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Anne-Sophie Le Gal, Jean-Yves Georges, Christine Sotin, Bruno Charrière,  
Olivier Verneau

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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 <sup>a,b,c</sup>, Jean-Yves Georges <sup>c</sup>, Christine Sotin <sup>a,b</sup>, Bruno Charrière <sup>a,b</sup>, Olivier  
6 Verneau <sup>a,b,d</sup>

7 <sup>a</sup> 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 <sup>b</sup> CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens, UMR  
11 5110, 52 Avenue Paul Alduy, F-66860 Perpignan cedex, France

12 <sup>c</sup> Université de Strasbourg, CNRS, IPHC UMR 7178, 23 rue du loess, 67000 Strasbourg, France

13 <sup>d</sup> 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](mailto: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 Southwest 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).

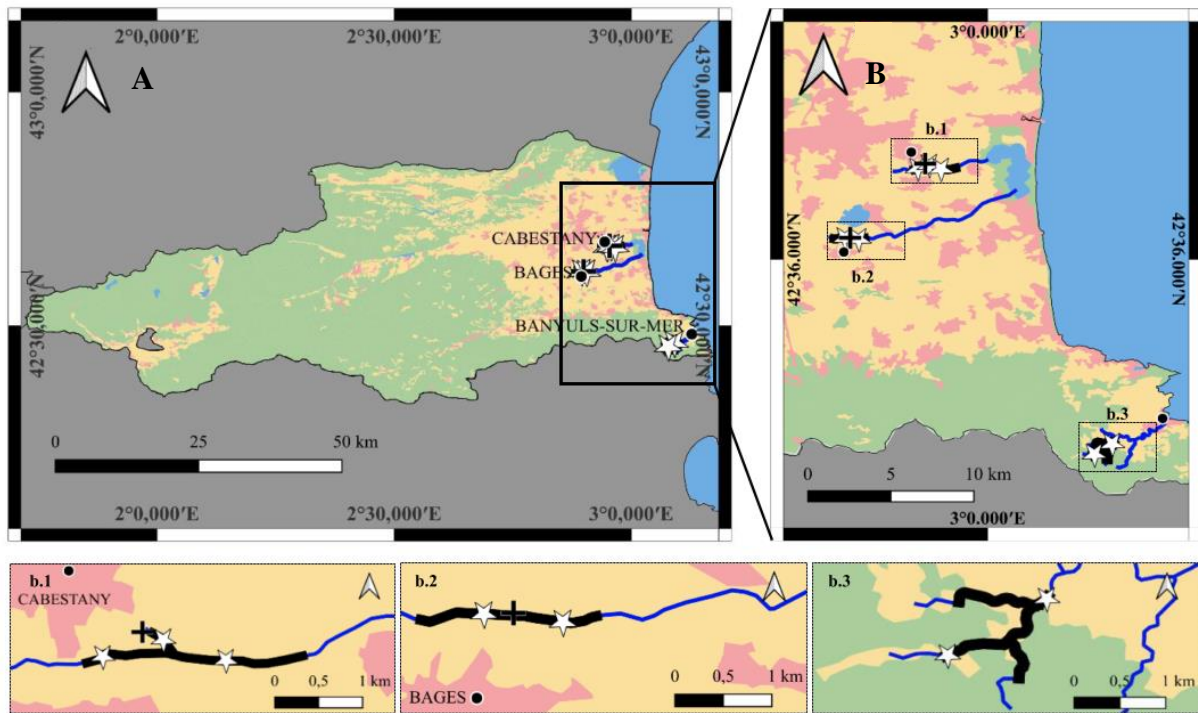
120 *Mauremys leprosa* ~~It~~ can ~~indeed~~ tolerate eutrophic waters (da Silva, 1995; Franch,  
121 2003), and has even been reported in highly polluted habitats (Naimi et al., 2012~~El Hassani et~~  
122 ~~al., 2019~~; Héritier et al., 2017; El Hassani et al., 2019~~Naimi et al., 2012~~). El Hassani 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 km<sup>2</sup>, 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 km<sup>2</sup>, 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 km<sup>2</sup>, 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 (BAI<sub>1</sub>: N 42°27'25" E 3°4'57") and lower (BAI<sub>2</sub>:  
240 N 42°27'45" E 3°5'26") reaches. On the Fosseille River, point sampling was done on the  
241 upstream (FOS<sub>1</sub>: N 42°40'2" E 2°56'53"), outfall (FOS<sub>2</sub>: N 42°40'11" E 2°57'26") and  
242 downstream (FOS<sub>3</sub>: 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 (AGO<sub>1</sub>: N 42°36'57.5" E 2°53'41") and  
244 downstream (AGO<sub>2</sub>: 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<sup>-1</sup>), and conductivity (σ, in μS.cm<sup>-1</sup>),  
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<sup>-1</sup>) 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<sup>-1</sup>), 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<sup>-1</sup>,  
256 1 ng.L<sup>-1</sup>, and 2 ng.L<sup>-1</sup> for POC, PON, and POP, respectively.

257

258 Nutrients, including nitrite ( $\text{NO}_2^-$ , in  $\text{mg.L}^{-1}$ ), ammonium ( $\text{NH}_4^+$ , in  $\text{mg.L}^{-1}$ ), nitrate  
259 ( $\text{NO}_3^-$ , in  $\text{mg.L}^{-1}$ ), and orthophosphate ( $\text{PO}_4^{3-}$ , in  $\text{mg.L}^{-1}$ ), 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  $\text{NO}_2^-$  and  $\text{NH}_4^+$  ( $0.01 \text{ mg.L}^{-1}$ ), the liquid  
264 chromatography assay for  $\text{NO}_3^-$  ( $0.2 \text{ mg.L}^{-1}$ ), and the spectrophotometry for  $\text{PO}_4^{3-}$  ( $0.02 \text{ mg.L}^{-1}$ ).  
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  $\text{BAI}_1$  and  $\text{BAI}_2$  of the Baillaury River, between  $\text{FOS}_1$  and  $\text{FOS}_2$ ,  $\text{FOS}_1$  and  $\text{FOS}_3$ , and  $\text{FOS}_2$  and  
276  $\text{FOS}_3$  of the Fosseille River, and between  $\text{AGO}_1$  and  $\text{AGO}_2$  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<sup>-1</sup>), 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 BAI<sub>1</sub>, and eight in BAI<sub>2</sub>. The pesticides  
358 with the highest concentrations were the hydroxy simazine (0.10 µg.L<sup>-1</sup>) and the AMPA (0.06  
359 µg.L<sup>-1</sup>). 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<sup>-1</sup>. 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<sup>-1</sup>) and the metformin (3.1 µg.L<sup>-1</sup>). 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<sup>-1</sup>) and the metformin (5.7 µg.L<sup>-1</sup>). 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 (BAI<sub>1</sub> and BAI<sub>2</sub>) and the upstream subsection of the Fosseille River (FOS<sub>1</sub>) present a  
387 low level of pollution and are classified as the first level; (ii) the outfall (FOS<sub>2</sub>) and the  
388 downstream (FOS<sub>3</sub>) 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 (AGO<sub>1</sub> and AGO<sub>2</sub>)  
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 (AGO<sub>2</sub>) from the Baillaury River section (BAI<sub>1</sub> and BAI<sub>2</sub>) (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* AGO<sub>2</sub>:  $0.009 \pm 0.001 \mu\text{g.L}^{-1}$ ), low  
396 conductivity ( $488.00 \pm 51.56 \mu\text{S.cm}^{-1}$ ; *versus* AGO<sub>2</sub>:  $990.00 \pm 22.00 \mu\text{S.cm}^{-1}$ ), and low  
397 temperature ( $17.78 \pm 0.48 \text{ }^\circ\text{C}$ ; *versus* AGO<sub>2</sub>:  $23.15 \pm 1.15 \text{ }^\circ\text{C}$ ) (Fig.3). AGO<sub>2</sub> 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 NO<sub>2</sub>- ( $0.49 \pm 0.09 \mu\text{g.L}^{-1}$ ; *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 (FOS<sub>2</sub>) and the downstream (FOS<sub>3</sub>) subsections  
403 of the Fosseille River from the Baillaury River section (BAI<sub>1</sub> and BAI<sub>2</sub>) (Fig.3). FOS<sub>2</sub> and FOS<sub>3</sub>  
404 were characterized by high conductivity ( $1412.00 \pm 78.33 \mu\text{S.cm}^{-1}$ ; *versus* BAI:  $488.00 \pm 51.56$

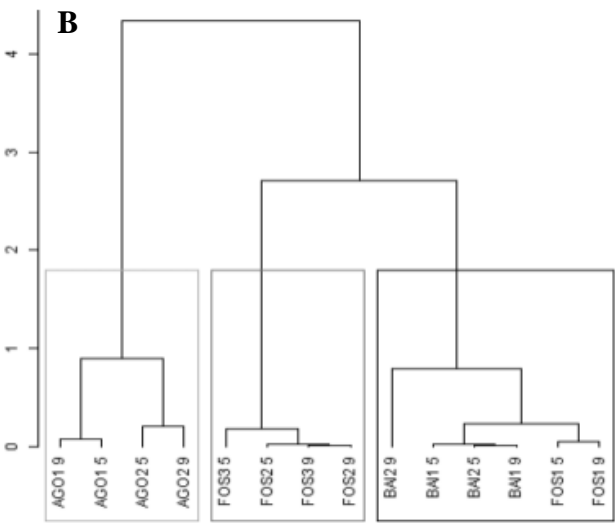
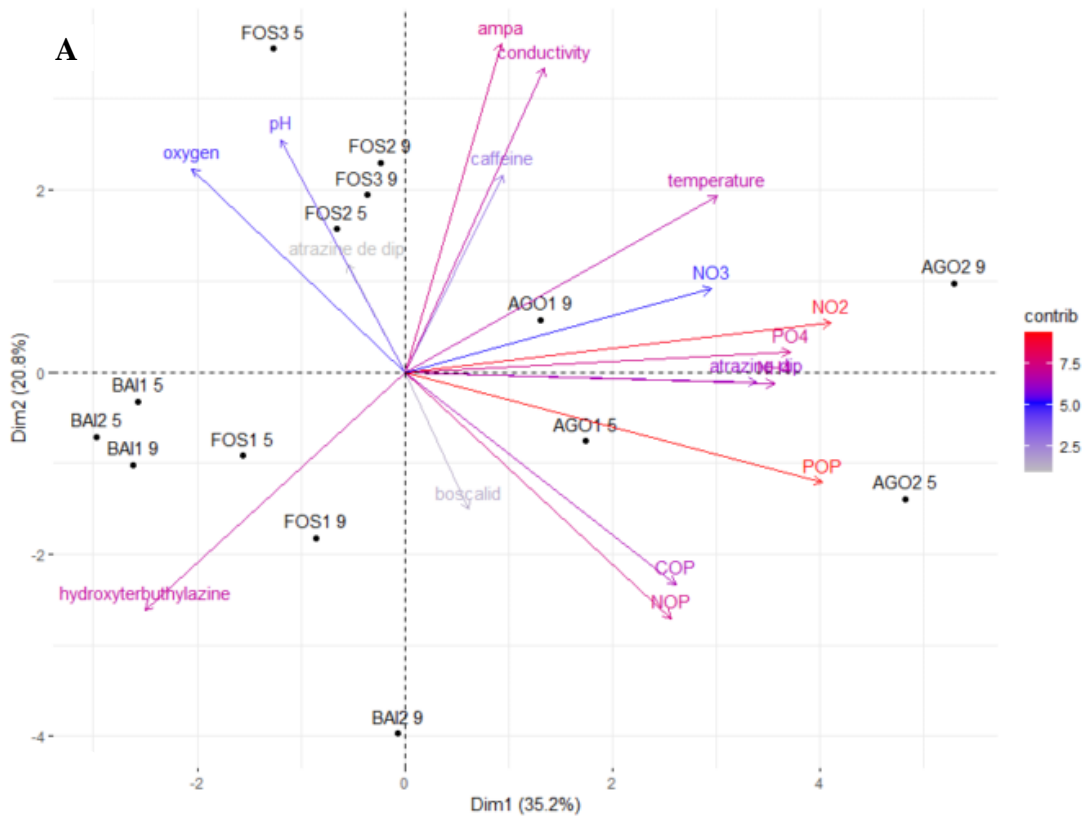
405  $\mu\text{S}\cdot\text{cm}^{-1}$ ), high concentrations of AMPA ( $7.93 \pm 0.76 \mu\text{g}\cdot\text{L}^{-1}$ ; *versus* BAI:  $0.028 \pm 0.017 \mu\text{g}\cdot\text{L}^{-1}$ )  
406  $^1$ ), and high concentrations of caffeine ( $0.17 \pm 0.05 \mu\text{g}\cdot\text{L}^{-1}$ ; *versus* BAI:  $0.040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

407         Because samples collected in BAI<sub>1</sub> and BAI<sub>2</sub> 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 FOS<sub>2</sub> and FOS<sub>3</sub> did not show significant differences in pollution levels,  
410 they were considered as a single subsection named hereafter FOS<sub>2+3</sub>. Five subsections were  
411 therefore considered for population structure analyses, namely BAI, FOS<sub>1</sub>, FOS<sub>2+3</sub>, AGO<sub>1</sub>, and  
412 AGO<sub>2</sub>.



413

414 **Figure 2.** Concentration of (A) pesticides and (B) pharmaceuticals detected in the water. BAI<sub>1</sub>  
 415 and BAI<sub>2</sub> represent the upper and lower water sampling points on the Baillaury River section;  
 416 FOS<sub>1</sub>, FOS<sub>2</sub>, and FOS<sub>3</sub> represent respectively the upstream, outfall, and downstream  
 417 subsections of the Fosseille River; AGO<sub>1</sub> and AGO<sub>2</sub> 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 FOS<sub>2+3</sub>  
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 FOS<sub>1</sub>, where it was balanced at 0.48 (Table 1). The adult population  
443 of the Baillaury River consisted of  $45 \pm 5$  turtles (95% CI = [40;64]) and had a density of 27  
444 turtles.km<sup>-1</sup>. On the Fosseille River, the adult population consisted of  $157 \pm 24$  turtles (95% CI  
445 = [121;223]) and density ranged from 63 to 68 turtles.km<sup>-1</sup>, 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<sup>-1</sup>, 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 FOS<sub>1</sub>), 12% in habitat of level 2 (FOS<sub>2+3</sub>), and 7-9% in  
450 habitats of level 3 (AGO<sub>1</sub> and AGO<sub>2</sub>).

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	<del>IMMJU</del> <u>V</u>	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS <sub>1</sub> )	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS <sub>2+3</sub> )	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 <sub>1</sub> )	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO <sub>2</sub> )	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

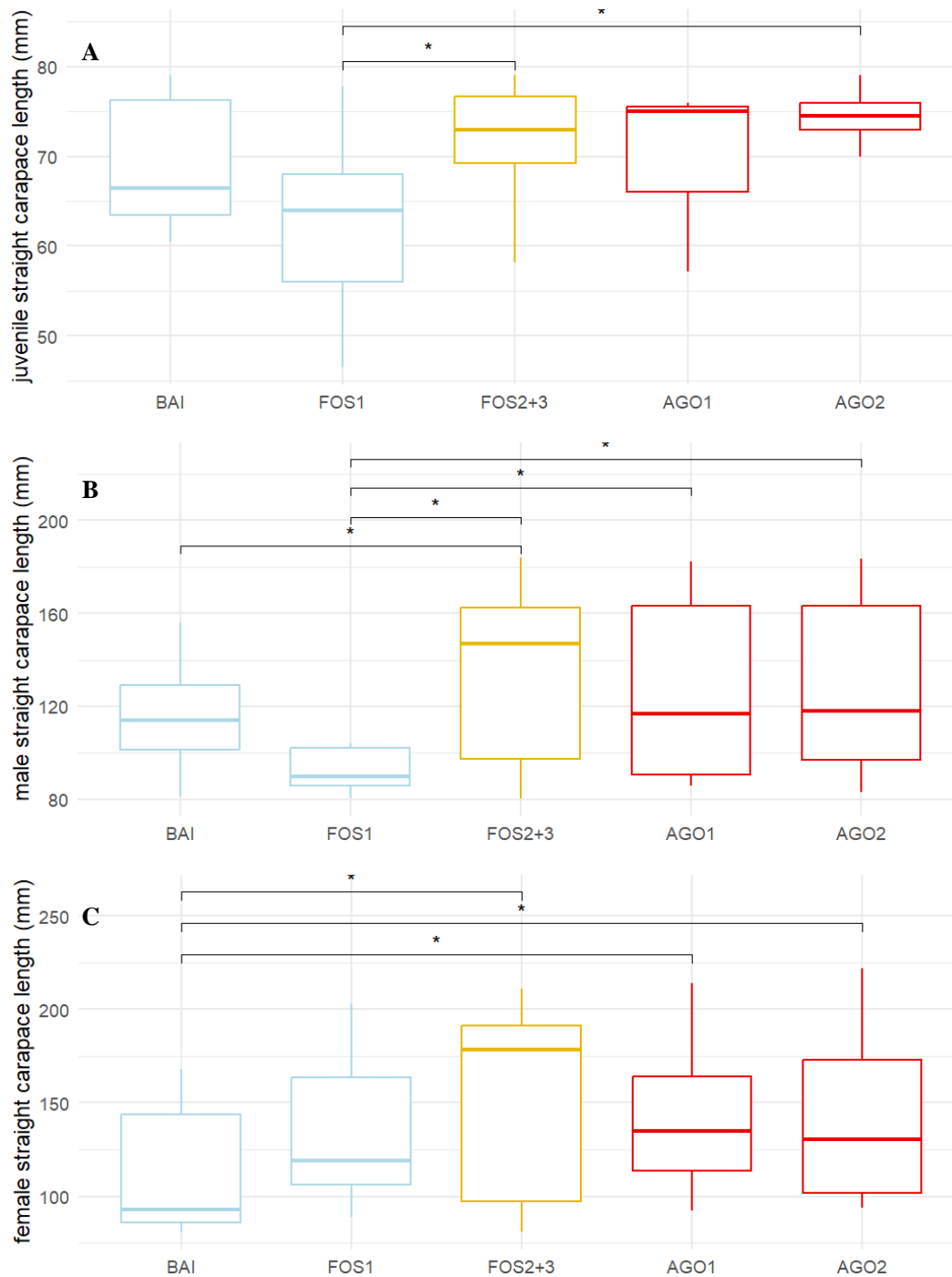
### 458 3.4. Turtles body metrics

459 Body size (SCL) and mass (BM) of adults were different across the five river subsections, i.e.,  
 460 BAI, FOS<sub>1</sub>, FOS<sub>2+3</sub>, AGO<sub>1</sub>, and AGO<sub>2</sub> (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 FOS<sub>2+3</sub> 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).

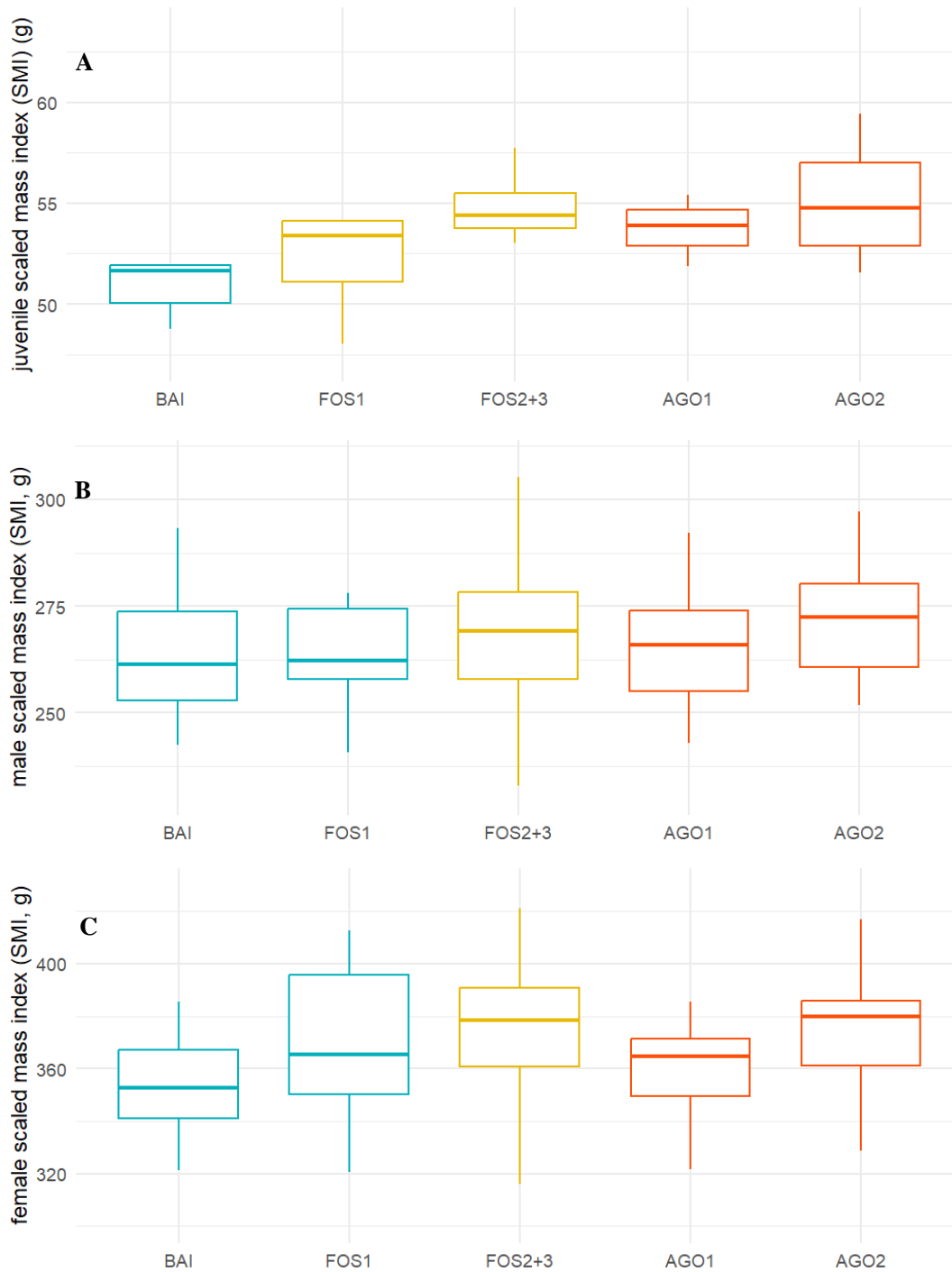
474 Growth rates were different across the five river subsections ~~sections~~ (Fig.6) and also  
475 differed according to turtle body size. Differences were significant in males (n = 172; ANOVA;  
476 SCL:  $F_{1,77} = 12.98$ ,  $p = 5.55e-04$ ; River subsection ~~section~~:  $F_{4,77} = 4.27$ ,  $p = 3.58e-03$ ; 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 (AGO<sub>1</sub> and AGO<sub>2</sub>) than in other habitats. However, females  
480 grew slower in the habitat of level 2 (FOS<sub>2+3</sub>) 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).





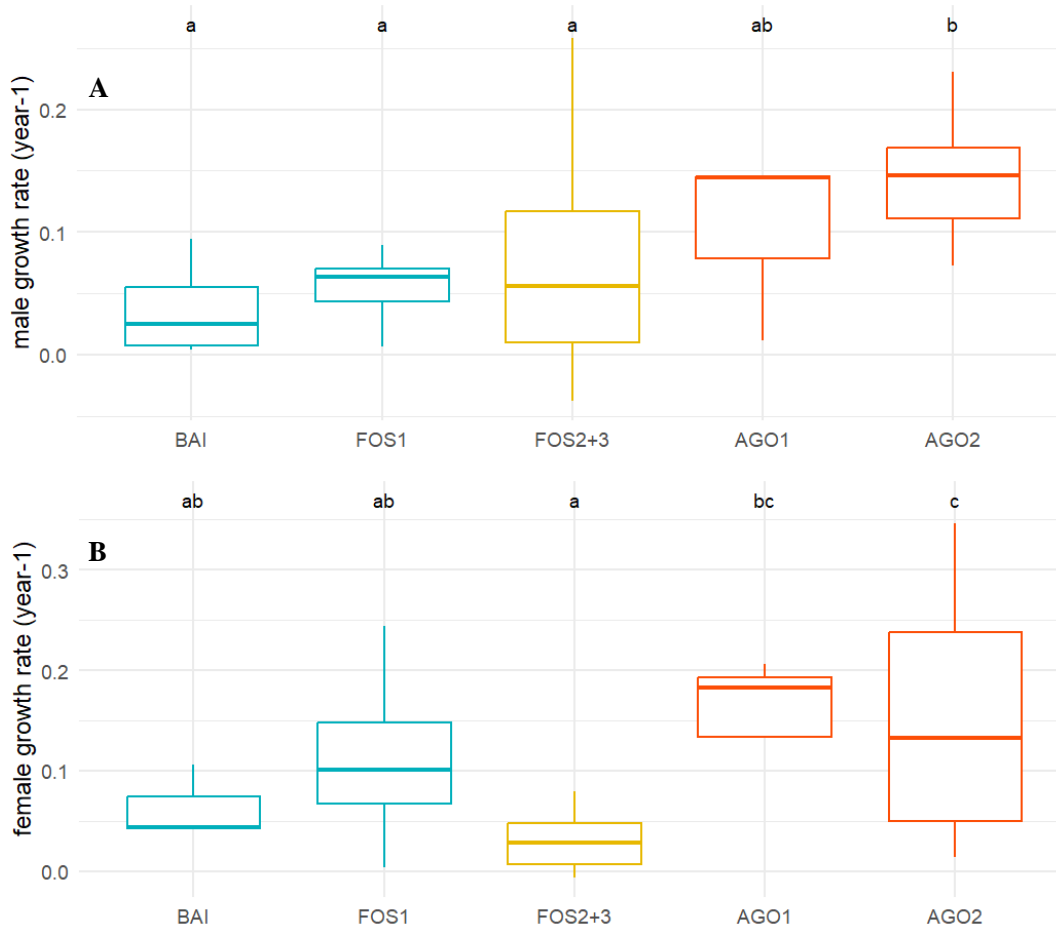
485

486 **Figure 4.** Body size of immature juveniles (A), males (B), and females (C) along the  
 487 investigated rivers: Baillaury River (BAI); upstream (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>)  
 488 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and  
 489 downstream (AGO<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>)  
 495 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and  
 496 downstream (AGO<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>) wastewater treatment plant (WWTP)  
 501 subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) 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 (BAI<sub>1</sub> and BAI<sub>2</sub>)  
508 and the upstream subsection of the Fosseille River (FOS<sub>17</sub>), BAI<sub>4</sub>, and BAI<sub>2</sub> that which  
509 exhibited the lowest diversity and concentrations of ~~pollutants~~ contaminants, ~~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 FOS<sub>1</sub> was higher than~~  
523 ~~in BAI<sub>1</sub> and BAI<sub>2</sub>, FOS<sub>1</sub> 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 amphibians ~~bio-indicators~~ such as the amphibians, ~~such as P.~~

530 *perezi* and *B. spinosus* ~~on the Baillaury River, which are absent on FOS<sub>1</sub> 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 FOS<sub>1</sub> was higher than in the Baillaury section, suggesting~~  
536 ~~that this subsection's quality was lower than that of the Baillaury River. FOS<sub>1</sub> 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 (FOS<sub>2</sub>)  
543 and the downstream WWTP (FOS<sub>3</sub>) subsections of the Fosseille River ~~(FOS<sub>2</sub> and FOS<sub>3</sub>)~~.  
544 They ~~it~~ 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 FOS<sub>1</sub>, no amphibians were  
561 detected in FOS<sub>2</sub> and FOS<sub>3</sub>, 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 (AGO<sub>1</sub> and AGO<sub>2</sub>). 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~~ AGO<sub>1</sub> 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 AGO<sub>2</sub> most likely originated from wastewater effluents. Similar  
570 as in FOS<sub>2+3</sub>, the most concentrated pollutants in AGO<sub>2</sub> 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<sup>-1</sup>) and Agouille de la Mar (44 to 50  
587 adults.km<sup>-1</sup>) rivers were higher than in the Baillaury River (22 adults.km<sup>-1</sup>), which was  
588 unexpected because of the ~~low-poor~~ water quality reported downstream of the Fosseille River  
589 (FOS<sub>2+3</sub>) and in both subsections of the Agouille de la Mar River (AGO<sub>1</sub> and AGO<sub>2</sub>). 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 ~~immaturejuveniles~~ 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 ~~immaturejuveniles~~ 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 ~~immaturejuveniles~~ 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~~ ~~h~~ 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 FOS<sub>1</sub>), 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 (FOS<sub>2+3</sub>) 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~~-immature juveniles 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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665

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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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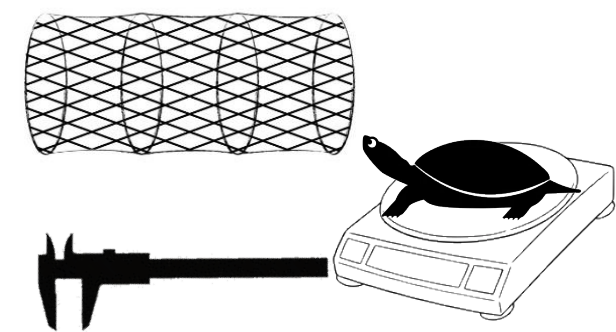
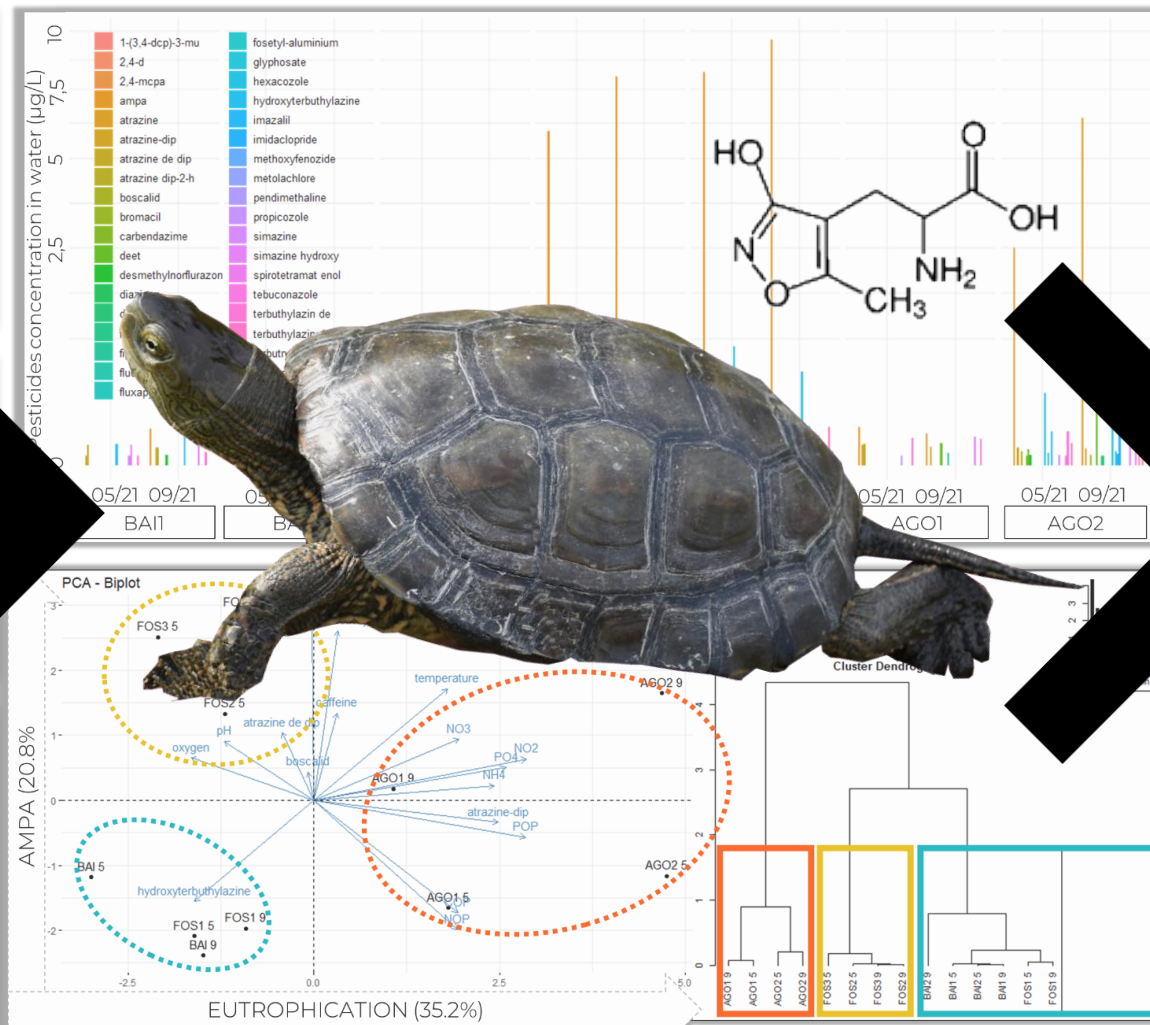
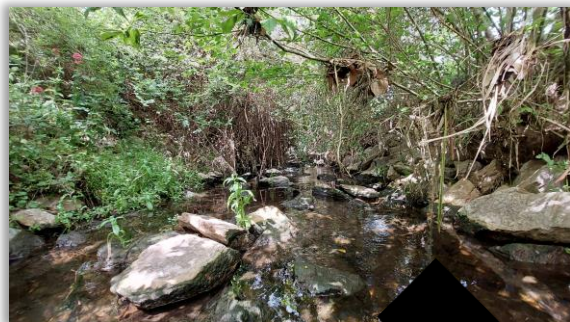
## 988 **Supplementary Materials**

989 **Supplementary Material A.** Physical and chemical water parameters assessed in 2021 in the  
990 upstream (FOS<sub>1</sub>), outfall (FOS<sub>2</sub>), and downstream (FOS<sub>3</sub>) wastewater treatment plant (WWTP)  
991 subsections of the Fosseille River; in the upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) WWTP  
992 subsections of the Agouille de la Mar River; in the upper (BAI<sub>1</sub>) and lower (BAI<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream  
997 (FOS<sub>2+3</sub>) wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream  
998 (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>) wastewater treatment plant (WWTP) subsections of the  
1005 Fosseille River; upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) WWTP subsections of the Agouille  
1006 de la Mar River.



Water samplings



Levels of habitat quality



Population structure and turtle's phenotype

- 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 <sup>a,b,c</sup>, Jean-Yves Georges <sup>c</sup>, Christine Sotin <sup>a,b</sup>, Bruno Charrière <sup>a,b</sup>, Olivier  
6 Verneau <sup>a,b,d</sup>

7 <sup>a</sup> 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 <sup>b</sup> CNRS, Centre de Formation et de Recherche sur les Environnements Méditerranéens, UMR  
11 5110, 52 Avenue Paul Alduy, F-66860 Perpignan cedex, France

12 <sup>c</sup> Université de Strasbourg, CNRS, IPHC UMR 7178, 23 rue du lœss, 67000 Strasbourg, France

13 <sup>d</sup> 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](mailto: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).

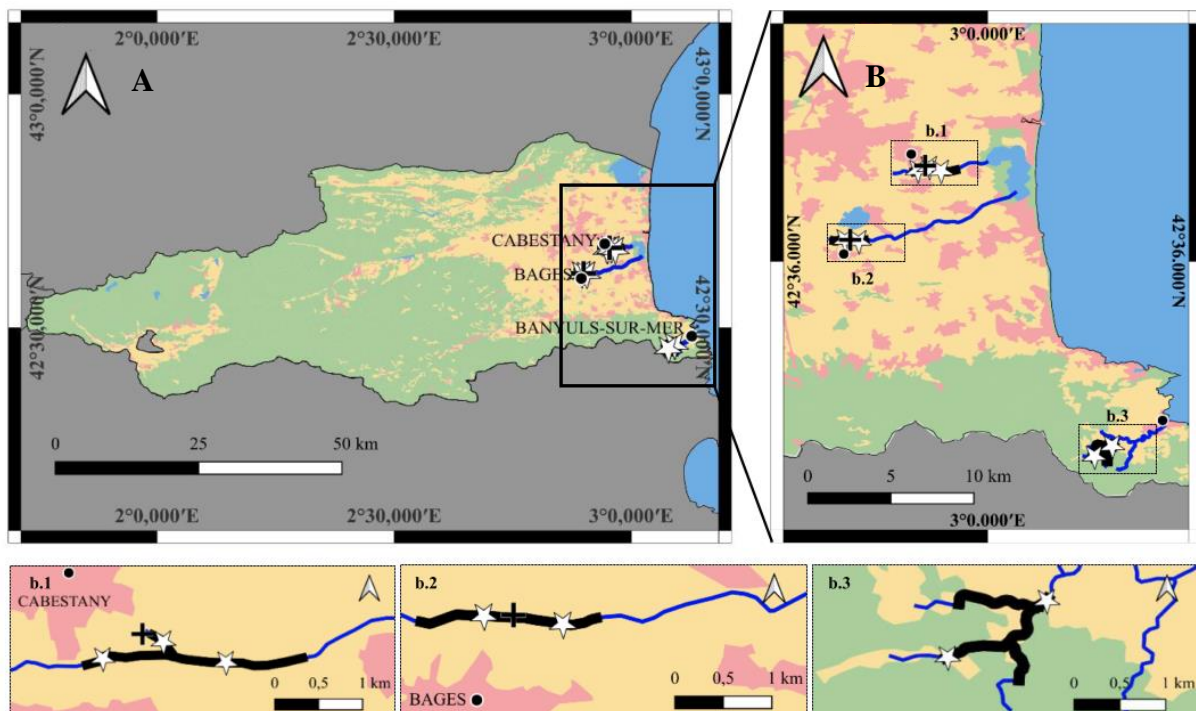
115 *Mauremys leprosa* can tolerate eutrophic waters (da Silva, 1995; Franch, 2003), and has  
116 even been reported in highly polluted habitats (Naimi et al., 2012; Hérítier et al., 2017; El  
117 Hassani et al., 2019). El Hassani et al. (2019) showed that turtles, in Northwestern Morocco,  
118 could 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 (BAI<sub>1</sub>: N 42°27'25" E 3°4'57") and lower (BAI<sub>2</sub>:  
192 N 42°27'45" E 3°5'26") reaches. On the Fosseille River, point sampling was done on the  
193 upstream (FOS<sub>1</sub>: N 42°40'2" E 2°56'53"), outfall (FOS<sub>2</sub>: N 42°40'11" E 2°57'26") and  
194 downstream (FOS<sub>3</sub>: 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 (AGO<sub>1</sub>: N 42°36'57.5" E 2°53'41") and  
196 downstream (AGO<sub>2</sub>: 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<sup>-1</sup>), and conductivity ( $\sigma$ , in  $\mu\text{S}\cdot\text{cm}^{-1}$ )  
198 <sup>1</sup>), were measured *in natura* with a HQ40D Portable Meter (Hach).

199 Water samples were filtered on 0.45  $\mu\text{m}$  (47 mm diameter) MF-Millipore membrane  
200 filters to quantify suspended particulate matter (SPM, in mg.L<sup>-1</sup>) at the Centre de Formation et  
201 de Recherche sur les Environnements Méditerranéens, and on 0.7  $\mu\text{m}$  (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<sup>-1</sup>), 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<sup>-1</sup>, 1 ng.L<sup>-1</sup>, and 2 ng.L<sup>-1</sup> for POC,  
208 PON, and POP, respectively.

209 Nutrients, including nitrite (NO<sub>2</sub><sup>-</sup>, in mg.L<sup>-1</sup>), ammonium (NH<sub>4</sub><sup>+</sup>, in mg.L<sup>-1</sup>), nitrate  
210 (NO<sub>3</sub><sup>-</sup>, in mg.L<sup>-1</sup>), and orthophosphate (PO<sub>4</sub><sup>3-</sup>, in mg.L<sup>-1</sup>), as well as pesticides (N = 477  
211 molecules ; in  $\mu\text{g}\cdot\text{L}^{-1}$ ) and pharmaceuticals (N = 23 molecules ; in  $\mu\text{g}\cdot\text{L}^{-1}$ ), 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  $\text{NO}_2^-$  and  $\text{NH}_4^+$  ( $0.01 \text{ mg.L}^{-1}$ ), the liquid  
215 chromatography assay for  $\text{NO}_3^-$  ( $0.2 \text{ mg.L}^{-1}$ ), and the spectrophotometry for  $\text{PO}_4^{3-}$  ( $0.02 \text{ mg.L}^{-1}$ ). 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.  
221

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  $\text{BAI}_1$  and  $\text{BAI}_2$  of the Baillaury River, between  $\text{FOS}_1$  and  $\text{FOS}_2$ ,  $\text{FOS}_1$  and  $\text{FOS}_3$ , and  $\text{FOS}_2$  and  
227  $\text{FOS}_3$  of the Fosseille River, and between  $\text{AGO}_1$  and  $\text{AGO}_2$  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<sup>-1</sup>), 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 BAI<sub>1</sub>, and eight in BAI<sub>2</sub>. 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,  $\chi^2 = 1.78$ ,  $\text{df} = 3$ ,  $p = 0.62$ ).

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,  $\chi^2 = 44.35$ ,  $df = 5$ ,  $p = 1.97\text{e-}08$ ).

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  $df = 3$ ,  $p = 2.06\text{e-}05$ ).

325

### 326 *3.2. Levels of water quality*

327 The hierarchical clustering revealed three levels of habitat quality: (i) the Baillaury River  
328 section ( $\text{BAI}_1$  and  $\text{BAI}_2$ ) and the upstream subsection of the Fosseille River ( $\text{FOS}_1$ ) present a  
329 low level of pollution and are classified as the first level; (ii) the outfall ( $\text{FOS}_2$ ) and the  
330 downstream ( $\text{FOS}_3$ ) 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 ( $\text{AGO}_1$  and  $\text{AGO}_2$ )

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 (AGO<sub>2</sub>) from the Baillaury River section (BAI<sub>1</sub> and BAI<sub>2</sub>) (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* AGO<sub>2</sub>:  $0.009 \pm 0.001 \mu\text{g.L}^{-1}$ ), low  
338 conductivity ( $488.00 \pm 51.56 \mu\text{S.cm}^{-1}$ ; *versus* AGO<sub>2</sub>:  $990.00 \pm 22.00 \mu\text{S.cm}^{-1}$ ), and low  
339 temperature ( $17.78 \pm 0.48 \text{ }^\circ\text{C}$ ; *versus* AGO<sub>2</sub>:  $23.15 \pm 1.15 \text{ }^\circ\text{C}$ ) (Fig.3). AGO<sub>2</sub> 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 NO<sub>2</sub>- ( $0.49 \pm 0.09 \mu\text{g.L}^{-1}$ ; *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 (FOS<sub>2</sub>) and the downstream (FOS<sub>3</sub>) subsections  
345 of the Fosseille River from the Baillaury River section (BAI<sub>1</sub> and BAI<sub>2</sub>) (Fig.3). FOS<sub>2</sub> and FOS<sub>3</sub>  
346 were characterized by high conductivity ( $1412.00 \pm 78.33 \mu\text{S.cm}^{-1}$ ; *versus* BAI:  $488.00 \pm 51.56$   
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 <sup>1</sup>), 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 BAI<sub>1</sub> and BAI<sub>2</sub> 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 FOS<sub>2</sub> and FOS<sub>3</sub> did not show significant differences in pollution levels,  
352 they were considered as a single subsection named hereafter FOS<sub>2+3</sub>. Five subsections were  
353 therefore considered for population structure analyses, namely BAI, FOS<sub>1</sub>, FOS<sub>2+3</sub>, AGO<sub>1</sub>, and  
354 AGO<sub>2</sub>.

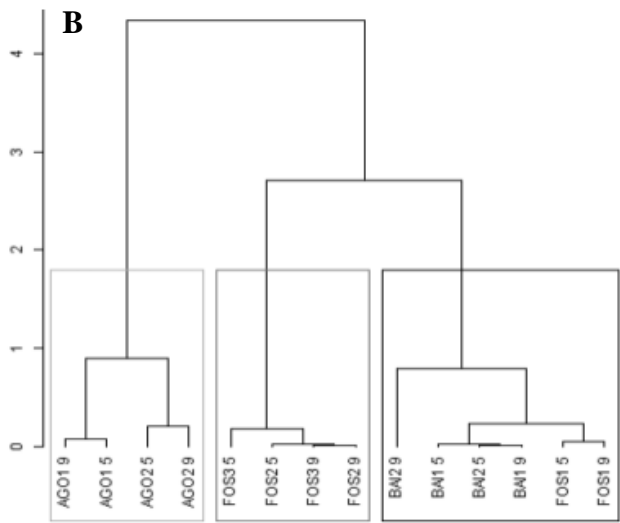
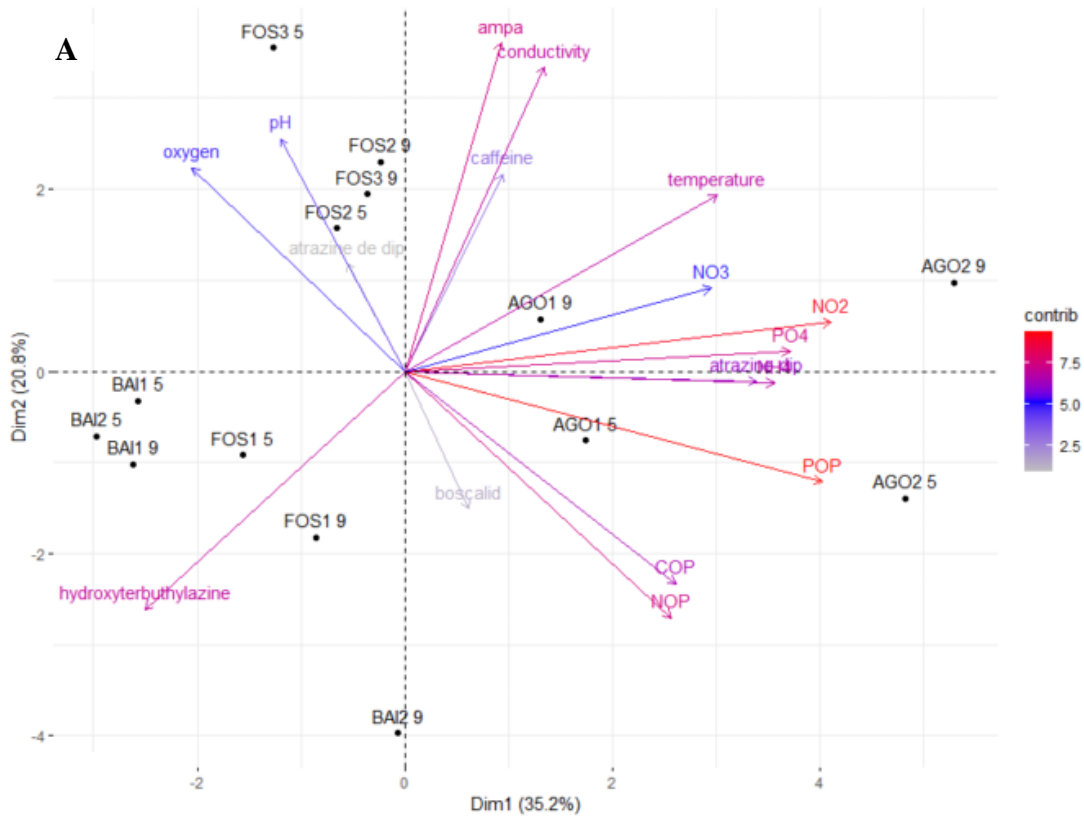
355



356

357 **Figure 2.** Concentration of (A) pesticides and (B) pharmaceuticals detected in the water. BAI<sub>1</sub>  
 358 and BAI<sub>2</sub> represent the upper and lower water sampling points on the Baillaury River section;  
 359 FOS<sub>1</sub>, FOS<sub>2</sub>, and FOS<sub>3</sub> represent respectively the upstream, outfall, and downstream  
 360 subsections of the Fosseille River; AGO<sub>1</sub> and AGO<sub>2</sub> 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:  $z_{lor} = 1.71$ ,  $df = 1$ ,  $p = 0.05$ ; females:  $z_{lor} = 0.07$ ,  
376  $df = 1$ ,  $p = 0.47$ ), but there was no transience on the Fosseille (males:  $z_{lor} = 0.75$ ,  $df = 1$ ,  $p$   
377  $= 0.23$ ; females:  $z_{lor} = 0.67$ ,  $df = 1$ ,  $p = 0.25$ ) and on the Agouille de la Mar (males:  $z_{lor} = 0.62$ ,  
378  $df = 1$ ,  $p = 0.27$ ; females:  $z_{lor} = 0.42$ ,  $df = 1$ ,  $p = 0.85$ ) 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 FOS<sub>2+3</sub>  
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 FOS<sub>1</sub>, where it was balanced at 0.48 (Table 1). The adult population  
386 of the Baillaury River consisted of  $45 \pm 5$  turtles (95% CI = [40;64]) and had a density of 27  
387 turtles.km<sup>-1</sup>. On the Fosseille River, the adult population consisted of  $157 \pm 24$  turtles (95% CI  
388 = [121;223]) and density ranged from 63 to 68 turtles.km<sup>-1</sup>, 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<sup>-1</sup>, 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 FOS<sub>1</sub>), 12% in habitat of level 2 (FOS<sub>2+3</sub>), and 7-9% in habitats  
393 of level 3 (AGO<sub>1</sub> and AGO<sub>2</sub>).

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 <sub>1</sub> )	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS <sub>2+3</sub> )	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 <sub>1</sub> )	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO <sub>2</sub> )	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

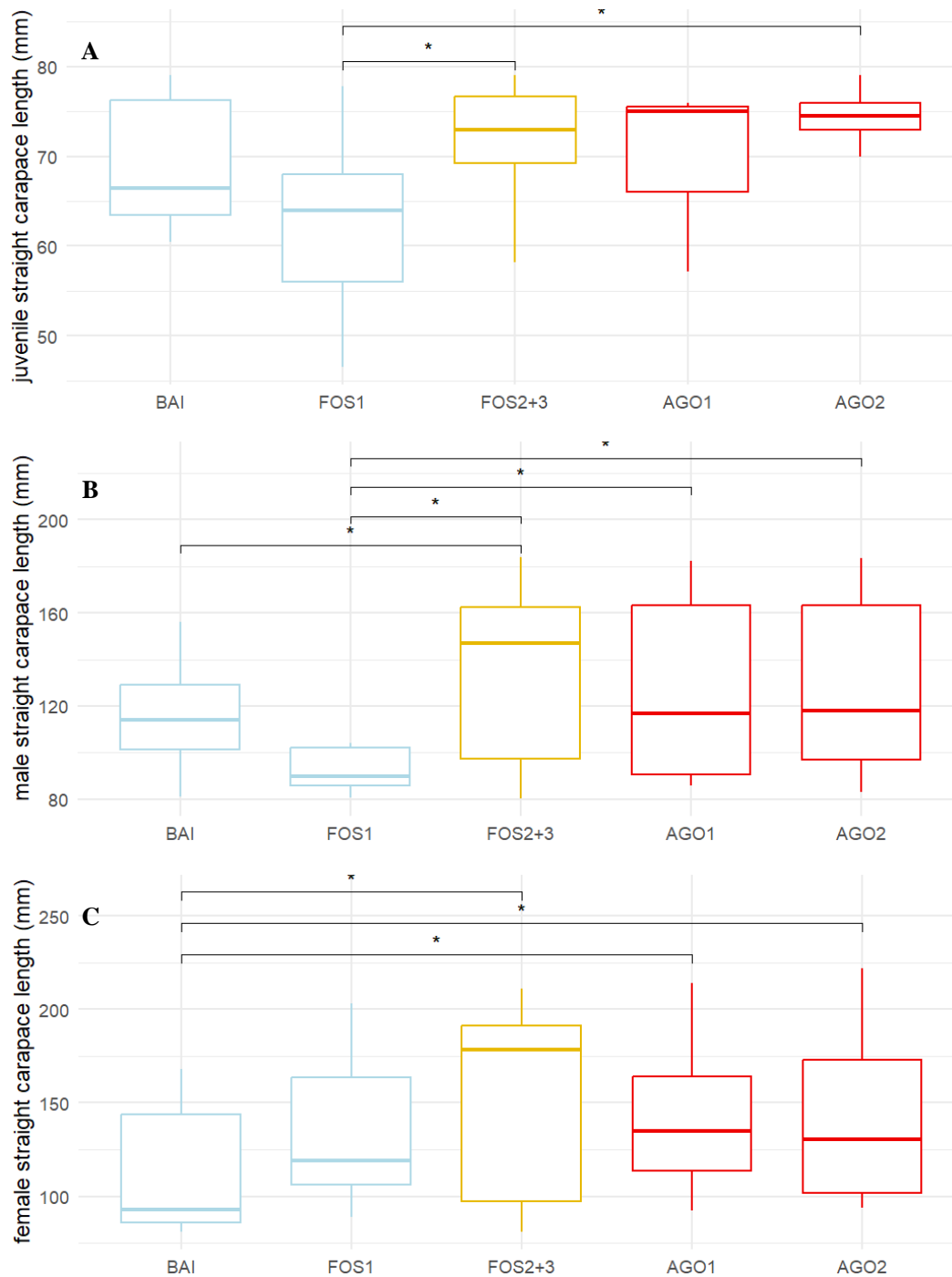
399

#### 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, FOS<sub>1</sub>, FOS<sub>2+3</sub>, AGO<sub>1</sub>, and AGO<sub>2</sub> (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 FOS<sub>2+3</sub> and in both subsections of the  
 407 Agouille de la Mar River (Fig.4; Supplementary Material B). However, the SMI of males did

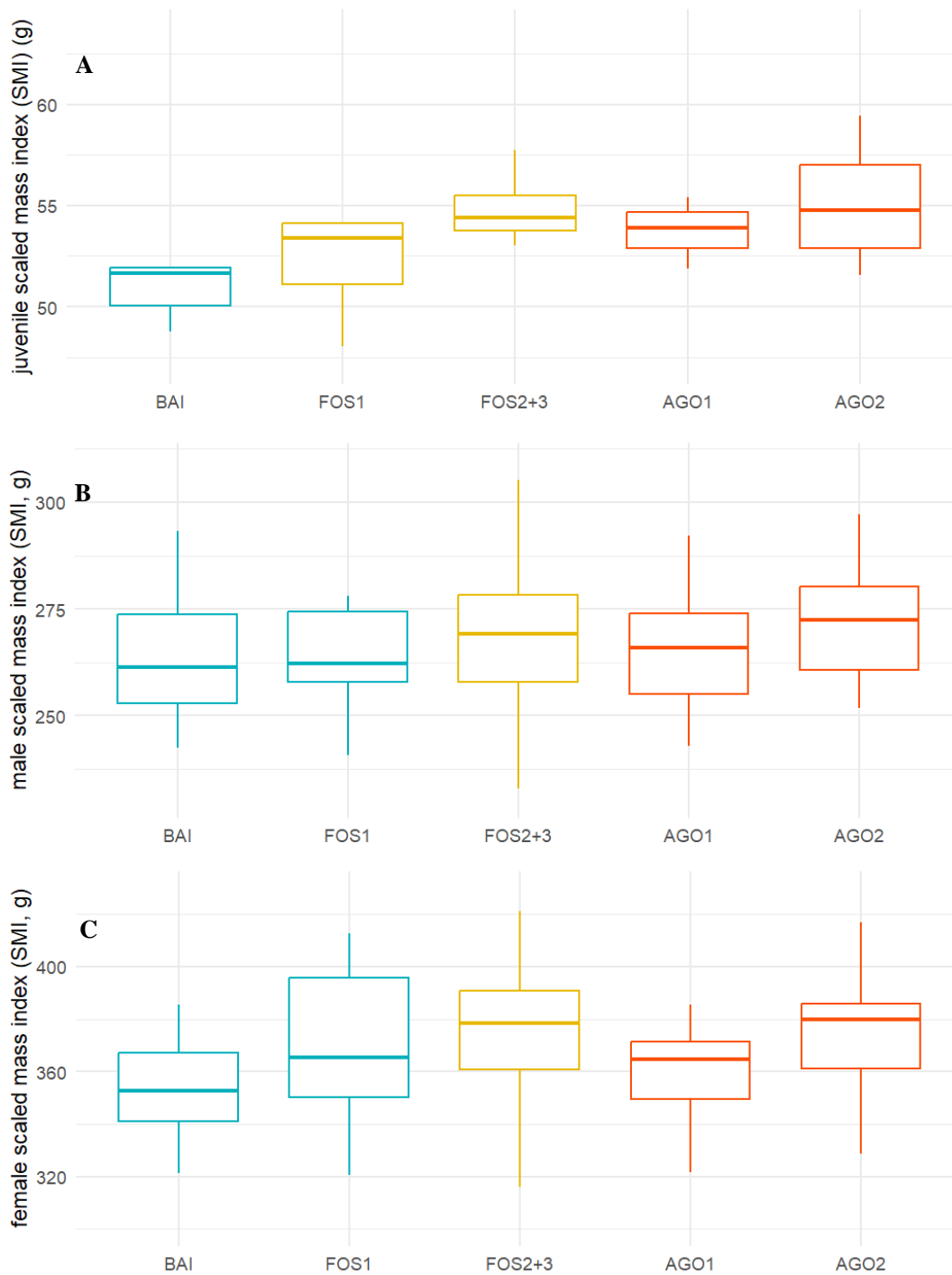
408 not differ between the five river subsections ( $n = 172$ ; ANOVA; SMI:  $F_{4,167} = 0.72$ ,  $p = 0.58$ ;  
409 Shapiro-Wilk:  $W = 0.99$ ,  $p = 0.08$ ), nor in females ( $n = 86$ ; ANOVA; SMI:  $F_{4,81} = 1.77$ ,  $p =$   
410  $0.14$ ; Shapiro-Wilk:  $W = 0.98$ ,  $p = 0.11$ ) (Fig.5). The 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 (AGO<sub>1</sub> and AGO<sub>2</sub>) than in other habitats. However, females grew slower in the habitat  
421 of level 2 (FOS<sub>2+3</sub>) 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).



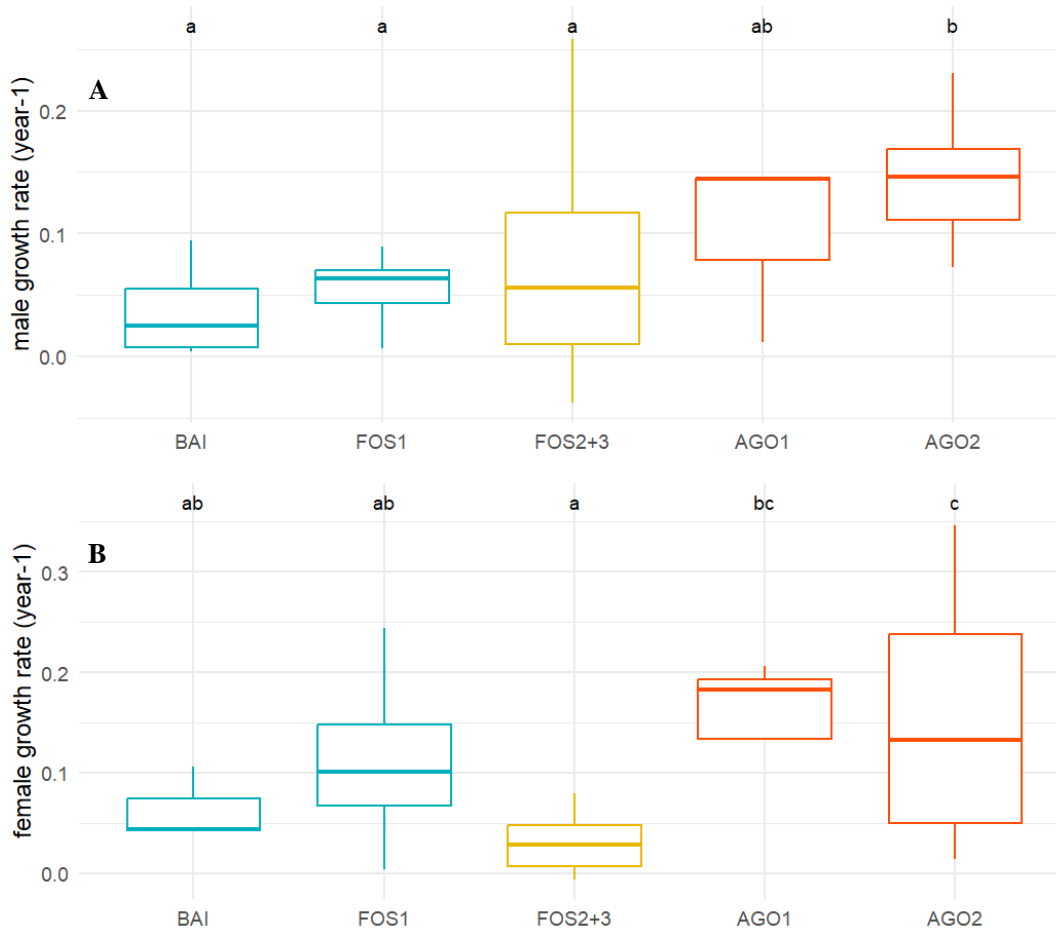
425

426 **Figure 4.** Body size of juveniles (A), males (B), and females (C) along the investigated rivers:  
 427 Baillaury River (BAI); upstream (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>) wastewater treatment plant  
 428 (WWTP) subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>)  
 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>)  
 435 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and  
 436 downstream (AGO<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>) wastewater treatment plant (WWTP)

441 subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) 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 (BAI<sub>1</sub> and BAI<sub>2</sub>) and the  
448 upstream subsection of the Fosseille River (FOS<sub>1</sub>), 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 FOS<sub>1</sub> was higher than in the Baillaury section, suggesting that this subsection's  
465 quality was lower than that of the Baillaury River. FOS<sub>1</sub> 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 (FOS<sub>2</sub>) and the  
471 downstream (FOS<sub>3</sub>) 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 FOS<sub>1</sub>, no amphibians were detected  
484 in FOS<sub>2</sub> and FOS<sub>3</sub>, 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 (AGO<sub>1</sub> and AGO<sub>2</sub>). 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 AGO<sub>1</sub> could also be  
490 the result of illegal discharges. Conversely, the high diversity and concentrations of  
491 contaminants detected in AGO<sub>2</sub> most likely originated from wastewater effluents. Similar as in  
492 FOS<sub>2+3</sub>, the most concentrated pollutants in AGO<sub>2</sub> 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<sup>-1</sup>) and Agouille de la Mar (44 to 50  
506 adults.km<sup>-1</sup>) rivers were higher than in the Baillaury River (22 adults.km<sup>-1</sup>), which was  
507 unexpected because of the poor water quality reported downstream of the Fosseille River  
508 (FOS<sub>2+3</sub>) and in both subsections of the Agouille de la Mar River (AGO<sub>1</sub> and AGO<sub>2</sub>). 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 FOS<sub>1</sub>), 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 (FOS<sub>2+3</sub>) 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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578

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587

#### 588 **Competition and interests**

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

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 (FOS<sub>1</sub>), outfall (FOS<sub>2</sub>), and downstream (FOS<sub>3</sub>) wastewater treatment plant (WWTP)  
898 subsections of the Fosseille River; in the upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) WWTP  
899 subsections of the Agouille de la Mar River; in the upper (BAI<sub>1</sub>) and lower (BAI<sub>2</sub>) 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 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>)  
904 wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO<sub>1</sub>) and  
905 downstream (AGO<sub>2</sub>) 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.

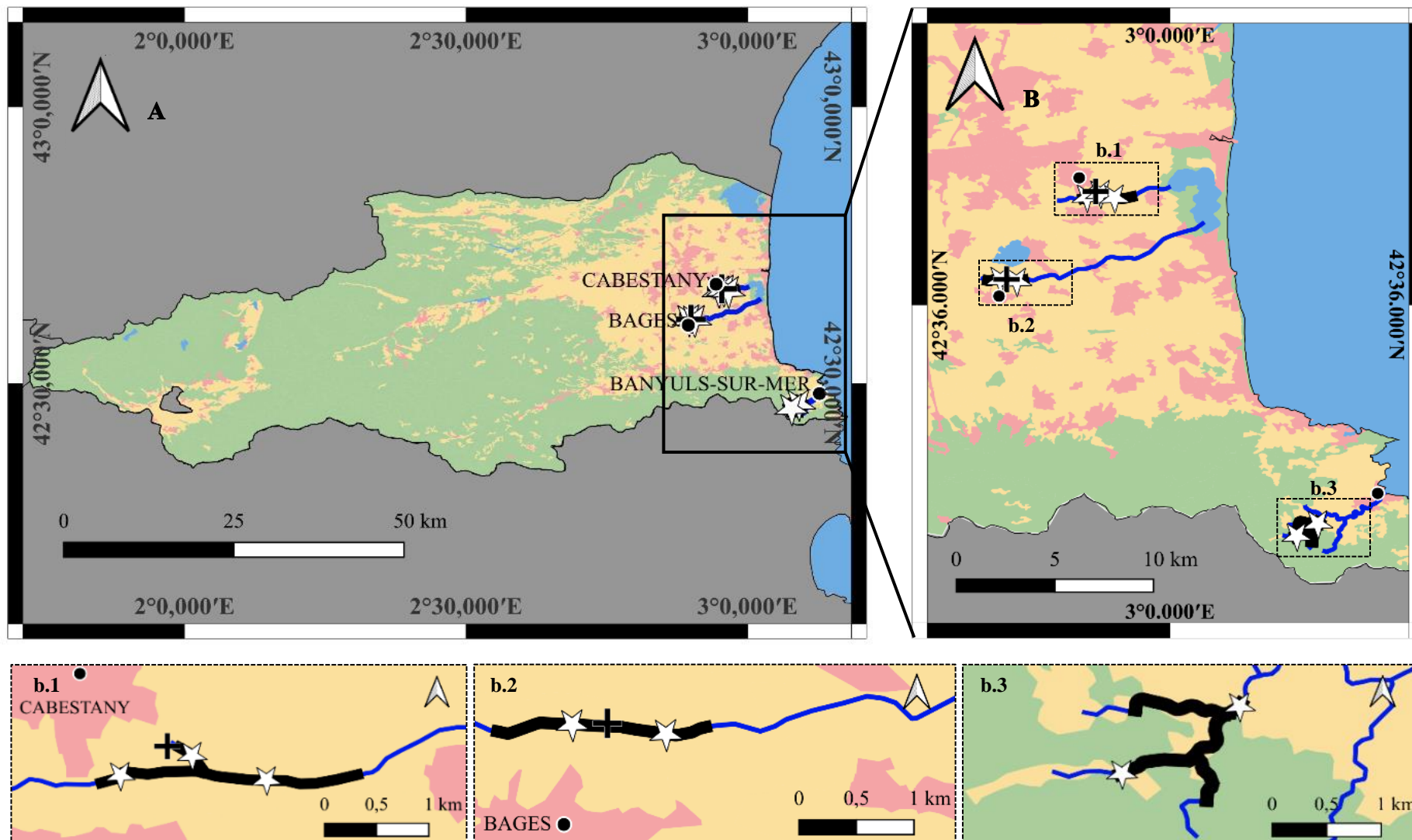
909 **Supplementary Material C.** Growth rates in males (a) and in females (b), relative to their body  
910 size. A unique symbol is displayed for each river subsection: Baillaury River (BAI); upstream  
911 (FOS<sub>1</sub>) and downstream (FOS<sub>2+3</sub>) wastewater treatment plant (WWTP) subsections of the  
912 Fosseille River; upstream (AGO<sub>1</sub>) and downstream (AGO<sub>2</sub>) WWTP subsections of the Agouille  
913 de la Mar River.

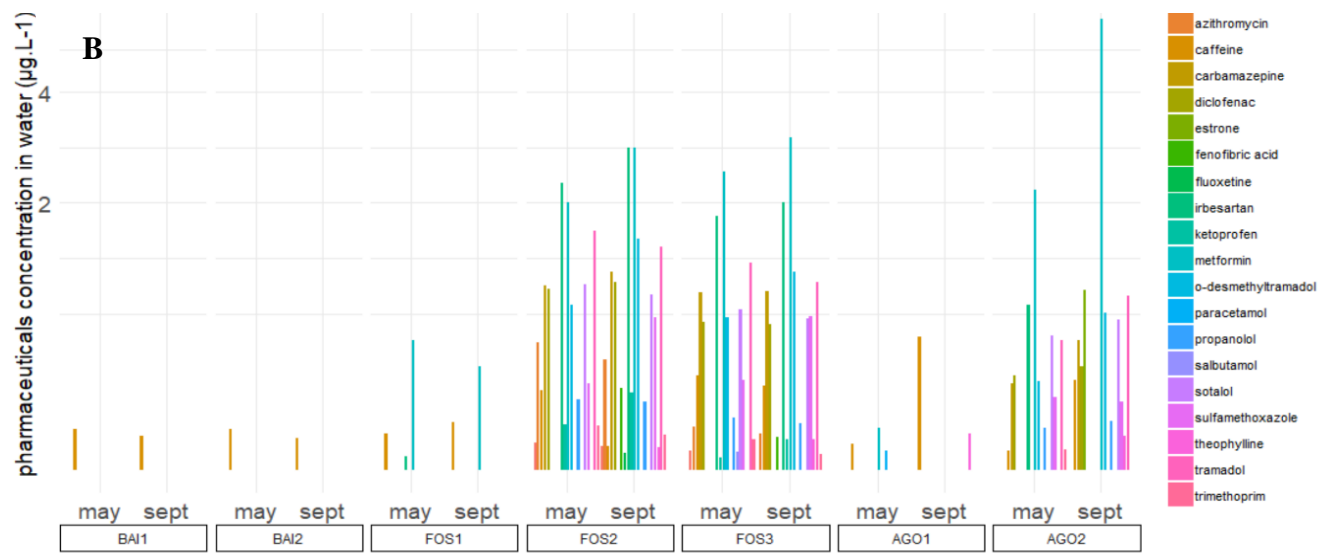
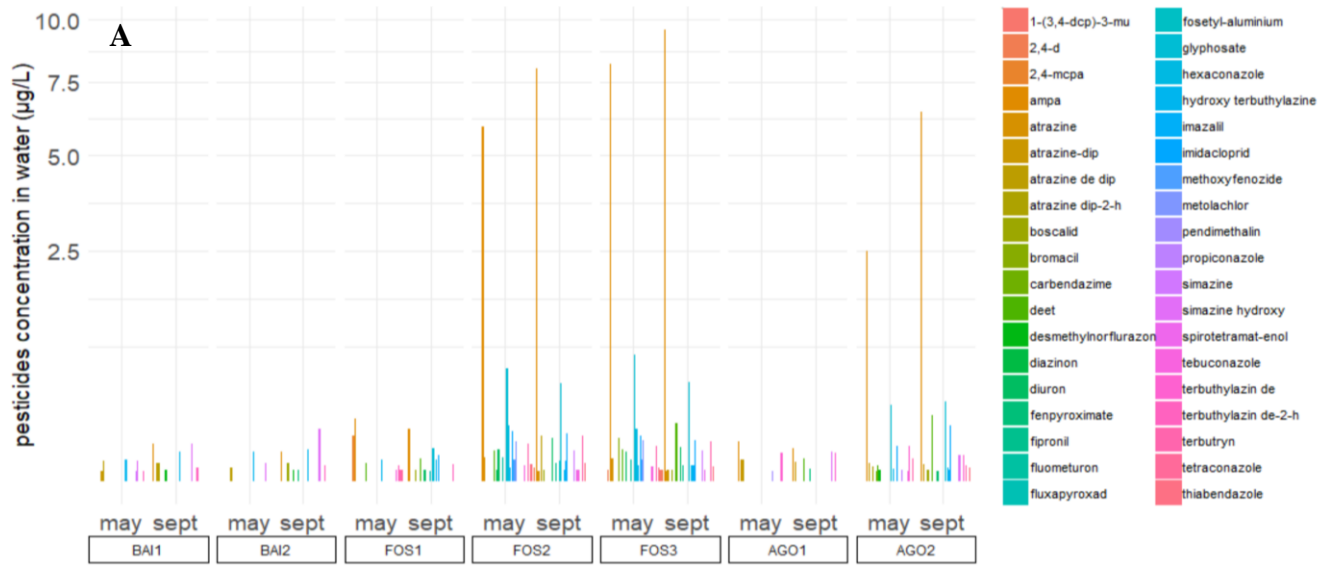


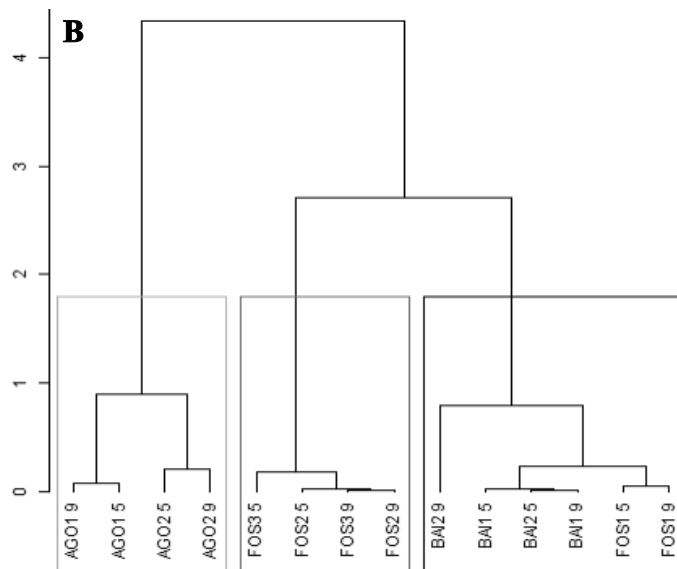
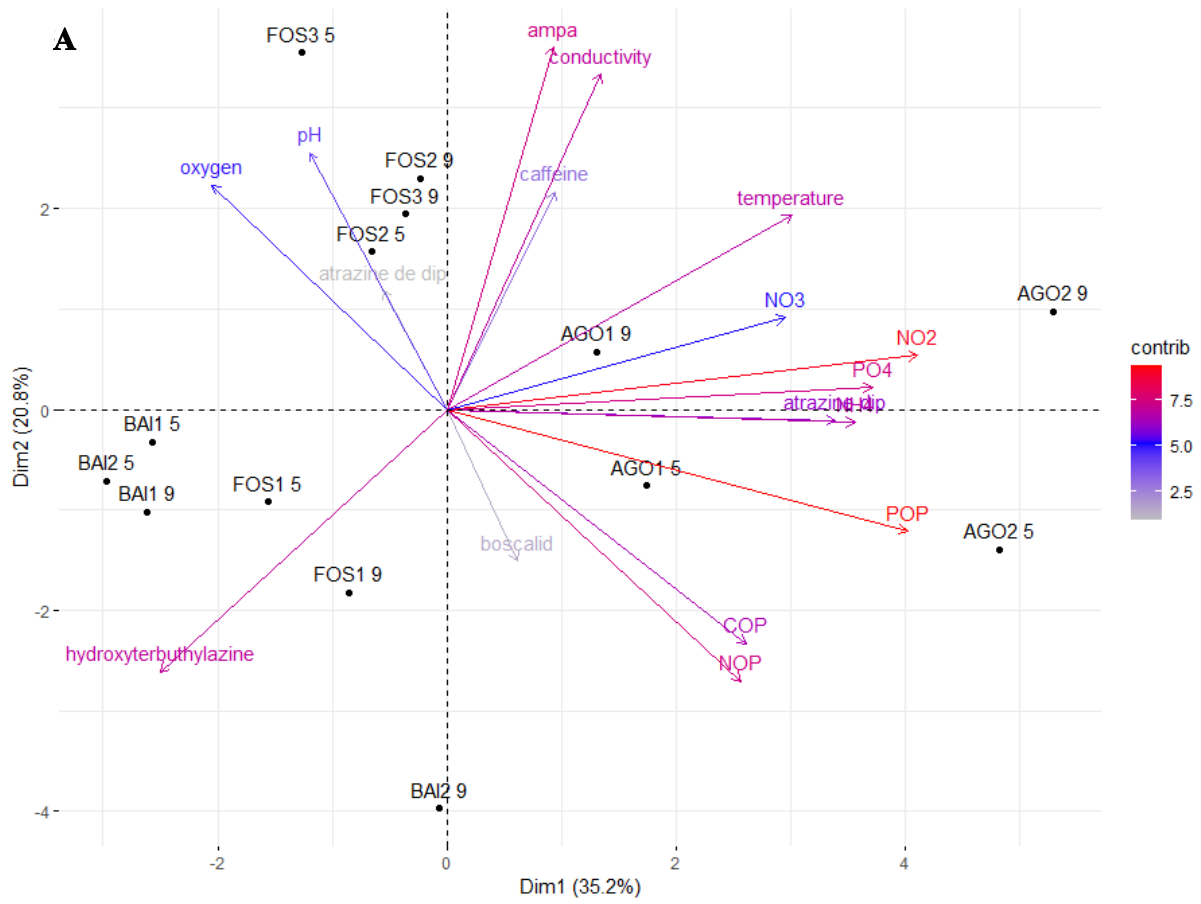
**Table 1.** Captures composition (males MAL, females FEM, and juveniles JUV) and operational sex ratio (OSR) of *M. leprosa* along the Baillaury River (BAI), the Fosseille River (FOS), and the Agouille de la Mar River (AGO). The total number of captures is indicated, with the number of distinct individuals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

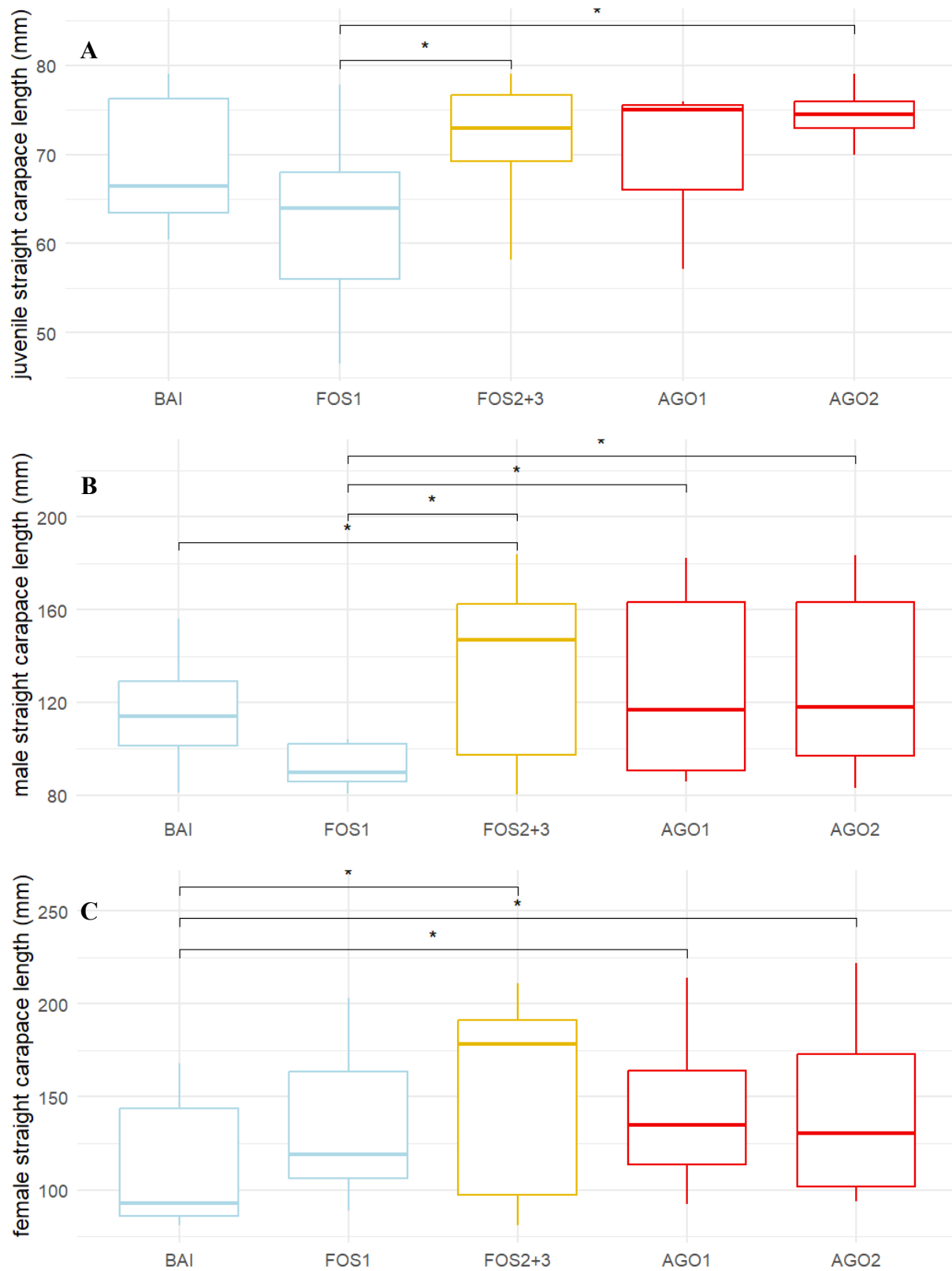
		MAL	FEM	JUV	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS <sub>1</sub> )	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS <sub>2+3</sub> )	172 (91)	39 (28)	28 (16)	239 (135)	0.88	0.76
	Full river	201 (101)	65 (39)	65 (33)	331 (173)	0.81	0.72
AGO	Upstream (AGO <sub>1</sub> )	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO <sub>2</sub> )	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

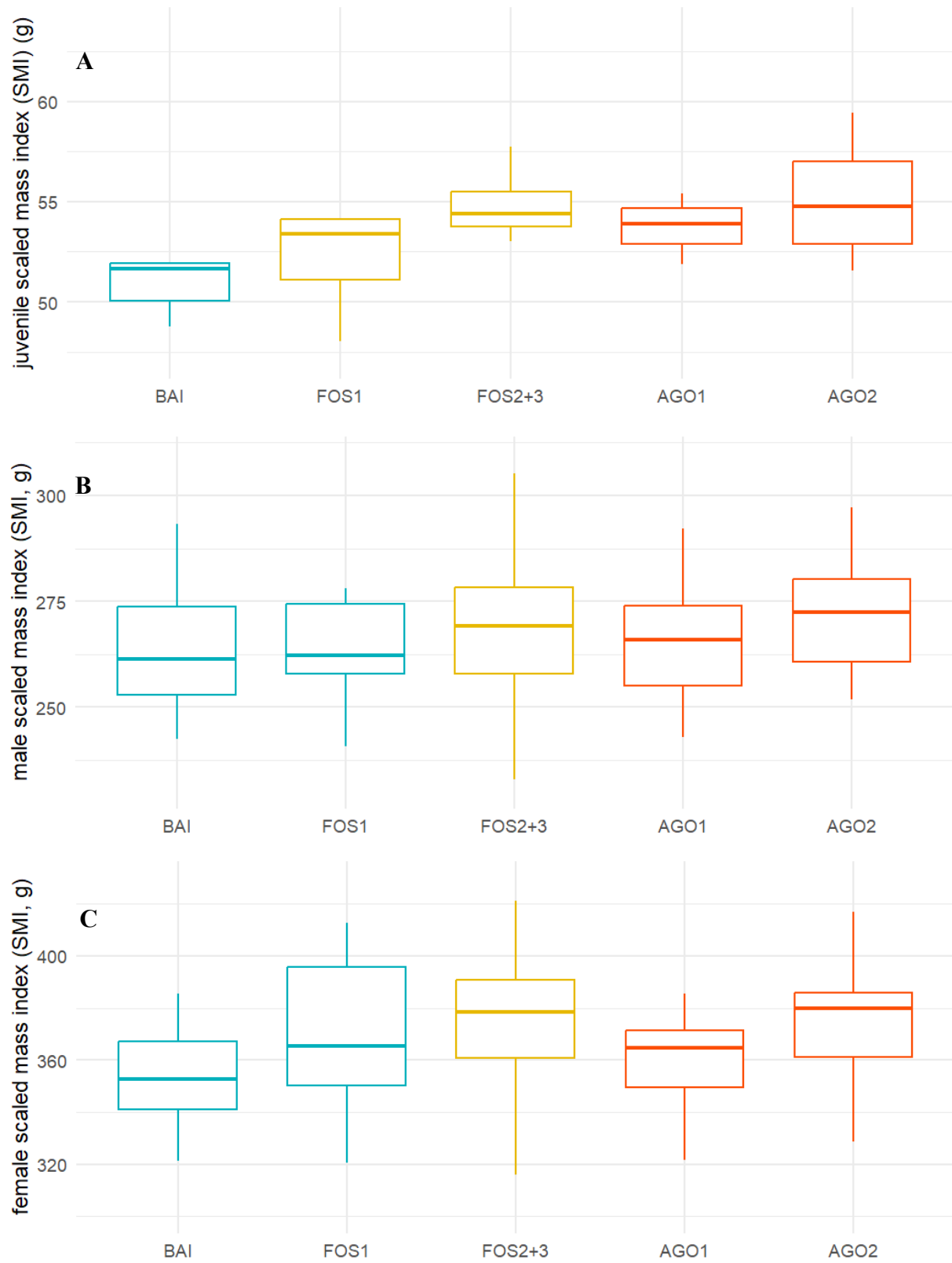
Figure 1

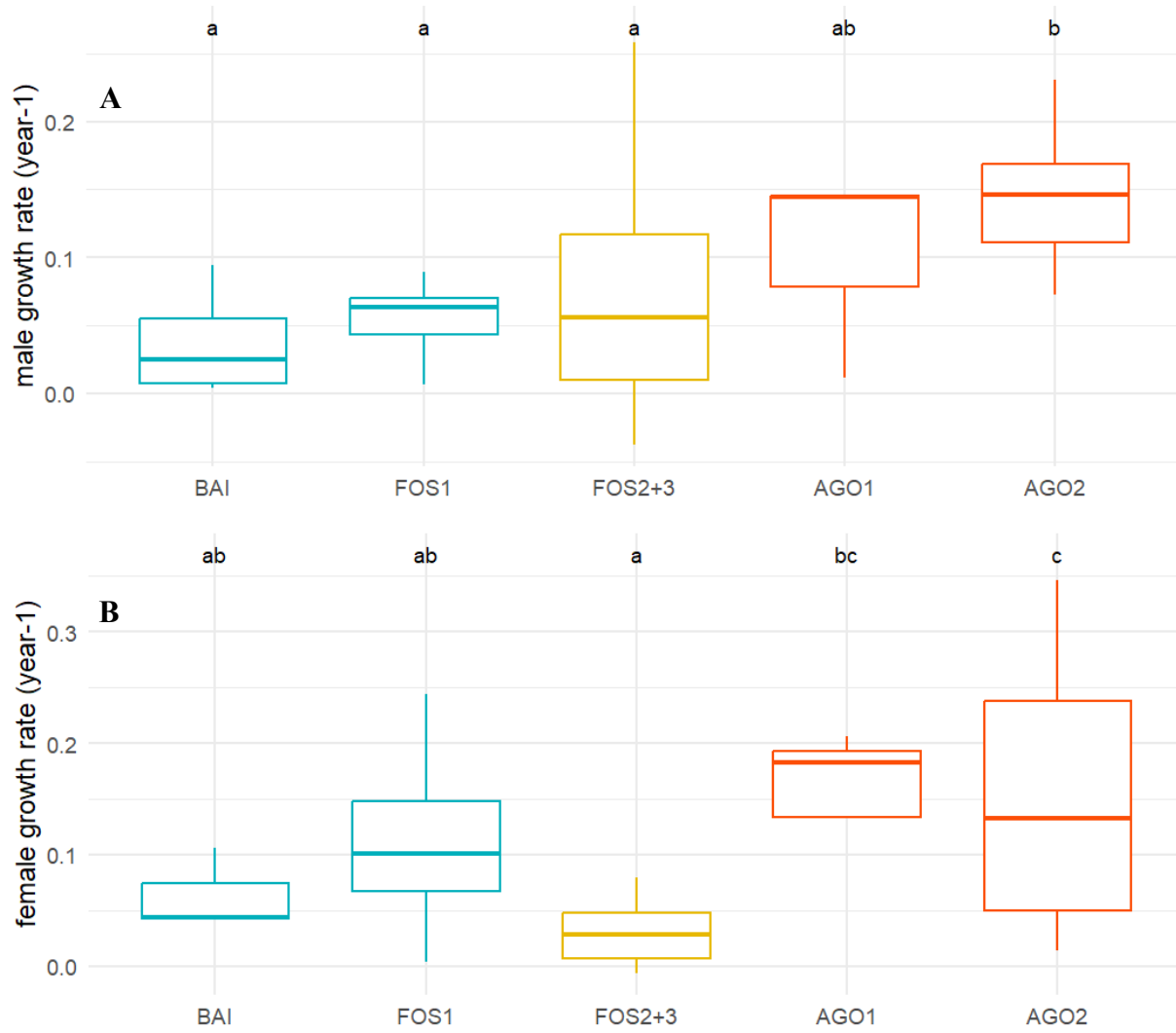














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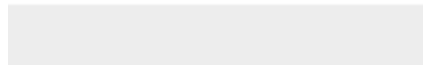


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**Declaration of interests**

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: