Following a model selection process using AIC in a stepwise algorithm, ANOVA was used to
338 search for mixed effects of SCL and river subsection on SMI and growth rate, respectively.
339 Durbin-Watson and Shapiro-Wilk tests were used to verify the assumptions of the ANOVA.
340 Tukey HSD tests were then used to determine the groups that exhibited statistically significant
341 differences. In view of the variations in body composition specific to age and sex, separate
342 analyses were performed for ~~immaturejuveniles~~, males, and females. All analyses were
343 performed on RStudio 3.6.2 (RStudio Team, 2020) with significance levels set at 5%; the
344 MASS package was used for model selection (Venables and Ripley, 2002); and the ggplot2
345 package was used for data visualization (Wickham, 2016).

346

347 **3. Results**

348 *3.1. Diversity and concentration of contaminants*

349 A total of 58 contaminants were detected in the water samples, including 38 pesticides and 20
350 pharmaceuticals (Fig.2). Pesticides were detected in all three rivers, while pharmaceuticals,
351 with the exception of caffeine (included in pharmaceuticals for convenience), were only
352 detected in the Fosseille and Agouille de la Mar rivers. The two most concentrated contaminants
353 detected overall were the glyphosate (0.05 to 0.75 $\mu\text{g.L}^{-1}$) and the AMPA (0.05 to 9.6 $\mu\text{g.L}^{-1}$).
354 The diversity of contaminants was similar between May and September 2021, regardless the
355 river.

356 In the Baillaury River, 11 pesticides and ~~the caffeine~~one pharmaceutical were detected in
357 the water. Among the pesticides, nine were detected in BAI₁, and eight in BAI₂. The pesticides
358 with the highest concentrations were the hydroxy simazine (0.10 µg.L⁻¹) and the AMPA (0.06
359 µg.L⁻¹). The other pesticides included three derivatives of atrazine, one derivative of
360 norflurazon, two derivatives of terbuthylazine, fenpyroximate, simazine and one of its
361 derivatives. Caffeine was the only pharmaceutical detected, with a concentration ranging from
362 0.03 to 0.05 µg.L⁻¹. The concentrations in contaminants did not differ between the two sampling
363 sites (Kruskal-Wallis rank sum test, $\chi^2 = 1.78$, df = 3, p = 0.62).

364 In the Fosseille River, 30 pesticides and 18 pharmaceuticals were detected in the water.
365 Among the pesticides, 13 were detected upstream, 23 at the outfall, and 22 downstream of the
366 WWTP. Among the pharmaceuticals, three were detected upstream (caffeine, irbesartan, and
367 metformin), 17 at the outfall, and 17 downstream of the WWTP. Contaminants with the highest
368 concentrations were the AMPA (9.6 µg.L⁻¹) and the metformin (3.1 µg.L⁻¹). The diversity of
369 contaminants was higher downstream (N = 39 contaminants) and at the outfall (N = 40
370 contaminants), than upstream of the WWTP (N = 16 contaminants). The concentrations of the
371 contaminants did not differ significantly between the outfall and the downstream river
372 subsections, regardless the period of sampling, i.e., May and September 2021. They were
373 however significantly higher than those reported upstream of the WWTP (Kruskal-Wallis rank
374 sum test, $\chi^2 = 44.35$, df = 5, p = 1.97e-08).

375 In the Agouille de la Mar River, a total of 21 pesticides and 14 pharmaceuticals were
376 detected in the water. Among the pesticides, eight were detected upstream and 19 downstream
377 of the WWTP. Among the pharmaceuticals, four were detected upstream (caffeine, metformin,
378 paracetamol, and theophylline), and 13 downstream of the WWTP. Contaminants with the
379 highest concentrations were the AMPA (6.4 µg.L⁻¹) and the metformin (5.7 µg.L⁻¹). Both the
380 diversity and the concentrations of contaminants were higher downstream than upstream of the

381 WWTP (N = 32 and 12 contaminants, respectively; Kruskal-Wallis rank sum test, $\chi^2 = 24.40$,
382 $df = 3$, $p = 2.06e-05$).

383

384 3.2. Levels of water quality

385 The hierarchical clustering revealed three levels of habitat quality: (i) the Baillaury River
386 section (BAI₁ and BAI₂) and the upstream subsection of the Fosseille River (FOS₁) present a
387 low level of pollution and are classified as the first level; (ii) the outfall (FOS₂) and the
388 downstream (FOS₃) subsections of the Fosseille River present a moderate level of pollution and
389 are classified as the second level; (iii) the Agouille de la Mar River section (AGO₁ and AGO₂)
390 presents the highest level of pollution and is classified as the third level (Fig.3; Supplementary
391 Material A).

392 The first dimension of the PCA distinguished the downstream subsection of the
393 Agouille de la Mar River (AGO₂) from the Baillaury River section (BAI₁ and BAI₂) (Fig.3).
394 The latter was characterized by low concentrations of contaminants to the exception of
395 hydroxy-terbutylazine ($0.040 \pm 0.006 \mu\text{g.L}^{-1}$; *versus* AGO₂: $0.009 \pm 0.001 \mu\text{g.L}^{-1}$), low
396 conductivity ($488.00 \pm 51.56 \mu\text{S.cm}^{-1}$; *versus* AGO₂: $990.00 \pm 22.00 \mu\text{S.cm}^{-1}$), and low
397 temperature ($17.78 \pm 0.48 \text{ }^\circ\text{C}$; *versus* AGO₂: $23.15 \pm 1.15 \text{ }^\circ\text{C}$) (Fig.3). AGO₂ was characterized
398 by high concentrations of particulate organic matter such as POP ($0.090 \pm 0.010 \mu\text{g.L}^{-1}$; *versus*
399 BAI: $0.010 \pm 0.010 \mu\text{g.L}^{-1}$), high nutrients levels such as NO₂- ($0.49 \pm 0.09 \mu\text{g.L}^{-1}$; *versus* BAI:
400 $< 0.01 \mu\text{g.L}^{-1}$), atrazine-dip ($0.016 \pm 0.001 \mu\text{g.L}^{-1}$; *versus* BAI: $0.001 \pm 0.001 \mu\text{g.L}^{-1}$), and high
401 water temperature ($23.15 \pm 1.15 \text{ }^\circ\text{C}$; *versus* BAI: $17.78 \pm 0.48 \text{ }^\circ\text{C}$) (Fig.3). The second
402 dimension of the PCA distinguished the outfall (FOS₂) and the downstream (FOS₃) subsections
403 of the Fosseille River from the Baillaury River section (BAI₁ and BAI₂) (Fig.3). FOS₂ and FOS₃
404 were characterized by high conductivity ($1412.00 \pm 78.33 \mu\text{S.cm}^{-1}$; *versus* BAI: 488.00 ± 51.56

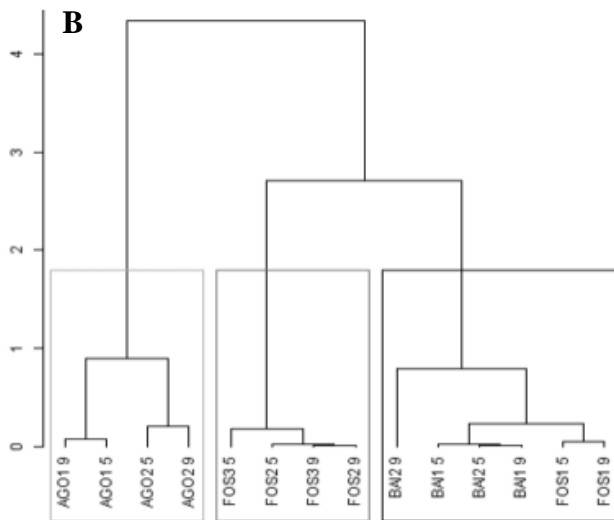
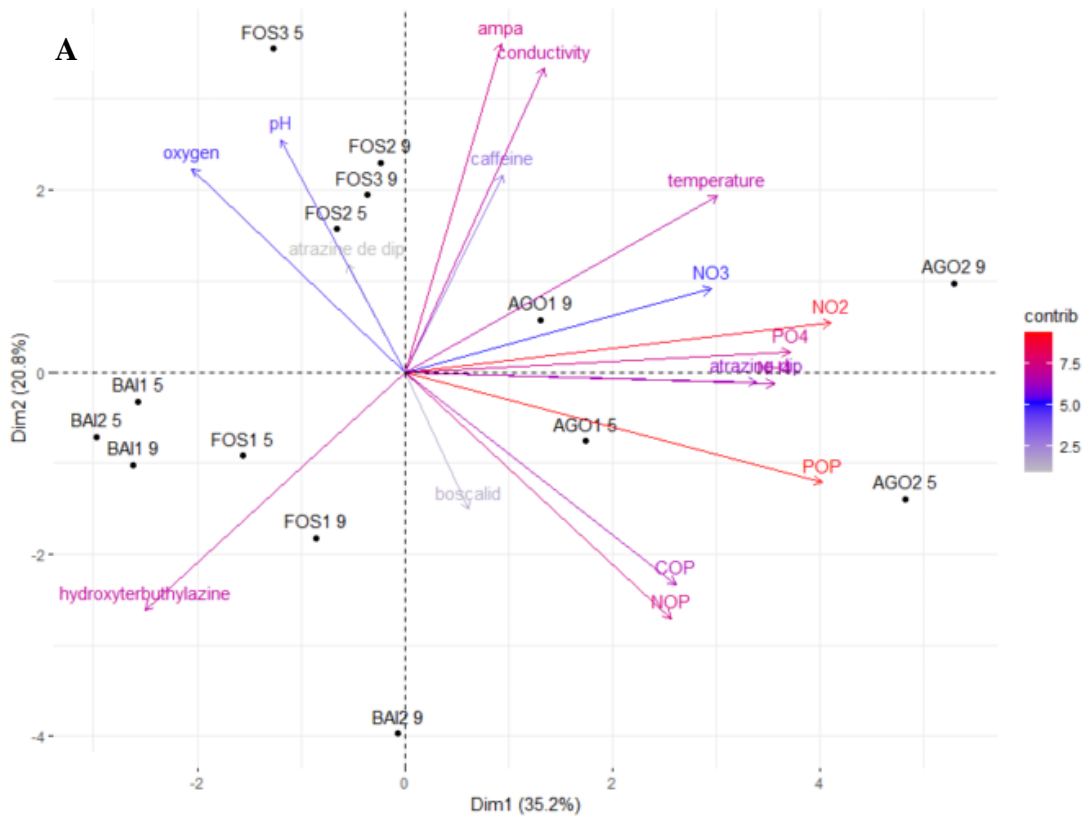
405 $\mu\text{S}\cdot\text{cm}^{-1}$), high concentrations of AMPA ($7.93 \pm 0.76 \mu\text{g}\cdot\text{L}^{-1}$; *versus* BAI: $0.028 \pm 0.017 \mu\text{g}\cdot\text{L}^{-1}$)
406 1), and high concentrations of caffeine ($0.17 \pm 0.05 \mu\text{g}\cdot\text{L}^{-1}$; *versus* BAI: $0.040 \pm 0.005 \mu\text{g}\cdot\text{L}^{-1}$).

407 Because samples collected in BAI₁ and BAI₂ did not differ in pollution levels, a single
408 river subsection (BAI) was considered for the Baillaury River when analyzing turtle population
409 structure. Similarly, as FOS₂ and FOS₃ did not show significant differences in pollution levels,
410 they were considered as a single subsection named hereafter FOS₂₊₃. Five subsections were
411 therefore considered for population structure analyses, namely BAI, FOS₁, FOS₂₊₃, AGO₁, and
412 AGO₂.



413

414 **Figure 2.** Concentration of (A) pesticides and (B) pharmaceuticals detected in the water. BAI₁
 415 and BAI₂ represent the upper and lower water sampling points on the Baillaury River section;
 416 FOS₁, FOS₂, and FOS₃ represent respectively the upstream, outfall, and downstream
 417 subsections of the Fosseille River; AGO₁ and AGO₂ represent respectively the upstream and
 418 downstream subsections of the Agouille de la Mar River. “may” is displayed for May 2021,
 419 and “sept” for September 2021.



420

421 **Figure 3.** (A) Biplot of the principal component analysis: the color gradient corresponds to the
 422 contribution of each variable to the construction of the plan (Dim1, Dim2). Each water sample
 423 is associated to a river (BAI for the Baillaury River section, FOS for the Fosseille River section,
 424 and AGO for the Aguille de la Mar River section), a sampling point (1:3), and a sampling
 425 month (5 for May, and 9 for September). (B) Hierarchical clustering tree: each box corresponds
 426 to a level of habitat quality.

427 *3.3. Population structure*

428 The closure test revealed that populations of the three rivers were closed in 2021, i.e.,
429 emigration and migration rates were negligible (Baillaury: $\chi^2 = 0.90$, $df = 1$, $p = 0.34$; Fosseille:
430 $\chi^2 = 2.48$, $df = 2$, $p = 0.29$; Agouille de la Mar: $\chi^2 = 1.97$, $df = 1$, $p = 0.37$), and no trap-
431 dependence was detected in the populations. Goodness of fit results indicated the presence of
432 transient males on the Baillaury River (males: $zlor = 1.71$, $df = 1$, $p = 0.05$; females: $zlor = 0.07$,
433 $df = 1$, $p = 0.47$), but there was no transience on the Fosseille (males: $zlor = 0.75$, $df = 1$, p
434 $= 0.23$; females: $zlor = 0.67$, $df = 1$, $p = 0.25$) and on the Agouille de la Mar (males: $zlor = 0.62$,
435 $df = 1$, $p = 0.27$; females: $zlor = 0.42$, $df = 1$, $p = 0.85$) rivers. On the Baillaury River, the capture
436 probability varied with time of capture (model $M(t)$) and was estimated at 0.26 (May 2021),
437 0.56 (June 2021), and 0.49 (July 2021). On the Fosseille and Agouille de la Mar rivers, the
438 probability of capture was constant (model $M(o)$) and estimated at 0.22 and 0.26 for each river,
439 respectively.

440 The number of individuals ranged from 43 turtles on BAI to 135 turtles on FOS₂₊₃
441 (Table 1). The OSR was male-biased in most of the river subsections and ranged from 0.56 to
442 0.76, with the exception of FOS₁, where it was balanced at 0.48 (Table 1). The adult population
443 of the Baillaury River consisted of 45 ± 5 turtles (95% CI = [40;64]) and had a density of 27
444 turtles.km⁻¹. On the Fosseille River, the adult population consisted of 157 ± 24 turtles (95% CI
445 = [121;223]) and density ranged from 63 to 68 turtles.km⁻¹, upstream to downstream of the
446 WWTP respectively. On the Agouille de la Mar River, the adult population consisted of $138 \pm$
447 18 turtles (95% CI = [112;186]), and density ranged from 44 to 50 turtles.km⁻¹, upstream to
448 downstream of the WWTP respectively. ~~Immature~~Juveniles accounted for 16-45% of the total
449 captures in habitats of level 1 (BAI and FOS₁), 12% in habitat of level 2 (FOS₂₊₃), and 7-9% in
450 habitats of level 3 (AGO₁ and AGO₂).

451

452 **Table 1.** Captures composition (males MAL, females FEM, and ~~immature juveniles IMMJU~~V)
 453 and operational sex ratio (OSR) of *M. leprosa* along the Baillaury River (BAI), the Fosseille
 454 River (FOS), and the Agouille de la Mar River (AGO). The total number of captures is
 455 indicated, with the -number of ~~distinct distinct~~ individuals ~~captured is indicated shown~~ in
 456 brackets.

		MAL	FEM	IMMJU <u>V</u>	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS ₁)	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS ₂₊₃)	172 (91)	39 (28)	28 (16)	239 (135)	0.88	0.76
	Full river	201 (101)	65 (39)	65 (33)	331 (173)	0.81	0.72
AGO	Upstream (AGO ₁)	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO ₂)	32 (23)	31 (18)	4 (4)	67 (45)	0.91	0.56
	Full river	71 (48)	53 (34)	8 (7)	132 (89)	0.92	0.59

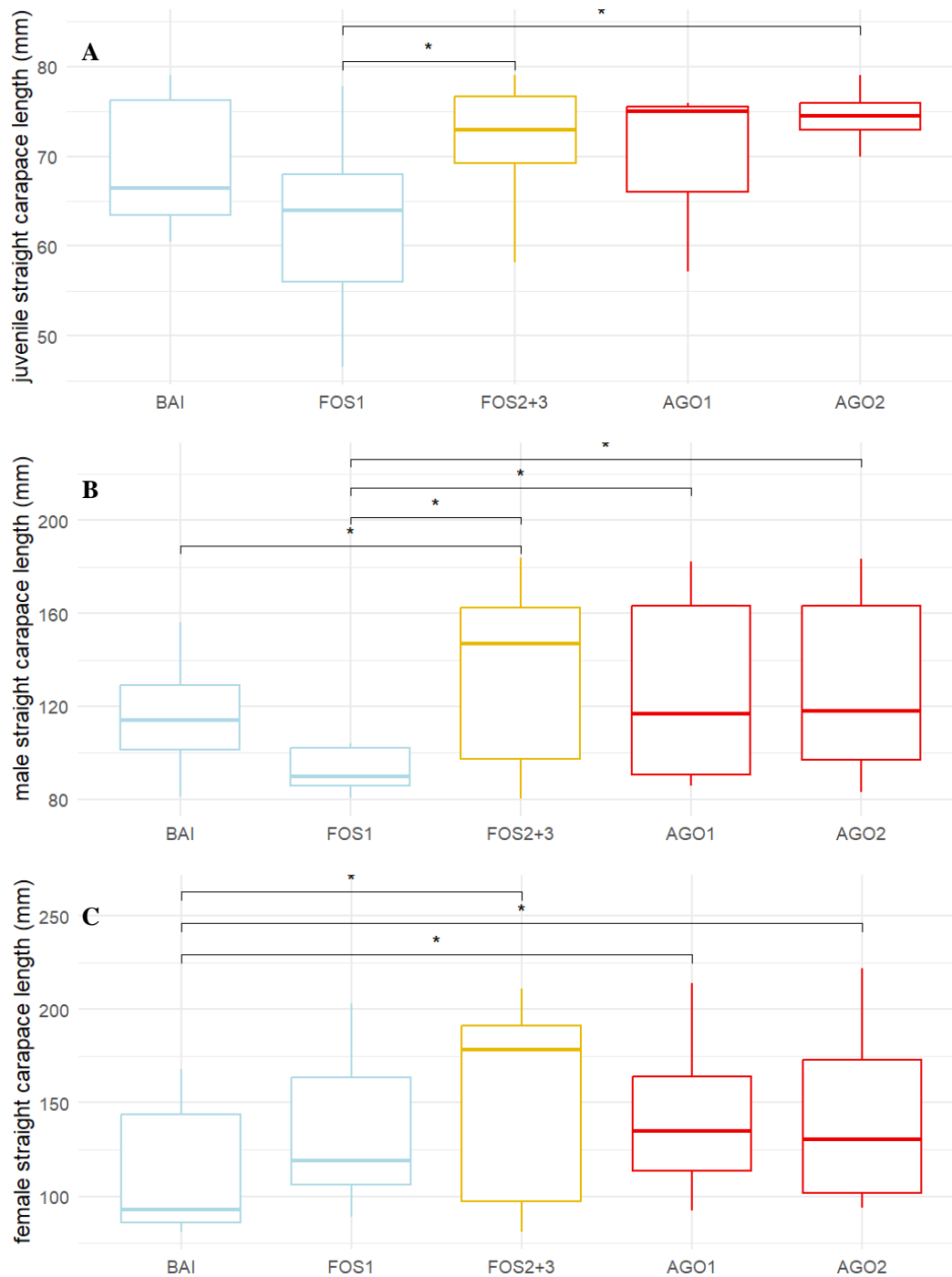
457

458 3.4. Turtles body metrics

459 Body size (SCL) and mass (BM) of adults were different across the five river subsections, i.e.,
 460 BAI, FOS₁, FOS₂₊₃, AGO₁, and AGO₂ (Fig.4; Supplementary Material B). Differences were
 461 significant in males (n = 172; Kruskal-Wallis rank sum test; SCL: $\chi^2 = 11.00$, df = 4, p = 0.03;
 462 BM: $\chi^2 = 11.62$, df = 4, p = 0.02), and near so in females (n = 86; Kruskal-Wallis rank sum test;
 463 SCL: $\chi^2 = 8.52$, df = 4, p = 0.07; BM: $\chi^2 = 9.71$, df = 4, p = 0.05). The largest and heaviest
 464 turtles were located in the most degraded habitats, i.e., in FOS₂₊₃ and in both subsections of the

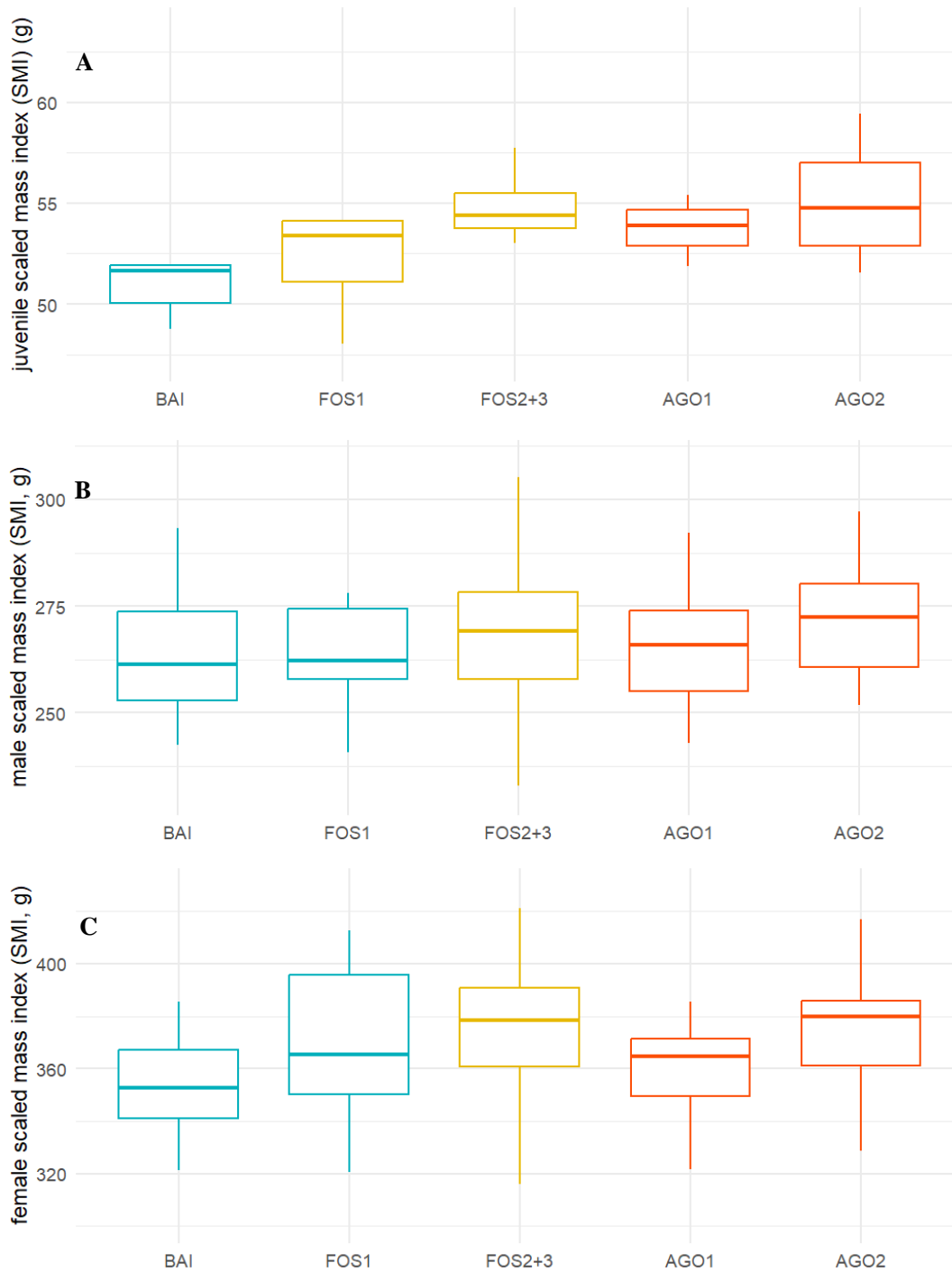
465 Agouille de la Mar River (Fig.4; Supplementary Material B). However, the ~~body condition~~SMI
466 of males did not differ between the five river subsections (n = 172; ANOVA; SMI: $F_{4,167} = 0.72$,
467 $p = 0.58$; Shapiro-Wilk: $W = 0.99$, $p = 0.08$), nor in females (n = 86; ANOVA; SMI: $F_{4,81} =$
468 1.77 , $p = 0.14$; Shapiro-Wilk: $W = 0.98$, $p = 0.11$) (Fig.5). The body size and SMI body
469 ~~condition~~ of immature juveniles were not different across the five river subsections (n = 47;
470 Kruskal-Wallis rank sum test; SCL: $\chi^2 = 7.49$, $df = 4$, $p = 0.11$; SMI: $\chi^2 = 7.62$, $df = 4$, $p = 0.11$)
471 (Fig.4-5). However, body mass tended to increase along the pollution gradient, from level 1 to
472 level 3 (n = 47; Kruskal-Wallis rank sum test; BM: $\chi^2 = 8.99$, $df = 4$, $p = 0.06$) (Supplementary
473 Material B).

474 Growth rates were different across the five river subsections ~~sections~~ (Fig.6) and also
475 differed according to turtle body size. Differences were significant in males (n = 172; ANOVA;
476 SCL: $F_{1,77} = 12.98$, $p = 5.55e-04$; River subsection ~~section~~: $F_{4,77} = 4.27$, $p = 3.58e-03$; Shapiro-
477 Wilk: $W = 0.98$; $p = 0.41$) and in females (n = 86; SCL: $F_{1,32} = 36.41$, $p = 9.84e-07$; River
478 subsection ~~section~~: $F_{4,32} = 7.28$, $p = 2.75e-04$; Shapiro-Wilk: $W = 0.95$; $p = 0.08$). Adults grew
479 faster in the habitats of level 3 (AGO₁ and AGO₂) than in other habitats. However, females
480 grew slower in the habitat of level 2 (FOS₂₊₃) than in other habitats (Fig.6). The inflexion point
481 of male growth rate was reached for a body size of 125 mm, whereas no such point was detected
482 in females (Supplementary Material C). The growth rate was close to zero for males with a
483 body size above 125 mm, and for females with a body size above 200 mm (Supplementary
484 Material C).



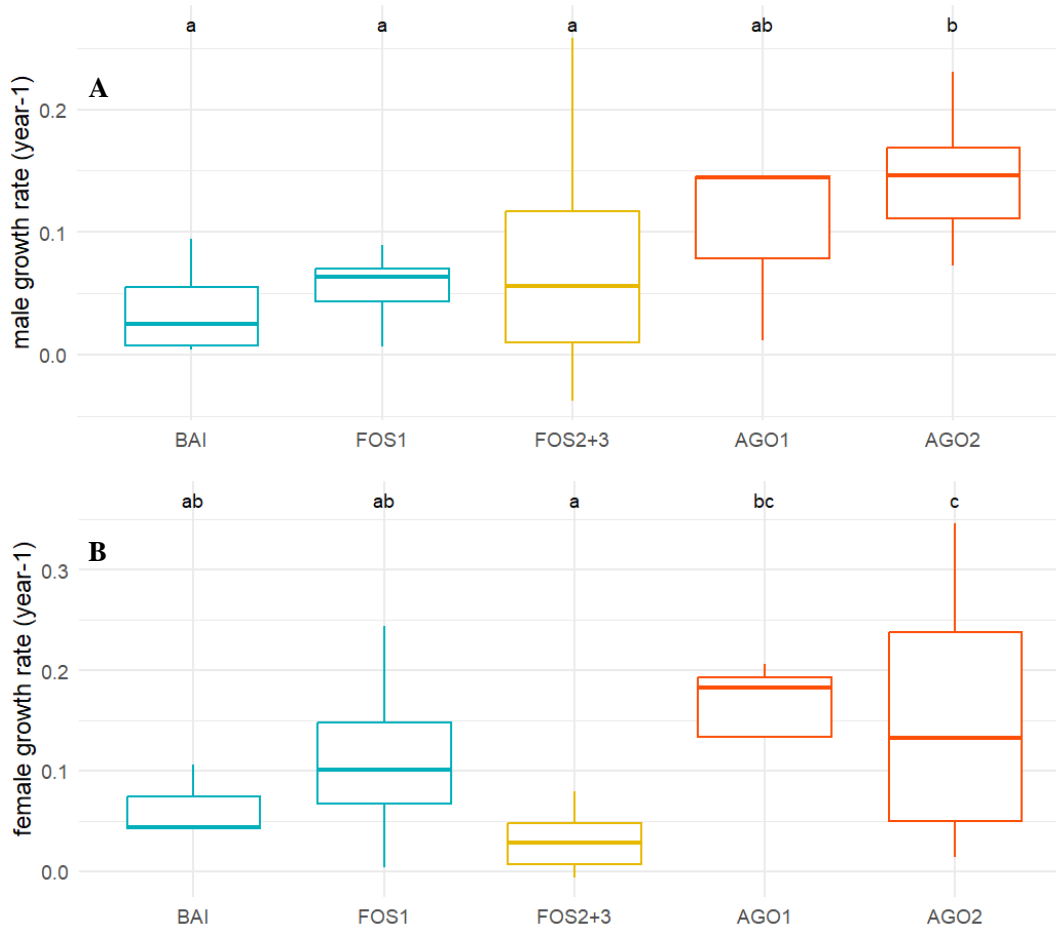
485

486 **Figure 4.** Body size of immature juveniles (A), males (B), and females (C) along the
 487 investigated rivers: Baillaury River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃)
 488 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO₁) and
 489 downstream (AGO₂) WWTP subsections of the Agouille de la Mar River. Each color represents
 490 a quality level, from blue to red. Pairwise Wilcoxon tests p-values are indicated (alpha = 5% *;
 491 1% **). The median of each group is displayed as a bold vertical line.



492

493 **Figure 5.** Scaled mass indices in immature juveniles (A), males (B), and females (C) along the
 494 investigated rivers: Baillaury River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃)
 495 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO₁) and
 496 downstream (AGO₂) WWTP subsections of the Agouille de la Mar River. Each color represents
 497 a quality level, from blue to red.



498

499 **Figure 6.** Growth rates in males (A) and females (B) along the investigated rivers: Baillaury
 500 River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃) wastewater treatment plant (WWTP)
 501 subsections of the Fosseille River; upstream (AGO₁) and downstream (AGO₂) WWTP
 502 subsections of the Agouille de la Mar River. Each color represents a quality level, from blue to
 503 red. Following ANOVA, Tukey test comparisons results are displayed as letters. Each color
 504 represents a quality level, from blue to red.

505 4. Discussion

506 4.1. Aquatic habitat quality

507 The least polluted habitats quality (level 1) encompassed the Baillaury River (BAI₁ and BAI₂)
508 and the upstream subsection of the Fosseille River (FOS₁₇), BAI₁, and BAI₂ that which
509 exhibited the lowest diversity and concentrations of pollutantscontaminants, the lowest
510 concentrations of nutrients of nutrients, the lowest conductivity, and the lowest water
511 temperatures. Whereas pesticides and caffeine detected in these subtwo sections might have
512 been drained by rain, they likely resulted from illegal discharge as they were assessed during
513 low flow periods. Caffeine is indeed a marker of anthropization and is the most detected
514 emerging contaminant in surface water (Korekar et al., 2020). Caffeine, which is obviously a
515 good marker of anthropization, is the most detected emerging contaminant in surface water
516 (Korekar et al., 2020). In the absence of WWTPs, such illegal discharge could therefore occur
517 via semi-rigid pipes connecting private plots to the river, as they which were often observed in
518 the field along the Baillaury River (A. S. Le Gal, personal observation). The contaminants with
519 the highest concentrations in these habitats were caffeine, glyphosate, and AMPA. AMPA,
520 which is the main metabolite of glyphosate and persistent in water, can have significant negative
521 effects on aquatic organisms (Daouk et al., 2013; Guilherme et al., 2014; Moore et al., 2014;
522 Tresnakova et al., 2021). Though the diversity of pollutants reported in FOS₁ was higher than
523 in BAI₁ and BAI₂, FOS₁ actually contains metformin which is detrimental to aquatic fauna with
524 notorious effects on endocrine function and fecundity (Niemuth and Klaper, 2015; Lee et al.,
525 2019). While the low DO concentration measured in the Baillaury River in September indicated
526 fairly poor water quality and anoxic conditions during drought periods The levels of pH and
527 DO that were measured in the permanent natural pools of the Baillaury River in September
528 2021 also reflected a low ecological quality (Supplementary Material A; Légifrance, 2010).
529 Nevertheless, the presence of amphibiansbio-indicators such as the amphibians, such as P.

530 *perezi* and *B. spinosus* ~~on the Baillaury River, which are absent on FOS₁ in that river~~, indicated
531 ~~pretty~~ good aquatic and terrestrial habitats (see Marques et al., 2008; Napoletano et al., 2023).
532 ~~Traditional agricultural practices, such as those used along the Baillaury environment, may be~~
533 ~~thereby more beneficial to species richness and diversity than the conventional agricultural~~
534 ~~practices used along the Fosseille environment (see Kazes et al., 2020). On the opposite, the~~
535 ~~diversity of pollutants reported in FOS₁ was higher than in the Baillaury section, suggesting~~
536 ~~that this subsection's quality was lower than that of the Baillaury River. FOS₁ also contained~~
537 ~~metformin which is detrimental to aquatic fauna with notorious effects on the endocrine~~
538 ~~function and fecundity of fish (Niemuth and Klaper, 2015; Lee et al., 2019). In contrast to the~~
539 ~~Baillaury River, the presence of alien species such as the crayfish *P. clarkii* in the~~ Fosseille
540 River ~~is home to several alien species such as the crayfish *P. clarkii*, which~~ could also explain
541 the absence of amphibians ~~following due to~~ predation (Gherardi et al., 2001).

542 The habitats of medium quality (level 2) ~~consisted of~~ encompassed both the outfall (FOS₂)
543 and the downstream ~~WWTP (FOS₃) subsections~~ of the Fosseille River ~~(FOS₂ and FOS₃)~~.
544 ~~They~~ exhibited high diversity and concentrations of pesticides and pharmaceuticals, high
545 concentrations of suspended particles, and high water temperatures compared to habitats of
546 level 1. Alterations in water quality were probably due to the presence of the Cabestany ~~'s~~
547 WWTP, which receives wastewater from the urban community, but also from agricultural plots.
548 Because not all pollutants are removed by WWTPs (Bruton et al., 2010; Deo, 2014), pesticides
549 and pharmaceuticals are commonly found in ~~the~~ water. The most concentrated pollutants were
550 indeed the AMPA, ~~i.e., the main degradation product of glyphosate which is persistent in water~~
551 ~~and can have significant negative effects on aquatic organisms (Daouk et al., 2013; Guilherme~~
552 ~~et al., 2014; Moore et al., 2014; Tresnakova et al., 2021)~~, and the metformin, which ~~is also~~ are
553 detrimental to wildlife ~~, as discussed previously since it can cause endocrine disruption and~~
554 ~~reduce fecundity as illustrated in fish (Niemuth and Klaper, 2015; Lee et al., 2019)~~. Among the

555 detected pharmaceuticals (Fig.2), carbamazepine was reported to negatively affect eggs
556 viability, female gonads, ~~antioxidant defense, neurotransmission,~~ and metabolism ~~of-in~~ the
557 zebrafish *Danio rerio* (Hamilton, 1822) (see Da Silva Santos et al., 2018), ~~as well as~~ but also
558 steroid production and reproductive output (Fraz et al., 2019). ~~Similarly, Fluoxetine,~~ which
559 was detected in these habitats, was reported to alter male mating strategy in the guppy fish
560 *Poecilia reticulata* (Peters, 1859) (see Fursdon et al., 2019). As in FOS₁, no amphibians were
561 detected in FOS₂ and FOS₃, which could be explained by the high diversity and concentrations
562 of pollutants in the water, but also by the presence of alien animal species such as *P. clarkii*.

563 The ~~worst habitat quality~~ most disturbed habitats (level 3) encompassed both subsections of
564 the Agouille de la Mar River (AGO₁ and AGO₂). They were characterized by high
565 concentrations of nutrients and organic matter, resulting in eutrophication of the watercourse.
566 Pesticides and pharmaceuticals (caffeine, metformin, paracetamol, and theophylline) that were
567 detected ~~in upstream of the WWTP~~ AGO₁ could also be the result of illegal discharges.
568 Conversely, the ~~wide-high~~ diversity and ~~high~~ concentrations of contaminants ~~that were~~ detected
569 ~~downstream of the WWTP~~ in AGO₂ most likely originated from wastewater effluents. Similar
570 as in FOS₂₊₃, the most concentrated pollutants in AGO₂ were the AMPA and metformin, both
571 known for their harmful effects on aquatic organisms. Carbamazepine, ~~with-which can have~~
572 ~~potential~~ detrimental effects on the physiology and reproduction of *D. rerio*; ~~as mentioned~~
573 ~~previously~~ (see Da Silva Santos et al., 2018), was also detected in that river (Fig.2). In addition
574 to ~~discharging~~ pollutant ~~dischargess~~, effluents ~~-~~ may have ~~enriched-dumped organic matter~~ the
575 in the downstream ~~section of the Agouille de la Mar~~ subsection ~~River with organic matter,~~ as as
576 the Bages WWTP ~~of Bages~~ has been exceeding its treatment limits for several years and is no
577 longer able to adequately treat incoming wastewater (Sandre, 2023). ~~Because-h~~ High organic
578 matter pollution, which consumes oxygen for oxidation processes, ~~may it~~ induces low DO
579 levels (Navarrete-Salgado et al., 2004); and high nitrite concentrations (Roberts and Palmeiro,

580 2008), ~~resulting in very poor water quality~~. ~~Consistent with this, the DO and nitrite~~
581 ~~concentrations that were measured downstream of the WWTP exceeded ecological quality~~
582 ~~standards~~ (Appendix A; Légifrance, 2010). As ~~for~~ in the Fosseille River, no amphibians were
583 detected ~~in this river~~, and while the alien species *P. clarkii* was still observed.

584

585 *Correlation between turtle demographics and habitat quality*

586 Turtle densities in the Fosseille (63 to 68 adults.km⁻¹) and Agouille de la Mar (44 to 50
587 adults.km⁻¹) rivers were higher than in the Baillaury River (22 adults.km⁻¹), which was
588 unexpected because of the ~~low-poor~~ water quality reported downstream of the Fosseille River
589 (FOS₂₊₃) and in both subsections of the Agouille de la Mar River (AGO₁ and AGO₂). These
590 contrasting results were not due to differences in capture rates of turtles across the three rivers,
591 as capture rates in the Baillaury River were similar, even higher (p = 0.26 in May, 0.56 in June,
592 and 0.49 in July, 2021) than in the Fosseille (p = 0.22) and Agouille de la Mar (p = 0.26) rivers.
593 Moreover, these rates were consistent yet slightly lower than those reported for the European
594 pond turtle *Emys orbicularis* (Linnaeus, 1758) (see ~~Escoriza et al., 2021~~; Olivier et al., 2010;
595 Owen-Jones et al., 2016; Escoriza et al., 2021). It should be mentioned that the Baillaury River
596 consists of several river sections, which are hydrologically interconnected. Therefore, the low
597 turtle density in the investigated section could be explained by temporary transitions between
598 sections.

599 Suprisingly, the proportion of ~~immature juveniles~~ in the Baillaury River was almost
600 twice higher than that reported on the two subsections of the Agouille de la Mar River. In
601 addition, the proportion of ~~immature juveniles~~ was also higher upstream than downstream of the
602 Fosseille River. The decrease in juveniles proportion could be related to the level of increase of
603 habitat pollution: from 16-45% of ~~immature juveniles~~ in ~~habitats of level 1~~ habitats of level 1,

604 to 12% in ~~habitat of level 2~~ habitats of level 2, and 7-9% in ~~habitats of level 3~~ habitats of level
605 3 (Table 1). Because ~~adults~~ capture rates were similar between the three rivers ~~of investigation~~,
606 this suggests poor breeding condition of adults and/or higher ~~rates of immature juveniles~~
607 mortalities in the most polluted rivers. In line with this, El Hassani et al. (2019) reported ~~for M.~~
608 ~~leprosa~~ low proportion of immature juveniles in highly polluted habitats (8% of the captures) in
609 comparison to pristine ones (34% of the captures) for the same turtle species. A similar similar
610 result was reported by Germano (2010) in the emydid *Actinemys marmorata* (Baird and Girard,
611 1852) inhabiting sewage-treatment facilities (6-7% of the captures). ~~Because~~ High
612 reproductive outputs were reported in freshwater turtles from highly polluted sites (Knight and
613 Gibbons, 1968; Lindeman, 1996), including *M. leprosa* (Gahmous et al., 2022), ~~it~~ which
614 suggests that high levels of pollution and eutrophication may have reduced the survival of
615 immature juveniles. Whereas high predation rates by birds or competition with *T. s. elegans* may
616 also impact survival of immature juveniles (Polo-Cavia et al., 2010), we did not assess these two
617 variables in the present study. However, because upstream and downstream subsections of the
618 Fosseille River are homogeneous in terms of riparian habitats, it is unlikely that differential
619 avian predation rates may explain differences in the proportion of immature juveniles.
620 Therefore, these differences are probably related to the quality of aquatic habitats.

621 *Correlation between phenotypic changes and habitat quality*

622 Our results showed that ~~the turtle~~ growth rate and body size ~~of turtles~~ increased with habitat
623 pollution. In the ~~habitats of best quality~~ least polluted habitats (BAI and FOS₁), the low growth
624 rates ~~of turtles~~ could be related to ~~cooler~~ chilly waters, as observed in the freshwater turtle A.
625 marmorata (Snover et al., 2015). The partial dryness of the Baillaury River during ~~summer~~
626 ~~times~~ drought could also adversely affect turtle growth, because of limited food resources.
627 Consistent with this, Powell et al. (2023) showed that drought could have ~~could have a negative~~
628 ~~effect on~~ slow down -growth ~~rate in~~ of the freshwater turtle C. picta. The Baillaury River and the

629 ~~WWTP~~-upstream subsection of the Fosseille River ~~therefore~~-appeared to be the least ~~favorable~~
630 supportive habitats for turtle growth and body size, due to chilly waters and altered flows. On
631 the opposite, the high nutrient levels reported in polluted and eutrophic habitats may have
632 enhanced growth rate and body size (Fig.4 and 6). Consistent with this, it has been shown that
633 *C. picta* exhibited higher growth rates in polluted lentic habitats (Knight and Gibbons, 1968) as
634 well as in wastewater lagoons (Lindeman, 1996), than in pristine ones. A similar result was also
635 reported by Germano (2010) for *A. marmorata*. While Knight and Gibbons (1968) already
636 pointed out that high growth rates in polluted environments could be the result of diet changes
637 in *C. picta*, Sidis and Gasith (1995) illustrated shifts from plant to detrital organic matter in the
638 diet of the Caspian turtle *Mauremys caspica* (Gmelin, 1774) when present in polluted habitats.
639 The low growth rate of females observed in the habitat of medium quality (FOS₂₊₃) may be
640 related to turtles age and reproductive status, as growth rate usually decreases after females
641 become reproductive (Bury, 1979; Galbraith et al., 1989) (Fig.6). Females may have stored
642 more reserves to compensate for low growth rates, resulting in higher ~~body-conditions~~SMI
643 (Fig.5) (Ashton et al., 2015). Finally, the high ~~body-conditions~~SMI of ~~immature~~juveniles
644 reported in the ~~worst~~-most disturbed habitats (Fig.5) ~~may~~-could reflect a poor health status
645 induced by pollution, as reported earlier for the Central American river turtle *Dermatemys*
646 *mawii* (Gray, 1847) (see Rangel-Mendoza et al., 2014).

647

648 **Conclusion**

649 Our results highlighted different ~~phenotypes~~-morphologies and population structures in *M.*
650 *leprosa* in relation to increased levels of pollution and eutrophication of aquatic habitats.
651 Growth rates as well as adult body sizes were the highest in the most polluted and eutrophic
652 habitats. However, ~~fewer~~-immaturejuveniles were under-represented in such habitats,
653 suggesting an issue with their survival or the adult reproduction. Although differences in

654 population structure may result from alterations in water quality, additional factors such as
655 predation and competition with invasive species, not investigated here, could also have
656 impacted the demography of *M. leprosa*.

657

658 **Credit author statement**

659 **Anne-Sophie Le Gal**: Conceptualization; Data curation; Methodology; Formal analysis;
660 Investigation; Writing - Original Draft; Writing - Review & Editing; Visualization; Funding
661 acquisition. **Jean-Yves Georges**: Conceptualization; Writing - Review & Editing; Supervision.
662 **Christine Sotin**: Investigation. **Bruno Charrière**: Investigation. **Olivier Verneau**:
663 Conceptualization; Writing - Review & Editing; Supervision; Project administration; Funding
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677

678 **Competition and interests**

679 The authors declare that they have no known competing financial interests or personal
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681

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987

988 **Supplementary Materials**

989 **Supplementary Material A.** Physical and chemical water parameters assessed in 2021 in the
990 upstream (FOS₁), outfall (FOS₂), and downstream (FOS₃) wastewater treatment plant (WWTP)
991 subsections of the Fosseille River; in the upstream (AGO₁) and downstream (AGO₂) WWTP
992 subsections of the Agouille de la Mar River; in the upper (BAI₁) and lower (BAI₂) sections of
993 the Baillaury River. Colors indicate the ecological quality of each parameter, from blue to red:
994 very good, good, average, poor (Légifrance, 2010).

995 **Supplementary Material B.** Body mass (BM) of immature juveniles (a), males (b), and females
996 (c) along the investigated rivers: Baillaury River (BAI); upstream (FOS₁) and downstream
997 (FOS₂₊₃) wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream
998 (AGO₁) and downstream (AGO₂) WWTP subsections of the Agouille de la Mar River. Each

999 color represents a quality level, from blue to red. Pairwise Wilcoxon tests p-values are indicated
1000 (alpha = 5% *; 1% **). The median of each group is displayed as a bold vertical line, and the
1001 mean of each group as a black dot.

1002 **Supplementary Material C.** Growth rates in males (a) and in females (b), relative to their body
1003 size. A unique symbol is displayed for each river subsection: Baillaury River (BAI); upstream
1004 (FOS₁) and downstream (FOS₂₊₃) wastewater treatment plant (WWTP) subsections of the
1005 Fosseille River; upstream (AGO₁) and downstream (AGO₂) WWTP subsections of the Agouille
1006 de la Mar River.

- 1 Phenotypes and population structure of *Mauremys leprosa* are influenced by water quality
- 2 Fast-growing turtles, as well as largest and heaviest males, are associated with the most polluted
- 3 habitats
- 4 Highest proportions of juveniles are associated with the least polluted habitats
- 5 Eutrophication affects turtle phenotype and may adversely affect reproductive success

1 **Morphological Variations And Demographic Responses Of The Mediterranean Pond**
2 **Turtle *Mauremys Leprosa* To Heterogeneous Aquatic Habitats**

3

4 **Author names and affiliations**

5 Anne-Sophie Le Gal ^{a,b,c}, Jean-Yves Georges ^c, Christine Sotin ^{a,b}, Bruno Charrière ^{a,b}, Olivier
6 Verneau ^{a,b,d}

7 ^a Université de Perpignan Via Domitia, Centre de Formation et de Recherche sur les
8 Environnements Méditerranéens, UMR 5110, 52 Avenue Paul Alduy, F-66860 Perpignan
9 cedex, France

10 ^b CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens, UMR
11 5110, 52 Avenue Paul Alduy, F-66860 Perpignan cedex, France

12 ^c Université de Strasbourg, CNRS, IPHC UMR 7178, 23 rue du lœss, 67000 Strasbourg, France

13 ^d Unit for Environmental Sciences and Management, North-West University, Potchefstroom
14 campus, Private Bag X6001, 20520 Potchefstroom, South Africa

15

16 **Corresponding author**

17 Anne-Sophie Le Gal

18 Université de Perpignan Via Domitia, Centre de Formation et de Recherche sur les
19 Environnements Méditerranéens, UMR 5110, 52 Avenue Paul Alduy, F-66860 Perpignan
20 cedex, France

21 asophielegal@gmail.com

22

23 **Highlights**

24 Phenotypes and population structure of *Mauremys leprosa* are influenced by water quality

25 Fast-growing turtles, as well as largest and heaviest males, are associated with the most polluted
26 habitats

27 Highest proportions of juveniles are associated with the least polluted habitats

28 Eutrophication affects turtle phenotype and may adversely affect reproductive success

29 **Abstract**

30 Human activities affect terrestrial and aquatic habitats leading to changes at both individual and
31 population levels in wild animal species. In this study, we investigated the phenotype and
32 demographics of the Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) in
33 contrasted environments of Southern France: two peri-urban rivers receiving effluents from
34 wastewater treatment plants (WWTP), and another one without sewage treatment plant. Our
35 findings revealed the presence of pesticides and pharmaceuticals in the three rivers of
36 investigation, the highest diversities and concentrations of pollutants being found in the river
37 subsections impacted by WWTP effluents. Principal component analysis and hierarchical
38 clustering identified three levels of habitat quality, with different pollutant concentrations,
39 thermal conditions, nutrient, and organic matter levels. The highest turtle densities, growth
40 rates, and body sizes were estimated in the most disturbed habitats, suggesting potential adult
41 benefits derived from harsh environmental conditions induced by pollution and eutrophication.
42 Conversely, juveniles were the most abundant in the least polluted habitats, suggesting adverse
43 effects of pollution on juvenile survival or adult reproduction. This study suggests that turtles
44 living in polluted habitats may benefit from enhanced growth and body size, at the expense of
45 reproductive success.

46

47 **Keywords**

48 Freshwater turtles; Wastewater treatment plant; Water pollution; Population structure

49 **Abbreviations**

50	AGO: Agouille de la Mar River	55	OSR: Operational sex ratio
51	AMPA: Aminomethylphosphonic acid	56	PCA: Principal component analysis
52	BAI: Baillaury River	57	SCL: Straight carapace length
53	BM: Body mass	58	SMI: Scaled mass index
54	FOS: Fosseille River	59	WWTP: Wastewater treatment plant

60

61 **1. Introduction**

62 Sauropsid reptiles have evolved over 300 million years, resulting in the emergence of several
63 morphs adapted to aquatic and terrestrial life (Reisz, 2005). They are unique among vertebrates,
64 encompassing various biological and behavioral characteristics, that include ectothermy,
65 delayed sexual maturity, migratory patterns, and cryptic behavior specially during early
66 developmental stages (Hopkins, 2000). They possess physical defenses such as scales and shells
67 that operate as protective armor against predation and desiccation, contributing to their
68 particularly long lifespan even in harsh conditions (Reinke et al., 2022). They need to
69 thermoregulate to reach their physiological optimum (Pettersen, 2020). In extreme
70 environments, however, they can slow down metabolism by entering into hibernation, which
71 limits energy expenditure and enhances survival (Holden et al., 2021). Nevertheless, major and
72 rapid environmental changes following anthropization are responsible for their decline
73 worldwide (Gibbons et al., 2000; Todd et al., 2010; Weir et al., 2010; Doherty et al., 2020).
74 Accordingly, environmental pollutions can affect animal survival (Trakimas and Sidaravičius,
75 2008; Owen-Jones et al., 2016) and population stability (Gibbons et al., 2000; Irwin and Irwin,
76 2006; Boissinot et al., 2015).

77 Reptiles can be exposed to pollutants such as pesticides, metals, and polychlorinated
78 biphenyls (PCBs) through ingestion, inhalation, dermal absorption, but also during maternal
79 transfer to the eggs (Freitas et al., 2020). In early life stages, micropollutants can interfere with
80 embryonic development and hatchling viability (Woodward et al., 1993; Willemsen and Hailey,
81 2001). Pesticides which are widely used for pest control in agriculture, can have adverse effects
82 (Weir et al., 2014; Wagner et al., 2015; Mingo et al., 2016). It was reported that organochlorines
83 (e.g., PCBs), triazines (e.g., atrazine, simazine), and phenylureas (e.g., diuron) can interfere
84 with sex hormones, damaging morphology and functionality of reproductive organs, with
85 implications for fertility (Guillette et al., 1994; Crain and Guillette, 1998). Even though the
86 lethal dose of a pollutant is not reached, chronic exposure can also harm animals at low
87 concentrations (Faburé et al., 2022). However, many questions still remain about effects of
88 chronic exposure to low concentrations of pollutants on animal population dynamics.

89 Among reptiles, aquatic turtles have the particularity of exploiting both aquatic and
90 terrestrial habitats. Because they are long-lived animals and prone to bioaccumulate pollutants,
91 they have been considered as relevant bio-indicators to monitor pollutants exposure (Golden
92 and Rattner, 2003; Hopkins et al., 2013; Slimani et al., 2018). When present in food, water,
93 and/or sediment, contaminants can impact turtles through dermal exposure and/or ingestion
94 (Leusch et al., 2021). Pollutants can affect metabolic profile (Meng et al., 2023), sexual
95 development (Matsumoto et al., 2014), growth rate (Holliday and Holliday, 2012), and increase
96 incidence of juvenile deformities and mortalities (Adams et al., 2016). Deleterious effects, such
97 as lethal deformities in embryos (Bell et al., 2006), but also skin lesions and physiological stress
98 (Leusch et al., 2021) can occur as a result of chronic exposure to a cocktail of pollutants.
99 Furthermore, changes in the thermal regime in combination with maternally-derived pollutants
100 can unbalance sex ratio in clutches (Thompson et al., 2018). However, despite the numerous
101 studies that were conducted on the effects of environmental pollution on turtle species, too few

102 to date have focused on the same species occurring in contrasted environments with varying
103 degrees of pollution and eutrophication.

104 The Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) is a freshwater
105 turtle whose range extends from Northwest Africa (Western Libya, Tunisia, Algeria, and
106 Morocco) to Southwest Europe (Spain, Portugal, and Southern France) (Bertolero and Busack,
107 2017). This species occurs mostly in permanent wetlands, living in stagnant water or slow-
108 flowing rivers (Keller, 1997; Díaz-Paniagua et al., 2015). It is adapted to watercourses
109 experiencing sharp seasonal changes and, consequently, large fluctuations in water quantity and
110 quality (Díaz-Paniagua et al., 2015). It is omnivorous, feeding mainly on plants and macro-
111 invertebrates yet with some scavenger behavior (Díaz-Paniagua et al., 2015). Because it is a
112 long-lived animal with a high somatic conversion rate of food, it can bioaccumulate pollutants
113 over a long period of time with various responses in contrasted environments (Martínez- López
114 et al., 2017; Slimani et al., 2018).

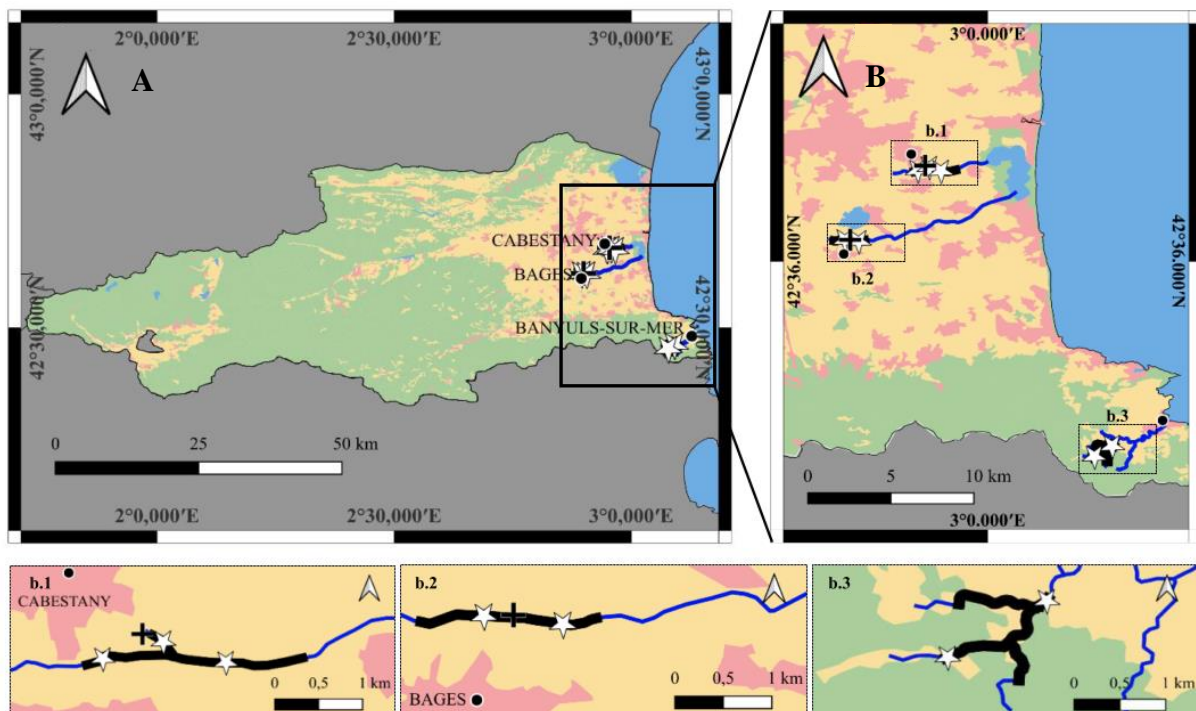
115 *Mauremys leprosa* can tolerate eutrophic waters (da Silva, 1995; Franch, 2003), and has
116 even been reported in highly polluted habitats (Naimi et al., 2012; Hérítier et al., 2017; El
117 Hassani et al., 2019). El Hassani et al. (2019) showed that turtles, in Northwestern Morocco,
118 could cope with habitat pollution without exhibiting physiological disorders. On the opposite,
119 Hérítier et al. (2017) showed that turtles living in aquatic habitats impacted from wastewater
120 treatment plant (hereafter referred as WWTP) effluents, in Southern France, exhibited high
121 oxidative stress. Despite a significant difference in pesticide pollution upstream and
122 downstream of the WWTP regarding effluents on the river, over a nearly ten years study was
123 revealed that turtles were concentrated preferentially downstream of the WWTP, which was
124 unexpected (Le Gal et al., 2023). Yet, additional comparative studies are necessary to better
125 understand the relationship between habitat alteration and the demographics of *M. leprosa*.

126 The objectives of our study were to determine how *M. leprosa* responds to habitat alteration,
127 including pollution and eutrophication. Whether high degrees of habitat degradation can
128 promote large turtles and dense populations, a male-biased sex ratio was observed in the most
129 polluted habitats (El Hassani et al., 2019; Le Gal et al., 2023), suggesting detrimental effects
130 on reproduction. In order to investigate the relationship between habitat alteration,
131 morphological and demographic variations, three populations of *M. leprosa* occurring along a
132 latitudinal gradient were studied. Our aims were to assess the population structure and
133 dynamics, and to correlate demographic parameters to the quality of habitats.

134

135 2. Materials and methods

136 2.1. Study sites



137

138 **Figure 1.** (A) Region of Pyrénées-Orientales in Southern France, with aquatic areas in blue,
139 pristine areas in green, bare soil in yellow, cropland in orange and urbanized areas in red. (B)
140 The studied rivers are in navy blue, and their configuration is shown at the bottom of the main

141 map, with the river sections in black, the wastewater treatment plants represented by black
142 crosses, and water sampling points by white stars: (b.1) Fosseille River, (b.2) Agouille de la
143 Mar River, and (b.3) Baillaury River.

144

145 2.1.1. *The Baillaury environment*

146 The Baillaury River flows from its source, 600 meters high, to the Mediterranean Sea, at
147 Banyuls-sur-Mer (Fig.1). It is characterized by a slow flow during the dry season, when it
148 partially dries up, and by a water regime increasing considerably during seasonal rainfall events.
149 The river section of investigation flows from an altitude of 120 to 60 meters, is 1.62 kilometers
150 long, 0.5 to five meters wide, and 0.5 meters deep. Its watershed, which is almost free of urban
151 activities, is traditionally cultivated with vineyards. The edge vegetation is mainly composed
152 of the giant reed *Arundo donax* (Linnaeus, 1753), the European ash *Fraxinus excelsior*
153 (Linnaeus, 1753), the cork oak *Quercus suber* (Linnaeus, 1753), scrubland, and maquis.
154 *Mauremys leprosa* is the only freshwater turtle species present here, and other frequently
155 encountered aquatic species are mostly native, among which the Mediterranean barb *Barbus*
156 *meridionalis* (Risso, 1827), the European eel *Anguilla anguilla* (Linnaeus, 1758), the viperine
157 water snake *Natrix maura* (Linnaeus, 1758), the Perez frog *Pelophylax perezi* (López-Seoane,
158 1885), and the spiny toad *Bufo spinosus* (Daudin, 1803).

159

160 2.1.2. *The Fosseille environment*

161 The Fosseille River is a lentic river, flowing from the peri-urban south of Perpignan to the Saint
162 Nazaire lagoon, which is connected to the Mediterranean Sea (Fig.1). The river section of
163 investigation flows from an altitude of 16 to two meters, is 2.6 kilometers long, one to four
164 meters wide, and two meters deep. It receives effluents from the WWTP of Cabestany, which

165 contributes to maintain the continuous water flow of the river year-round. It consists of three
166 subsections according to the position of the WWTP: the upstream, outfall, and downstream
167 river subsections (Fig.1). Its watershed is highly anthropized by urban and agricultural activities
168 such as vine growing. The riparian zone is homogeneous, with sparse border vegetation
169 dominated by *A. donax*, the common reed *Phragmites australis* (Cavanilles) Trinius ex Steudel,
170 1840, the lakeshore bulrush *Schoenoplectus lacustris* (Linnaeus) Palla, 1888, and the yellow
171 flag *Iris pseudacorus* (Linnaeus, 1753). The frequently encountered aquatic species were *M.*
172 *leprosa*, the European roach *Rutilus rutilus* (Linnaeus, 1758), *Squalius* spp., *A. anguilla*, and *N.*
173 *maura*. Alien species were also encountered, among which the red-eared slider *Trachemys*
174 *scripta elegans* (Wied, 1838) and the red swamp crayfish *Procambarus clarkii* (Girard, 1852).

175

176 2.1.3. *The Agouille de la Mar environment*

177 The Agouille de la Mar River is a lentic river, flowing from the vicinity of Bages to the Saint
178 Nazaire lagoon (Fig.1). The river of investigation flows at an altitude of about 15 meters, is 1.9
179 kilometers long, three to eight meters wide, and two meters deep. Two subsections were defined
180 according to the position of the Bages WWTP: the upstream and the downstream subsections
181 (Fig.1). Its watershed is highly anthropized as the Fosseille River, because of urban and
182 agricultural activities. The riparian zone is homogeneous all along the study section, with sparse
183 border vegetation dominated by *A. donax*, *P. australis*, *S. lacustris*, and *I. pseudacorus*. The
184 encountered aquatic species are the same as for the Fosseille River and consist of native species,
185 including *M. leprosa*, and alien species such as *T. s. elegans* and *P. clarkii*.

186

187 2.2. *Habitat quality*

188 2.2.1. *Environmental parameters of investigation*

189 A total of 14 water samples were collected to assess water quality of the three rivers, among
190 which seven were collected on 2021/05/07, and seven on 2021/09/27 (Fig.1). On the Baillaury
191 River, point sampling was done on the upper (BAI₁: N 42°27'25" E 3°4'57") and lower (BAI₂:
192 N 42°27'45" E 3°5'26") reaches. On the Fosseille River, point sampling was done on the
193 upstream (FOS₁: N 42°40'2" E 2°56'53"), outfall (FOS₂: N 42°40'11" E 2°57'26") and
194 downstream (FOS₃: N 42°40'1" E 2°58'1") subsections. Finally, on the Agouille de la Mar
195 River, point sampling was conducted on the upstream (AGO₁: N 42°36'57.5" E 2°53'41") and
196 downstream (AGO₂: N 42°36'53.6" E 2°54'24") subsections. Environmental parameters, such
197 as temperature (T, in °C), pH, dissolved oxygen (DO, in mg.L⁻¹), and conductivity (σ, in μS.cm⁻¹)
198 ¹, were measured *in natura* with a HQ40D Portable Meter (Hach).

199 Water samples were filtered on 0.45 μm (47 mm diameter) MF-Millipore membrane
200 filters to quantify suspended particulate matter (SPM, in mg.L⁻¹) at the Centre de Formation et
201 de Recherche sur les Environnements Méditerranéens, and on 0.7 μm (47 mm diameter)
202 Whatman GF/F glass fiber filters (precombusted at 500 °C for four hours) to quantify particulate
203 organic matter, i.e., particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP)
204 (in mg.L⁻¹), at the Mediterranean Institute of Oceanography (MIO ; Marseille, France). Glass
205 fiber filters were dried at 60 °C and processed by the analytical platform for the chemistry of
206 marine environments at the MIO, with a SEAL analytical chain, using the wet-oxidation technic
207 (Raimbault et al., 1999). The detection limits were 120 ng.L⁻¹, 1 ng.L⁻¹, and 2 ng.L⁻¹ for POC,
208 PON, and POP, respectively.

209 Nutrients, including nitrite (NO₂⁻, in mg.L⁻¹), ammonium (NH₄⁺, in mg.L⁻¹), nitrate
210 (NO₃⁻, in mg.L⁻¹), and orthophosphate (PO₄³⁻, in mg.L⁻¹), as well as pesticides (N = 477
211 molecules ; in μg.L⁻¹) and pharmaceuticals (N = 23 molecules ; in μg.L⁻¹), were quantified from
212 the water samples by the Centre d'Analyses Méditerranée Pyrénées (Perpignan, France). The
213 analytical methods used to quantify the nutrients, with their detection limits, were the flow

214 analysis and spectrometric detection for NO_2^- and NH_4^+ (0.01 mg.L^{-1}), the liquid
215 chromatography assay for NO_3^- (0.2 mg.L^{-1}), and the spectrophotometry for PO_4^{3-} (0.02 mg.L^{-1}). The liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and the gas
216 chromatography-mass spectrometry (GC-MS/MS), were used to quantify pesticides. LC-
217 MS/MS was also used to quantify pharmaceuticals. The detection limits for contaminants
218 depended on the molecules and varied from $0.005 \text{ }\mu\text{g.L}^{-1}$ to $0.1 \text{ }\mu\text{g.L}^{-1}$. Methods used for
219 nutrients and contaminants quantification were in compliance with the LAB GTA 05
220 accreditation program.

222

223 2.2.2. *Statistical analyses of water quality*

224 Concentrations of contaminants were compared between samples collected along the same
225 river, using Kruskal-Wallis rank sum and Dunn post hoc tests. Comparisons were done between
226 BAI_1 and BAI_2 of the Baillaury River, between FOS_1 and FOS_2 , FOS_1 and FOS_3 , and FOS_2 and
227 FOS_3 of the Fosseille River, and between AGO_1 and AGO_2 of the Agouille de la Mar River, for
228 both periods of sampling. The water quality parameters were also compared between samples
229 collected from the three rivers. A principal component analysis (PCA) was performed on the
230 water quality dataset, including the values of the physicochemical parameters, organic matter,
231 nutrients, and contaminants. To distinguish the different levels of habitat quality among the
232 seven river subsections sampled in May and September, a hierarchical clustering (HC) was used
233 on the 14 water samples. Statistical tests were done on RStudio 3.6.2 (RStudio Team, 2020)
234 with significance levels set at 5%. The Factoshiny R package (Lê et al., 2008) was used for
235 PCA and HC analyses, and the ggplot2 R package for data visualization (Wickham, 2016).

236 2.3. *Turtles monitoring*

237 2.3.1. *Animals handling*

238 Three sessions of three days of capture-mark-recapture were conducted during the peak activity
239 of turtles, in May, June, and July 2021 in the three rivers of investigation. Baited crayfish traps
240 (60 x 30 cm and 80 x 35 cm), equipped with empty water bottles to avoid drowning of animals,
241 were deployed every 50 meters along each river. The yearly sampling effort was 450 traps.days
242 in the Baillaury River, 450 traps.days in the Fosseille River, and 288 traps.days in the Agouille
243 de la Mar River. Turtles were identified by a unique code following indentations on the
244 marginal scutes (Cagle, 1939). Sex of each turtle was determined from apparent secondary
245 sexual characters (MAL for males, and FEM for females), namely the concavity of the plastron
246 and the distance between the cloaca opening and the plastron notch (Pérez et al., 1979; Busack
247 and Ernst, 1980; Meek, 1987; Fritz, 2003). Because these traits are only apparent in turtles over
248 80 mm of size in the populations of interest, turtles smaller than 80 mm were considered as
249 juveniles (JUV). The body mass (BM) and the straight carapace length (SCL) were measured
250 with a scale (precision = 0.1 g) and a Vernier caliper (precision = 1 mm). After measurements,
251 turtles were immediately released at the place of capture. To estimate growth rates, unpublished
252 data acquired following turtles monitoring in the Baillaury River (2012-2021), Agouille de la
253 Mar River (2014-2021), and Fosseille River (2013-2021), as well as data retrieved from Le Gal
254 et al. (2023) were also used. A ministerial authorization was granted to the handlers of turtles
255 by the DREAL Occitanie, and experiments were carried out in accordance with the U.K.
256 Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU
257 for animal experiments.

258

259 2.3.2. *Population structure*

260 Population size, density, and operational sex ratio (OSR), were estimated from the data gathered
261 in 2021. Because turtle population stability mainly depends on the adults (Crouse et al., 1987;
262 Galbraith and Brooks, 1987; Frazer, 1992; Heppell, 1998; Blamires and Spencer, 2013), the
263 population structure was estimated after excluding juveniles from the dataset. Prior to modeling,
264 population closure was tested with the CloseTest software (Stanley and Burnham, 1999), trap-
265 dependence was tested on the CAPTURE program implemented in MARK 6.2 (White and
266 Burnham, 1999), and transience was analyzed using a goodness-of-fit test (Pradel et al., 2005)
267 performed on U-Care 2.3.2 (Pradel et al., 2010). The population size was then estimated from
268 CAPTURE (White and Burnham, 1999). Finally, the population density (in adults.km⁻¹), and
269 the OSR (MAL/(MAL+FEM)) were inferred from the raw data captures.

270 Differences in BM and SCL along the river subsections were assessed using Kruskal-
271 Wallis rank sum comparison tests, followed by Dunn post-hoc tests. Then, body condition, and
272 growth rate were studied, with only the first annual capture being considered to account for
273 pseudo replication. For body condition, the scaled mass index (SMI) was calculated following
274 the procedure developed by Peig and Green (2009). The SMI is a hypothetical mass that allows
275 for consideration of the relationship between body mass and body length within the group of
276 interest, and the impact of individual variability and measurement errors on BM and SCL. The
277 growth rate (GR) was calculated following the following equation designed by us: $GR_i =$
278 $(SCL_{2021} - SCL_y) / [(2021 - y) * SCL_y]$, with *i* the turtle, and *y* the first year of capture.
279 Following a model selection process using AIC in a stepwise algorithm, ANOVA was used to
280 search for mixed effects of SCL and river subsection on SMI and growth rate, respectively.
281 Durbin-Watson and Shapiro-Wilk tests were used to verify the assumptions of the ANOVA.
282 Tukey HSD tests were then used to determine the groups that exhibited statistically significant
283 differences. In view of the variations in body composition specific to age and sex, separate
284 analyses were performed for juveniles, males, and females. All analyses were performed on

285 RStudio 3.6.2 (RStudio Team, 2020) with significance levels set at 5%; the MASS package was
286 used for model selection (Venables and Ripley, 2002); and the ggplot2 package was used for
287 data visualization (Wickham, 2016).

288

289 **3. Results**

290 *3.1. Diversity and concentration of contaminants*

291 A total of 58 contaminants were detected in the water samples, including 38 pesticides and 20
292 pharmaceuticals (Fig.2). Pesticides were detected in all three rivers, while pharmaceuticals,
293 with the exception of caffeine (included in pharmaceuticals for convenience), were only
294 detected in the Fosseille and Agouille de la Mar rivers. The two most concentrated contaminants
295 detected overall were the glyphosate (0.05 to 0.75 $\mu\text{g.L}^{-1}$) and the AMPA (0.05 to 9.6 $\mu\text{g.L}^{-1}$).
296 The diversity of contaminants was similar between May and September 2021, regardless the
297 river.

298 In the Baillaury River, 11 pesticides and one pharmaceutical were detected in the water.
299 Among the pesticides, nine were detected in BAI₁, and eight in BAI₂. The pesticides with the
300 highest concentrations were the hydroxy simazine (0.10 $\mu\text{g.L}^{-1}$) and the AMPA (0.06 $\mu\text{g.L}^{-1}$).
301 The other pesticides included three derivatives of atrazine, one derivative of norflurazon, two
302 derivatives of terbuthylazine, fenpyroximate, simazine and one of its derivatives. Caffeine was
303 the only pharmaceutical detected, with a concentration ranging from 0.03 to 0.05 $\mu\text{g.L}^{-1}$. The
304 concentrations in contaminants did not differ between the two sampling sites (Kruskal-Wallis
305 rank sum test, $\chi^2 = 1.78$, $df = 3$, $p = 0.62$).

306 In the Fosseille River, 30 pesticides and 18 pharmaceuticals were detected in the water.
307 Among the pesticides, 13 were detected upstream, 23 at the outfall, and 22 downstream of the
308 WWTP. Among the pharmaceuticals, three were detected upstream (caffeine, irbesartan, and

309 metformin), 17 at the outfall, and 17 downstream of the WWTP. Contaminants with the highest
310 concentrations were the AMPA (9.6 $\mu\text{g.L}^{-1}$) and the metformin (3.1 $\mu\text{g.L}^{-1}$). The diversity of
311 contaminants was higher downstream (N = 39 contaminants) and at the outfall (N = 40
312 contaminants), than upstream of the WWTP (N = 16 contaminants). The concentrations of the
313 contaminants did not differ significantly between the outfall and the downstream river
314 subsections, regardless the period of sampling, i.e., May and September 2021. They were
315 however significantly higher than those reported upstream of the WWTP (Kruskal-Wallis rank
316 sum test, $\chi^2 = 44.35$, $\text{df} = 5$, $p = 1.97\text{e-}08$).

317 In the Agouille de la Mar River, a total of 21 pesticides and 14 pharmaceuticals were
318 detected in the water. Among the pesticides, eight were detected upstream and 19 downstream
319 of the WWTP. Among the pharmaceuticals, four were detected upstream (caffeine, metformin,
320 paracetamol, and theophylline), and 13 downstream of the WWTP. Contaminants with the
321 highest concentrations were the AMPA (6.4 $\mu\text{g.L}^{-1}$) and the metformin (5.7 $\mu\text{g.L}^{-1}$). Both the
322 diversity and the concentrations of contaminants were higher downstream than upstream of the
323 WWTP (N = 32 and 12 contaminants, respectively; Kruskal-Wallis rank sum test, $\chi^2 = 24.40$,
324 $\text{df} = 3$, $p = 2.06\text{e-}05$).

325

326 *3.2. Levels of water quality*

327 The hierarchical clustering revealed three levels of habitat quality: (i) the Baillaury River
328 section (BAI₁ and BAI₂) and the upstream subsection of the Fosseille River (FOS₁) present a
329 low level of pollution and are classified as the first level; (ii) the outfall (FOS₂) and the
330 downstream (FOS₃) subsections of the Fosseille River present a moderate level of pollution and
331 are classified as the second level; (iii) the Agouille de la Mar River section (AGO₁ and AGO₂)

332 presents the highest level of pollution and is classified as the third level (Fig.3; Supplementary
333 Material A).

334 The first dimension of the PCA distinguished the downstream subsection of the
335 Agouille de la Mar River (AGO₂) from the Baillaury River section (BAI₁ and BAI₂) (Fig.3).
336 The latter was characterized by low concentrations of contaminants to the exception of
337 hydroxy-terbutylazine ($0.040 \pm 0.006 \mu\text{g.L}^{-1}$; *versus* AGO₂: $0.009 \pm 0.001 \mu\text{g.L}^{-1}$), low
338 conductivity ($488.00 \pm 51.56 \mu\text{S.cm}^{-1}$; *versus* AGO₂: $990.00 \pm 22.00 \mu\text{S.cm}^{-1}$), and low
339 temperature ($17.78 \pm 0.48 \text{ }^\circ\text{C}$; *versus* AGO₂: $23.15 \pm 1.15 \text{ }^\circ\text{C}$) (Fig.3). AGO₂ was characterized
340 by high concentrations of particulate organic matter such as POP ($0.090 \pm 0.010 \mu\text{g.L}^{-1}$; *versus*
341 BAI: $0.010 \pm 0.010 \mu\text{g.L}^{-1}$), high nutrients levels such as NO₂- ($0.49 \pm 0.09 \mu\text{g.L}^{-1}$; *versus* BAI:
342 $< 0.01 \mu\text{g.L}^{-1}$), atrazine-dip ($0.016 \pm 0.001 \mu\text{g.L}^{-1}$; *versus* BAI: $0.001 \pm 0.001 \mu\text{g.L}^{-1}$), and high
343 water temperature ($23.15 \pm 1.15 \text{ }^\circ\text{C}$; *versus* BAI: $17.78 \pm 0.48 \text{ }^\circ\text{C}$) (Fig.3). The second
344 dimension of the PCA distinguished the outfall (FOS₂) and the downstream (FOS₃) subsections
345 of the Fosseille River from the Baillaury River section (BAI₁ and BAI₂) (Fig.3). FOS₂ and FOS₃
346 were characterized by high conductivity ($1412.00 \pm 78.33 \mu\text{S.cm}^{-1}$; *versus* BAI: 488.00 ± 51.56
347 $\mu\text{S.cm}^{-1}$), high concentrations of AMPA ($7.93 \pm 0.76 \mu\text{g.L}^{-1}$; *versus* BAI: $0.028 \pm 0.017 \mu\text{g.L}^{-1}$)
348 ¹), and high concentrations of caffeine ($0.17 \pm 0.05 \mu\text{g.L}^{-1}$; *versus* BAI: $0.040 \pm 0.005 \mu\text{g.L}^{-1}$).

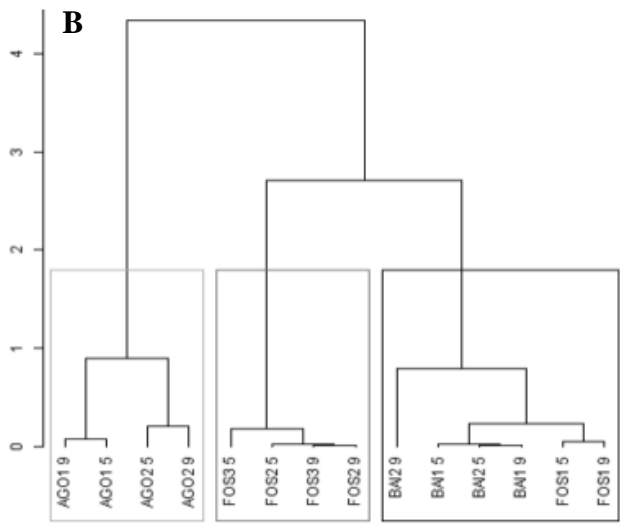
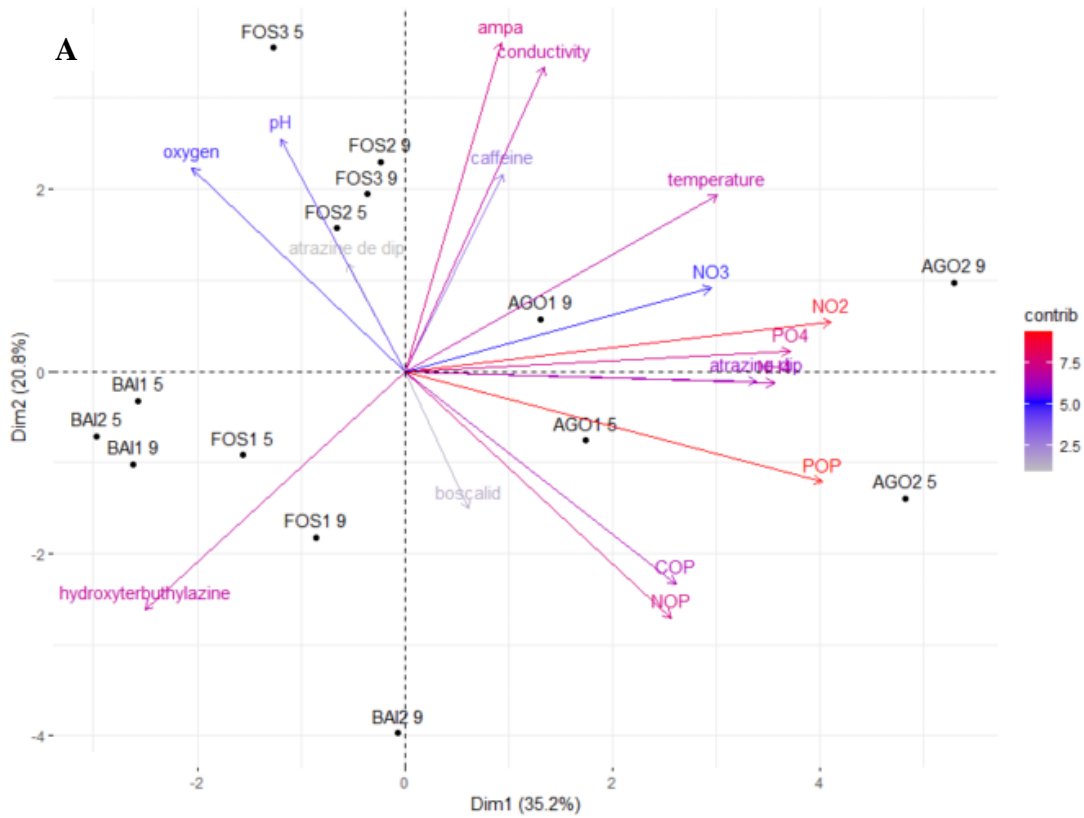
349 Because samples collected in BAI₁ and BAI₂ did not differ in pollution levels, a single
350 river subsection (BAI) was considered for the Baillaury River when analyzing turtle population
351 structure. Similarly, as FOS₂ and FOS₃ did not show significant differences in pollution levels,
352 they were considered as a single subsection named hereafter FOS₂₊₃. Five subsections were
353 therefore considered for population structure analyses, namely BAI, FOS₁, FOS₂₊₃, AGO₁, and
354 AGO₂.

355



356

357 **Figure 2.** Concentration of (A) pesticides and (B) pharmaceuticals detected in the water. BAI₁
 358 and BAI₂ represent the upper and lower water sampling points on the Baillaury River section;
 359 FOS₁, FOS₂, and FOS₃ represent respectively the upstream, outfall, and downstream
 360 subsections of the Fosseille River; AGO₁ and AGO₂ represent respectively the upstream and
 361 downstream subsections of the Agouille de la Mar River. “may” is displayed for May 2021,
 362 and “sept” for September 2021.



363

364 **Figure 3.** (A) Biplot of the principal component analysis: the color gradient corresponds to the
 365 contribution of each variable to the construction of the plan (Dim1, Dim2). Each water sample
 366 is associated to a river (BAI for the Baillaury River section, FOS for the Fosseille River section,
 367 and AGO for the Agouille de la Mar River section), a sampling point (1:3), and a sampling
 368 month (5 for May, and 9 for September). (B) Hierarchical clustering tree: each box corresponds
 369 to a level of habitat quality.

370 *3.3. Population structure*

371 The closure test revealed that populations of the three rivers were closed in 2021, i.e.,
372 emigration and migration rates were negligible (Baillaury: $\chi^2 = 0.90$, $df = 1$, $p = 0.34$; Fosseille:
373 $\chi^2 = 2.48$, $df = 2$, $p = 0.29$; Agouille de la Mar: $\chi^2 = 1.97$, $df = 1$, $p = 0.37$), and no trap-
374 dependence was detected in the populations. Goodness of fit results indicated the presence of
375 transient males on the Baillaury River (males: $zlor = 1.71$, $df = 1$, $p = 0.05$; females: $zlor = 0.07$,
376 $df = 1$, $p = 0.47$), but there was no transience on the Fosseille (males: $zlor = 0.75$, $df = 1$, p
377 $= 0.23$; females: $zlor = 0.67$, $df = 1$, $p = 0.25$) and on the Agouille de la Mar (males: $zlor = 0.62$,
378 $df = 1$, $p = 0.27$; females: $zlor = 0.42$, $df = 1$, $p = 0.85$) rivers. On the Baillaury River, the capture
379 probability varied with time of capture (model $M(t)$) and was estimated at 0.26 (May 2021),
380 0.56 (June 2021), and 0.49 (July 2021). On the Fosseille and Agouille de la Mar rivers, the
381 probability of capture was constant (model $M(o)$) and estimated at 0.22 and 0.26 for each river,
382 respectively.

383 The number of individuals ranged from 43 turtles on BAI to 135 turtles on FOS₂₊₃
384 (Table 1). The OSR was male-biased in most of the river subsections and ranged from 0.56 to
385 0.76, with the exception of FOS₁, where it was balanced at 0.48 (Table 1). The adult population
386 of the Baillaury River consisted of 45 ± 5 turtles (95% CI = [40;64]) and had a density of 27
387 turtles.km⁻¹. On the Fosseille River, the adult population consisted of 157 ± 24 turtles (95% CI
388 = [121;223]) and density ranged from 63 to 68 turtles.km⁻¹, upstream to downstream of the
389 WWTP respectively. On the Agouille de la Mar River, the adult population consisted of $138 \pm$
390 18 turtles (95% CI = [112;186]), and density ranged from 44 to 50 turtles.km⁻¹, upstream to
391 downstream of the WWTP respectively. Juveniles accounted for 16-45% of the total captures
392 in habitats of level 1 (BAI and FOS₁), 12% in habitat of level 2 (FOS₂₊₃), and 7-9% in habitats
393 of level 3 (AGO₁ and AGO₂).

394

395 **Table 1.** Captures composition (males MAL, females FEM, and juveniles JUV) and operational
 396 sex ratio (OSR) of *M. leprosa* along the Baillaury River (BAI), the Fosseille River (FOS), and
 397 the Agouille de la Mar River (AGO). The total number of captures is indicated, with the number
 398 of distinct individuals shown in brackets.

		MAL	FEM	JUV	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS ₁)	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS ₂₊₃)	172 (91)	39 (28)	28 (16)	239 (135)	0.88	0.76
	Full river	201 (101)	65 (39)	65 (33)	331 (173)	0.81	0.72
AGO	Upstream (AGO ₁)	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO ₂)	32 (23)	31 (18)	4 (4)	67 (45)	0.91	0.56
	Full river	71 (48)	53 (34)	8 (7)	132 (89)	0.92	0.59

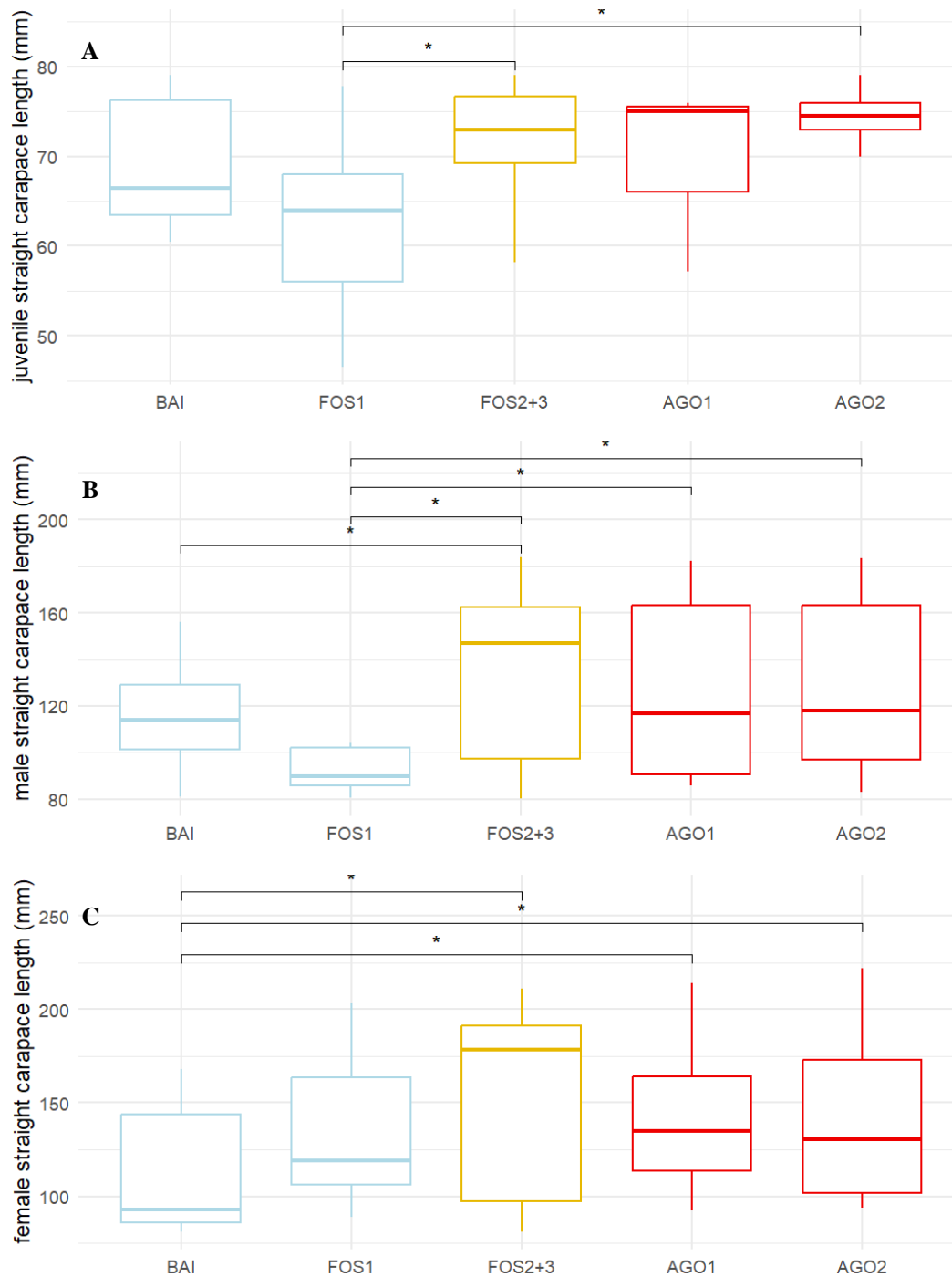
399

400 3.4. Turtles body metrics

401 Body size (SCL) and mass (BM) of adults were different across the five river subsections, i.e.,
 402 BAI, FOS₁, FOS₂₊₃, AGO₁, and AGO₂ (Fig.4; Supplementary Material B). Differences were
 403 significant in males (n = 172; Kruskal-Wallis rank sum test; SCL: $\chi^2 = 11.00$, df = 4, p = 0.03;
 404 BM: $\chi^2 = 11.62$, df = 4, p = 0.02), and near so in females (n = 86; Kruskal-Wallis rank sum test;
 405 SCL: $\chi^2 = 8.52$, df = 4, p = 0.07; BM: $\chi^2 = 9.71$, df = 4, p = 0.05). The largest and heaviest
 406 turtles were located in the most degraded habitats, i.e., in FOS₂₊₃ and in both subsections of the
 407 Agouille de la Mar River (Fig.4; Supplementary Material B). However, the SMI of males did

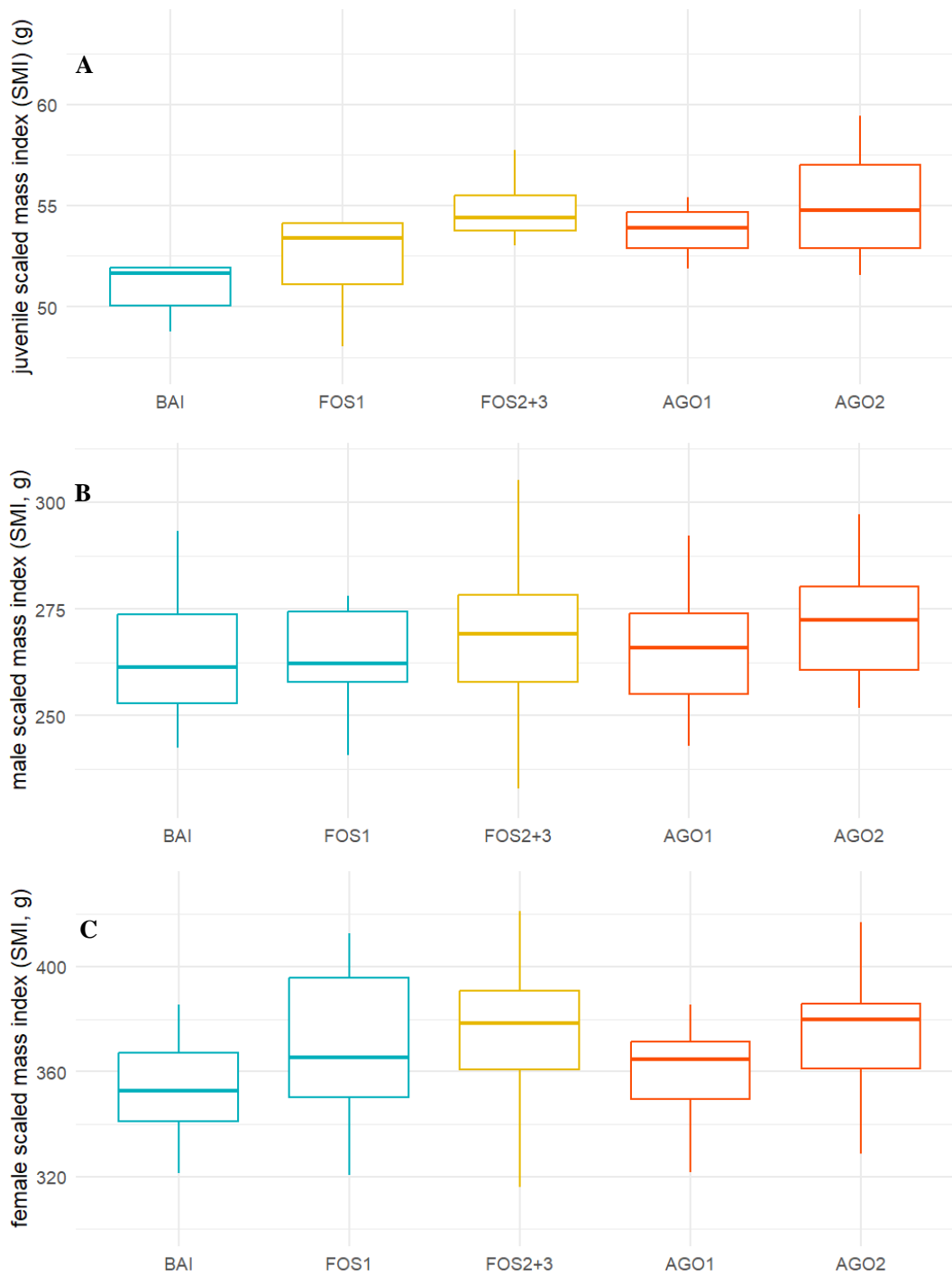
408 not differ between the five river subsections ($n = 172$; ANOVA; SMI: $F_{4,167} = 0.72$, $p = 0.58$;
409 Shapiro-Wilk: $W = 0.99$, $p = 0.08$), nor in females ($n = 86$; ANOVA; SMI: $F_{4,81} = 1.77$, $p =$
410 0.14 ; Shapiro-Wilk: $W = 0.98$, $p = 0.11$) (Fig.5). The body size and SMI of juveniles were not
411 different across the five river subsections ($n = 47$; Kruskal-Wallis rank sum test; SCL: $\chi^2 = 7.49$,
412 $df = 4$, $p = 0.11$; SMI: $\chi^2 = 7.62$, $df = 4$, $p = 0.11$) (Fig.4-5). However, body mass tended to
413 increase along the pollution gradient, from level 1 to level 3 ($n = 47$; Kruskal-Wallis rank sum
414 test; BM: $\chi^2 = 8.99$, $df = 4$, $p = 0.06$) (Supplementary Material B).

415 Growth rates were different across the five river subsections (Fig.6) and also differed
416 according to turtle body size. Differences were significant in males ($n = 172$; ANOVA; SCL:
417 $F_{1,77} = 12.98$, $p = 5.55e-04$; River subsection: $F_{4,77} = 4.27$, $p = 3.58e-03$; Shapiro-Wilk: $W =$
418 0.98 ; $p = 0.41$) and in females ($n = 86$; SCL: $F_{1,32} = 36.41$, $p = 9.84e-07$; River subsection: $F_{4,$
419 $32} = 7.28$, $p = 2.75e-04$; Shapiro-Wilk: $W = 0.95$; $p = 0.08$). Adults grew faster in the habitats
420 of level 3 (AGO₁ and AGO₂) than in other habitats. However, females grew slower in the habitat
421 of level 2 (FOS₂₊₃) than in other habitats (Fig.6). The inflexion point of male growth rate was
422 reached for a body size of 125 mm, whereas no such point was detected in females
423 (Supplementary Material C). The growth rate was close to zero for males with a body size above
424 125 mm, and for females with a body size above 200 mm (Supplementary Material C).



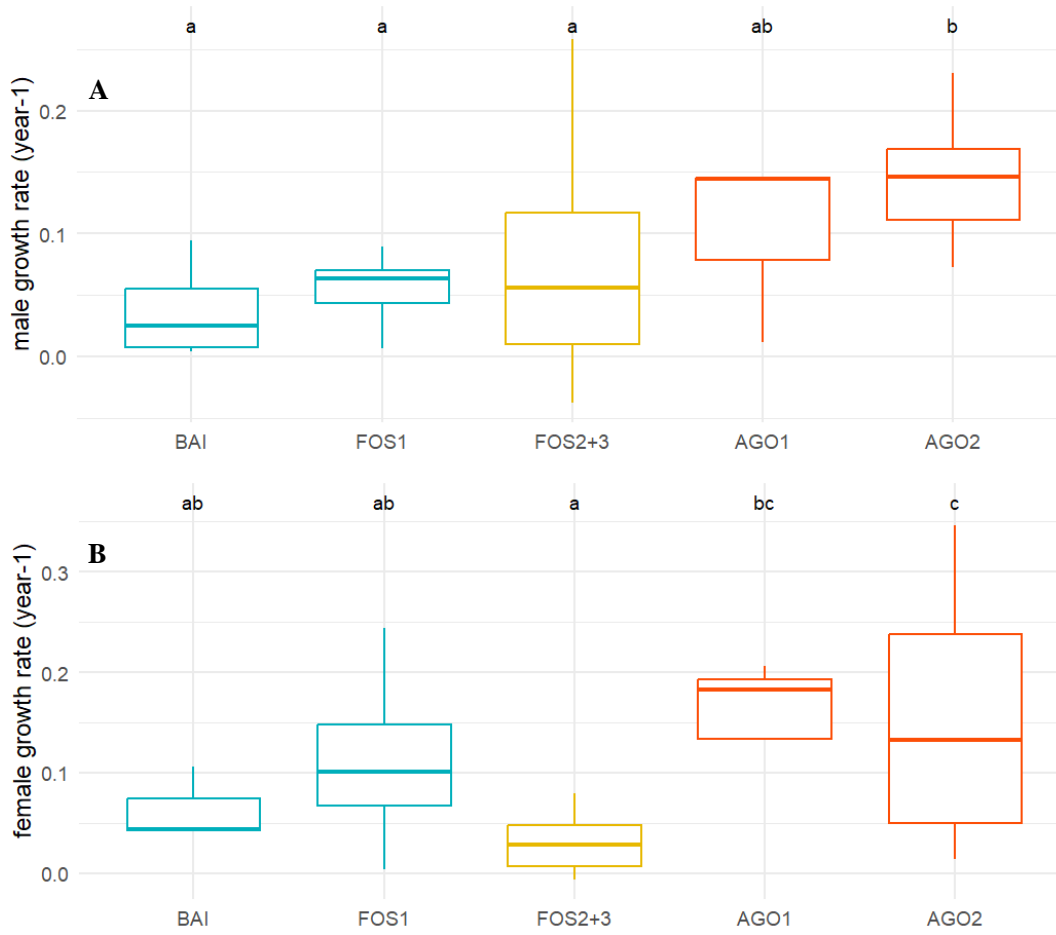
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426 **Figure 4.** Body size of juveniles (A), males (B), and females (C) along the investigated rivers:
 427 Baillaury River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃) wastewater treatment plant
 428 (WWTP) subsections of the Fosseille River; upstream (AGO₁) and downstream (AGO₂)
 429 WWTP subsections of the Agouille de la Mar River. Each color represents a quality level, from
 430 blue to red. Pairwise Wilcoxon tests p-values are indicated (alpha = 5% *; 1% **). The median
 431 of each group is displayed as a bold vertical line.



432

433 **Figure 5.** Scaled mass indices in juveniles (A), males (B), and females (C) along the
 434 investigated rivers: Baillaury River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃)
 435 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO₁) and
 436 downstream (AGO₂) WWTP subsections of the Agouille de la Mar River. Each color represents
 437 a quality level, from blue to red.



438

439 **Figure 6.** Growth rates in males (A) and females (B) along the investigated rivers: Baillaury

440 River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃) wastewater treatment plant (WWTP)

441 subsections of the Fosseille River; upstream (AGO₁) and downstream (AGO₂) WWTP

442 subsections of the Agouille de la Mar River. Each color represents a quality level, from blue to

443 red. Following ANOVA, Tukey test comparisons results are displayed as letters. Each color

444 represents a quality level, from blue to red.

445 **4. Discussion**

446 *4.1. Aquatic habitat quality*

447 The least polluted habitats (level 1) encompassed the Baillaury River (BAI₁ and BAI₂) and the
448 upstream subsection of the Fosseille River (FOS₁), which exhibited the lowest diversity and
449 concentrations of contaminants, the lowest concentrations of nutrients, the lowest conductivity,
450 and the lowest water temperatures. Whereas pesticides and caffeine detected in these
451 subsections might have been drained by rain, they likely resulted from illegal discharge as they
452 were assessed during low flow periods. Caffeine is indeed a marker of anthropization and is the
453 most detected emerging contaminant in surface water (Korekar et al., 2020). In the absence of
454 WWTP, such discharge could occur via semi-rigid pipes connecting private plots to the river,
455 which were observed along the Baillaury River. The contaminants with the highest
456 concentrations in these habitats were caffeine, glyphosate, and AMPA. AMPA, which is the
457 main metabolite of glyphosate and persistent in water, can have significant negative effects on
458 aquatic organisms (Daouk et al., 2013; Guilherme et al., 2014; Moore et al., 2014; Tresnakova
459 et al., 2021). While the low DO concentration measured in the Baillaury River in September
460 indicated fairly poor water quality and anoxic conditions during drought periods
461 (Supplementary Material A; Légifrance, 2010), the presence of bio-indicators such as the
462 amphibians *P. perezii* and *B. spinosus* in that river indicated good aquatic and terrestrial habitats
463 (see Marques et al., 2008; Napoletano et al., 2023). On the opposite, the diversity of pollutants
464 reported in FOS₁ was higher than in the Baillaury section, suggesting that this subsection's
465 quality was lower than that of the Baillaury River. FOS₁ also contained metformin which is
466 detrimental to aquatic fauna with notorious effects on the endocrine function and fecundity of
467 fish (Niemuth and Klaper, 2015; Lee et al., 2019). The presence of alien species such as the
468 crayfish *P. clarkii* in the Fosseille River could also explain the absence of amphibians due to
469 predation (Gherardi et al., 2001).

470 The habitats of medium quality (level 2) encompassed both the outfall (FOS₂) and the
471 downstream (FOS₃) subsections of the Fosseille River. They exhibited high diversity and
472 concentrations of pesticides and pharmaceuticals, high concentrations of suspended particles,
473 and high water temperatures compared to habitats of level 1. Alterations in water quality were
474 probably due to the presence of the Cabestany WWTP, which receives wastewater from the
475 urban community, but also from agricultural plots. Because not all pollutants are removed by
476 WWTPs (Bruton et al., 2010; Deo, 2014), pesticides and pharmaceuticals are commonly found
477 in water. The most concentrated pollutants were indeed the AMPA and the metformin, which
478 are detrimental to wildlife, as discussed previously. Among the detected pharmaceuticals
479 (Fig.2), carbamazepine was reported to negatively affect eggs viability, female gonads, and
480 metabolism in the zebrafish *Danio rerio* (Hamilton, 1822) (see Da Silva Santos et al., 2018),
481 but also steroid production and reproductive output (Fraz et al., 2019). Fluoxetine, which was
482 detected in these habitats, was reported to alter male mating strategy in the guppy fish *Poecilia*
483 *reticulata* (Peters, 1859) (see Fursdon et al., 2019). As in FOS₁, no amphibians were detected
484 in FOS₂ and FOS₃, which could be explained by the high diversity and concentrations of
485 pollutants in the water, but also by the presence of alien animal species such as *P. clarkii*.

486 The most disturbed habitats (level 3) encompassed both subsections of the Agouille de la
487 Mar River (AGO₁ and AGO₂). They were characterized by high concentrations of nutrients and
488 organic matter, resulting in eutrophication of the watercourse. Pesticides and pharmaceuticals
489 (caffeine, metformin, paracetamol, and theophylline) that were detected in AGO₁ could also be
490 the result of illegal discharges. Conversely, the high diversity and concentrations of
491 contaminants detected in AGO₂ most likely originated from wastewater effluents. Similar as in
492 FOS₂₊₃, the most concentrated pollutants in AGO₂ were the AMPA and metformin, both known
493 for their harmful effects on aquatic organisms. Carbamazepine, which can have detrimental
494 effects on the physiology and reproduction of *D. rerio* as mentioned previously (see Da Silva

495 Santos et al., 2018), was also detected in that river (Fig.2). In addition to pollutant discharges,
496 effluents may have dumped organic matter in the downstream subsection, as the Bages WWTP
497 has been exceeding its treatment limits for several years and is no longer able to adequately
498 treat incoming wastewater (Sandre, 2023). High organic matter pollution, which consumes
499 oxygen for oxidation processes, may induce low DO levels (Navarrete-Salgado et al., 2004)
500 and high nitrite concentrations (Roberts and Palmeiro, 2008), resulting in very poor water
501 quality (Appendix A; Légifrance, 2010). As in the Fosseille River, no amphibians were
502 detected, while the alien species *P. clarkii* was still observed.

503

504 *Correlation between turtle demographics and habitat quality*

505 Turtle densities in the Fosseille (63 to 68 adults.km⁻¹) and Agouille de la Mar (44 to 50
506 adults.km⁻¹) rivers were higher than in the Baillaury River (22 adults.km⁻¹), which was
507 unexpected because of the poor water quality reported downstream of the Fosseille River
508 (FOS₂₊₃) and in both subsections of the Agouille de la Mar River (AGO₁ and AGO₂). These
509 contrasting results were not due to differences in capture rates of turtles across the three rivers,
510 as capture rates in the Baillaury River were similar, even higher ($p = 0.26$ in May, 0.56 in June,
511 and 0.49 in July, 2021) than in the Fosseille ($p = 0.22$) and Agouille de la Mar ($p = 0.26$) rivers.
512 Moreover, these rates were consistent yet slightly lower than those reported for the European
513 pond turtle *Emys orbicularis* (Linnaeus, 1758) (see Olivier et al., 2010; Owen-Jones et al., 2016;
514 Escoriza et al., 2021). It should be mentioned that the Baillaury River consists of several river
515 sections, which are hydrologically interconnected. Therefore, the low turtle density in the
516 investigated section could be explained by temporary transitions between sections.

517 Suprisingly, the proportion of juveniles in the Baillaury River was almost twice higher
518 than that reported on the two subsections of the Agouille de la Mar River. In addition, the

519 proportion of juveniles was also higher upstream than downstream of the Fosseille River. The
520 decrease in juveniles proportion could be related to the level of habitat pollution: from 16-45%
521 of juveniles in habitats of level 1, to 12% in habitats of level 2, and 7-9% in habitats of level 3
522 (Table 1). Because capture rates were similar between the three rivers, this suggests poor
523 breeding condition of adults and/or higher juveniles mortalities in the most polluted rivers. In
524 line with this, El Hassani et al. (2019) reported low proportion of juveniles in highly polluted
525 habitats (8% of the captures) in comparison to pristine ones (34% of the captures) for the same
526 turtle species. A similar similar result was reported by Germano (2010) in the emydid *Actinemys*
527 *marmorata* (Baird and Girard, 1852) inhabiting sewage-treatment facilities (6-7% of the
528 captures). High reproductive outputs were reported in freshwater turtles from highly polluted
529 sites (Knight and Gibbons, 1968; Lindeman, 1996), including *M. leprosa* (Gahmous et al.,
530 2022), which suggests that high levels of pollution and eutrophication may have reduced the
531 survival of juveniles. Whereas high predation rates by birds or competition with *T. s. elegans*
532 may also impact survival of juveniles (Polo-Cavia et al., 2010), we did not assess these two
533 variables in the present study. However, because upstream and downstream subsections of the
534 Fosseille River are homogeneous in terms of riparian habitats, it is unlikely that differential
535 avian predation rates may explain differences in the proportion of juveniles. Therefore, these
536 differences are probably related to the quality of aquatic habitats.

537 *Correlation between phenotypic changes and habitat quality*

538 Our results showed that turtle growth rate and body size increased with habitat pollution. In the
539 least polluted habitats (BAI and FOS₁), the low growth rates could be related to chilly waters,
540 as observed in the freshwater turtle *A. marmorata* (Snover et al., 2015). The partial dryness of
541 the Baillaury River during drought could also adversely affect turtle growth, because of limited
542 food resources. Consistent with this, Powell et al. (2023) showed that drought could have slow
543 down growth of the freshwater turtle *C. picta*. The Baillaury River and the upstream subsection

544 of the Fosseille River appeared to be the least supportive habitats for turtle growth and body
545 size, due to chilly waters and altered flows. On the opposite, the high nutrient levels reported
546 in polluted and eutrophic habitats may have enhanced growth rate and body size (Fig.4 and 6).
547 Consistent with this, it has been shown that *C. picta* exhibited higher growth rates in polluted
548 lentic habitats (Knight and Gibbons, 1968) as well as in wastewater lagoons (Lindeman, 1996),
549 than in pristine ones. A similar result was also reported by Germano (2010) for *A. marmorata*.
550 While Knight and Gibbons (1968) already pointed out that high growth rates in polluted
551 environments could be the result of diet changes in *C. picta*, Sidis and Gasith (1995) illustrated
552 shifts from plant to detrital organic matter in the diet of the Caspian turtle *Mauremys caspica*
553 (Gmelin, 1774) when present in polluted habitats. The low growth rate of females observed in
554 the habitat of medium quality (FOS₂₊₃) may be related to turtles age and reproductive status, as
555 growth rate usually decreases after females become reproductive (Bury, 1979; Galbraith et al.,
556 1989) (Fig.6). Females may have stored more reserves to compensate for low growth rates,
557 resulting in higher SMI (Fig.5) (Ashton et al., 2015). Finally, the high SMI of juveniles reported
558 in the most disturbed habitats (Fig.5) could reflect a poor health status induced by pollution, as
559 reported earlier for the Central American river turtle *Dermatemys mawii* (Gray, 1847) (see
560 Rangel-Mendoza et al., 2014).

561

562 **Conclusion**

563 Our results highlighted different morphologies and population structures in *M. leprosa* in
564 relation to increased levels of pollution and eutrophication of aquatic habitats. Growth rate as
565 well as adult body size were the highest in the most polluted and eutrophic habitats. However,
566 juveniles were under-represented in such habitats, suggesting an issue with their survival or the
567 adult reproduction. Although differences in population structure may result from alterations in

568 water quality, additional factors such as predation and competition with invasive species, not
569 investigated here, could also have impacted the demography of *M. leprosa*.

570

571 **Credit author statement**

572 **Anne-Sophie Le Gal:** Conceptualization; Data curation; Methodology; Formal analysis;
573 Investigation; Writing - Original Draft; Writing - Review & Editing; Visualization; Funding
574 acquisition. **Jean-Yves Georges:** Conceptualization; Writing - Review & Editing; Supervision.
575 **Christine Sotin:** Investigation. **Bruno Charrière:** Investigation. **Olivier Verneau:**
576 Conceptualization; Writing - Review & Editing; Supervision; Project administration; Funding
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587

588 **Competition and interests**

589 The authors declare that they have no known competing financial interests or personal
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591

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894

895 **Supplementary Materials**

896 **Supplementary Material A.** Physical and chemical water parameters assessed in 2021 in the
897 upstream (FOS₁), outfall (FOS₂), and downstream (FOS₃) wastewater treatment plant (WWTP)
898 subsections of the Fosseille River; in the upstream (AGO₁) and downstream (AGO₂) WWTP
899 subsections of the Agouille de la Mar River; in the upper (BAI₁) and lower (BAI₂) sections of
900 the Baillaury River. Colors indicate the ecological quality of each parameter, from blue to red:
901 very good, good, average, poor (Légifrance, 2010).

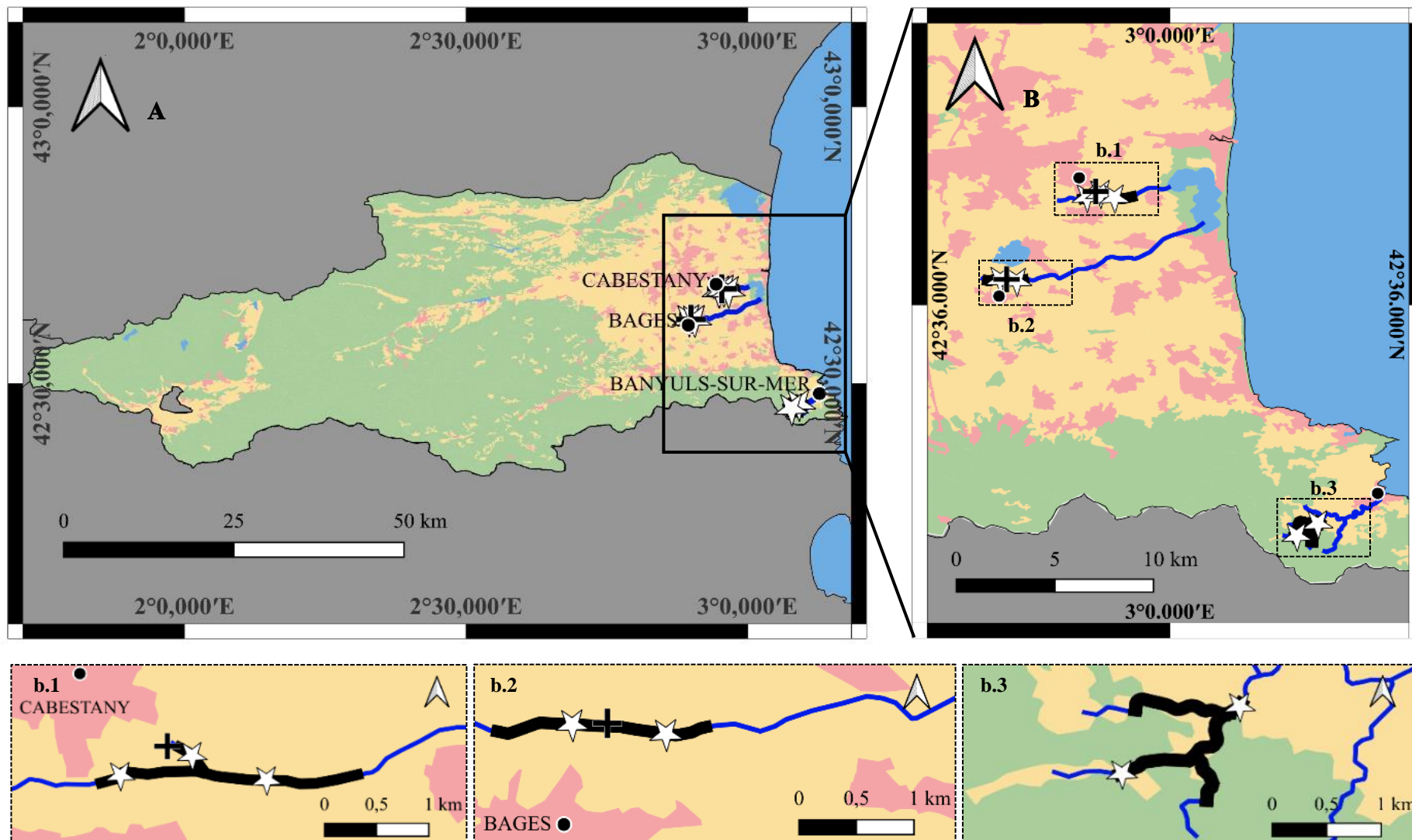
902 **Supplementary Material B.** Body mass (BM) of juveniles (a), males (b), and females (c) along
903 the investigated rivers: Baillaury River (BAI); upstream (FOS₁) and downstream (FOS₂₊₃)
904 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO₁) and
905 downstream (AGO₂) WWTP subsections of the Agouille de la Mar River. Each color represents
906 a quality level, from blue to red. Pairwise Wilcoxon tests p-values are indicated (alpha = 5% *;
907 1% **). The median of each group is displayed as a bold vertical line, and the mean of each
908 group as a black dot.

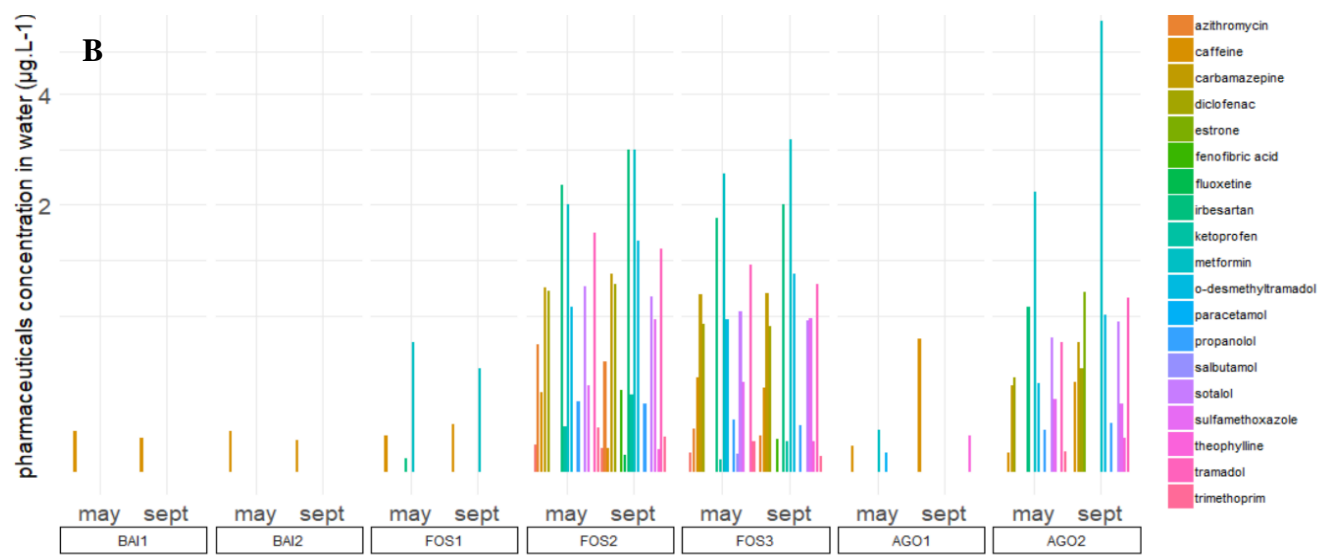
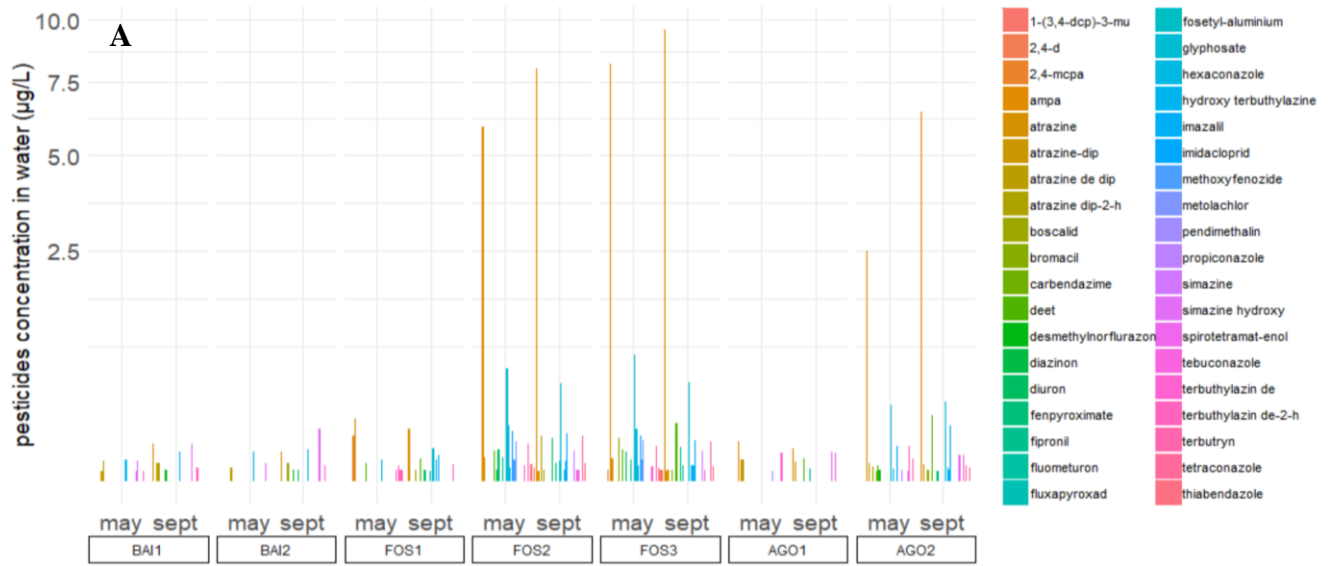
909 **Supplementary Material C.** Growth rates in males (a) and in females (b), relative to their body
910 size. A unique symbol is displayed for each river subsection: Baillaury River (BAI); upstream
911 (FOS₁) and downstream (FOS₂₊₃) wastewater treatment plant (WWTP) subsections of the
912 Fosseille River; upstream (AGO₁) and downstream (AGO₂) WWTP subsections of the Agouille
913 de la Mar River.

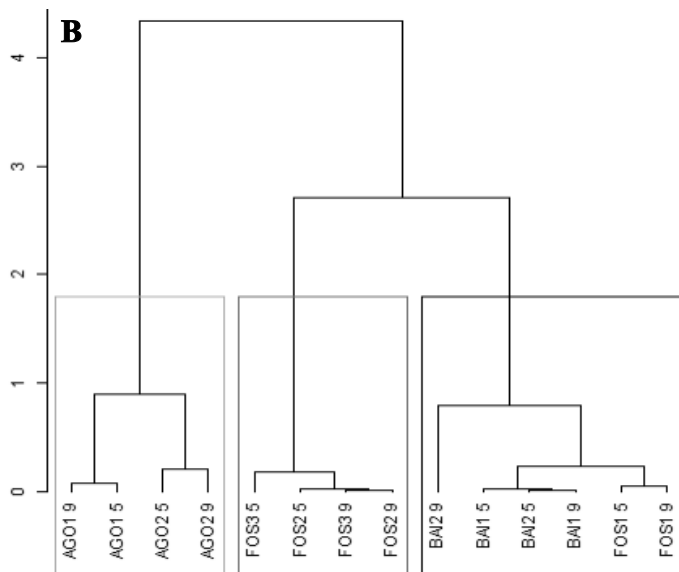
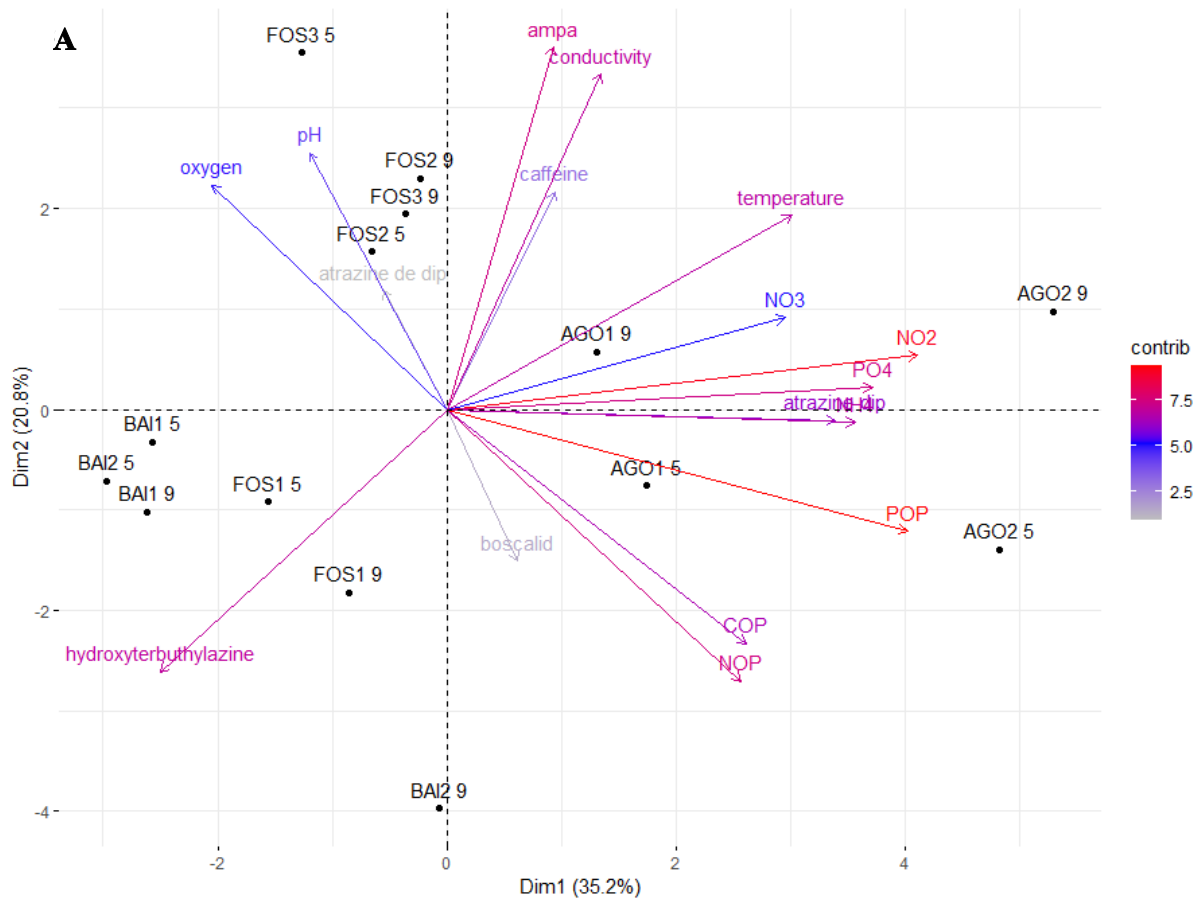
Table 1. Captures composition (males MAL, females FEM, and juveniles JUV) and operational sex ratio (OSR) of *M. leprosa* along the Baillaury River (BAI), the Fosseille River (FOS), and the Agouille de la Mar River (AGO). The total number of captures is indicated, with the number of distinct individuals shown in brackets.

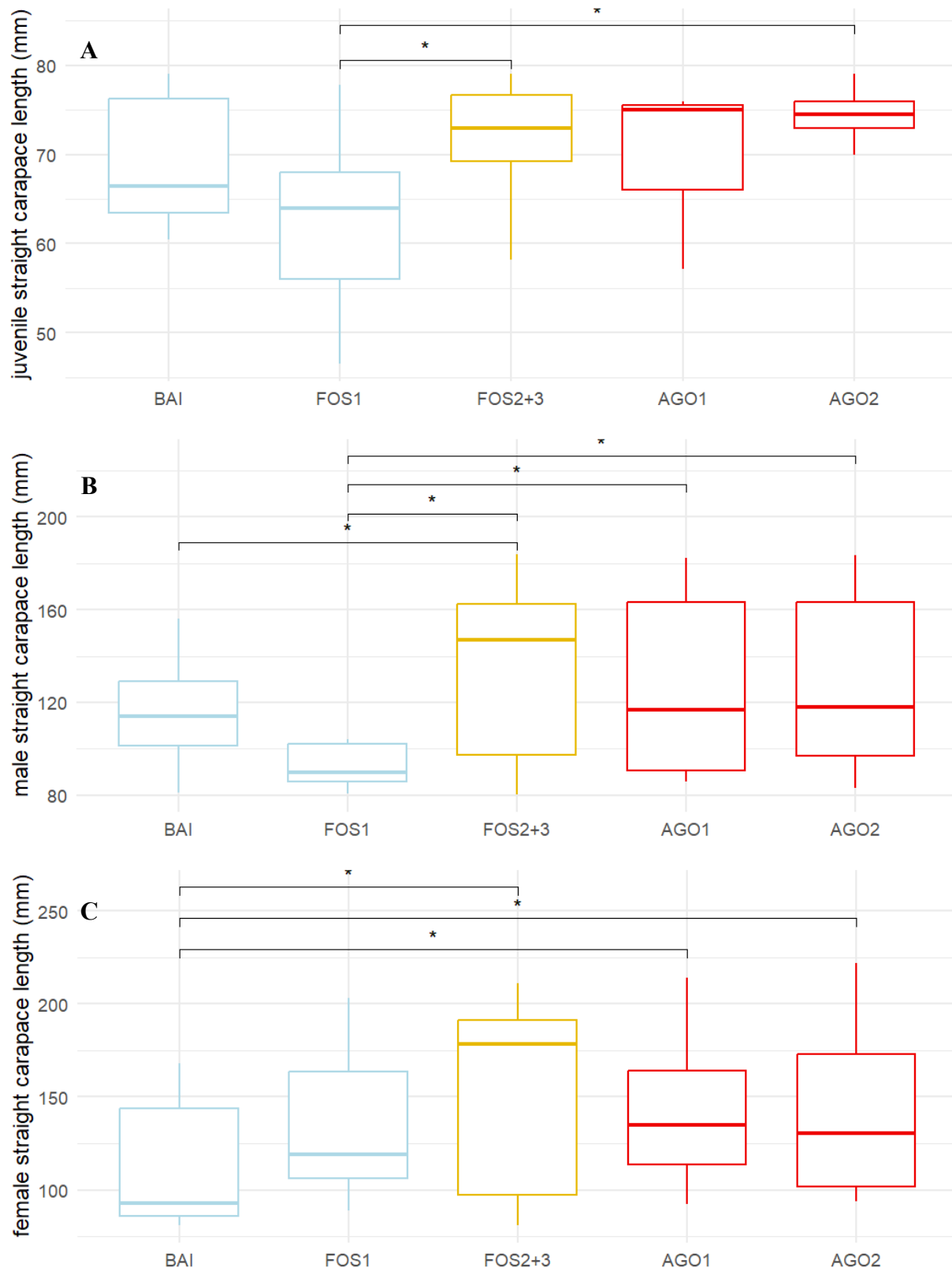
		MAL	FEM	JUV	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS ₁)	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS ₂₊₃)	172 (91)	39 (28)	28 (16)	239 (135)	0.88	0.76
	Full river	201 (101)	65 (39)	65 (33)	331 (173)	0.81	0.72
AGO	Upstream (AGO ₁)	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO ₂)	32 (23)	31 (18)	4 (4)	67 (45)	0.91	0.56
	Full river	71 (48)	53 (34)	8 (7)	132 (89)	0.92	0.59

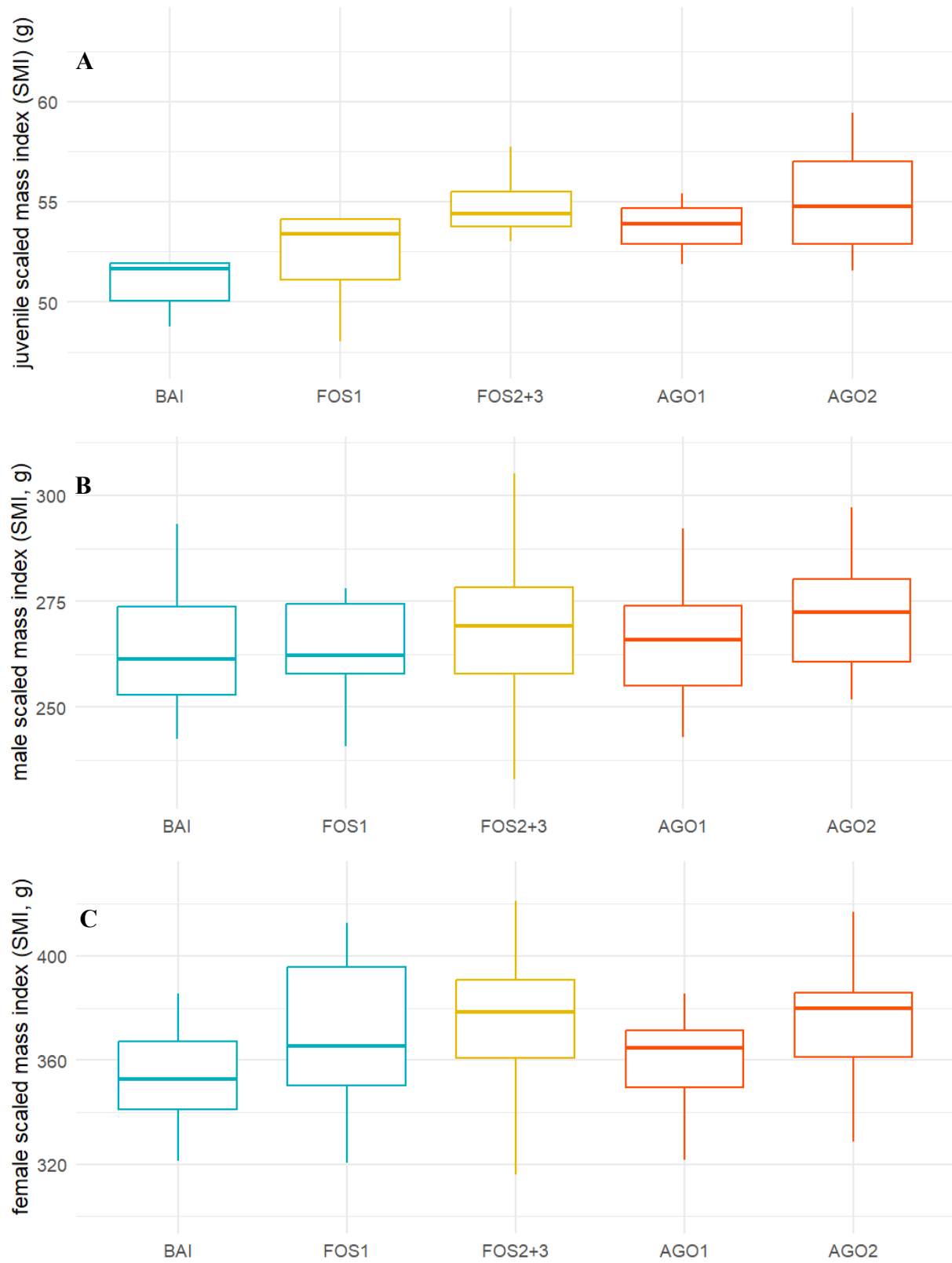
Figure 1

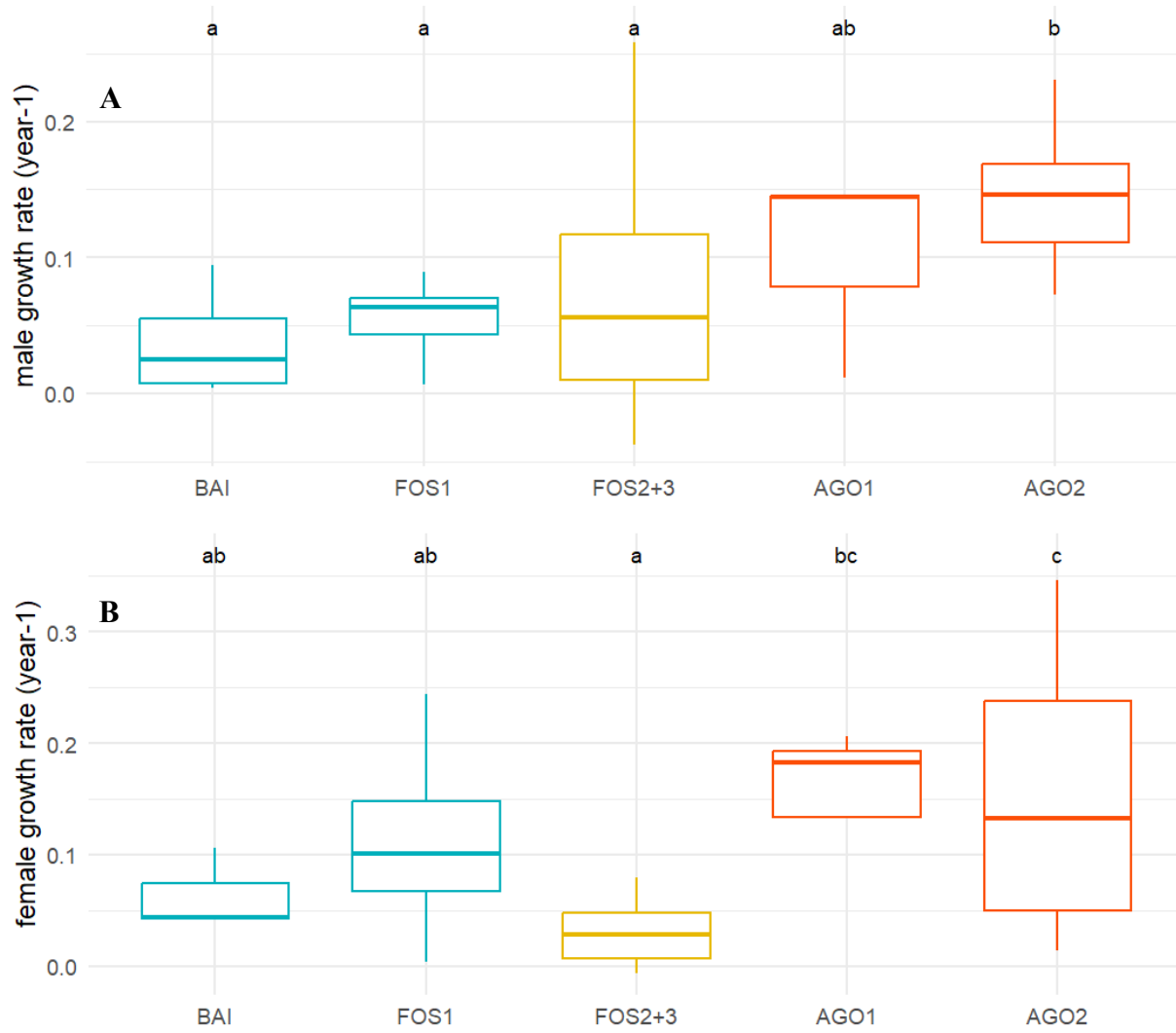














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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: