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Morphological variations and demographic responses of the Mediterranean pond turtle *Mauremys leprosa* to heterogeneous aquatic habitats

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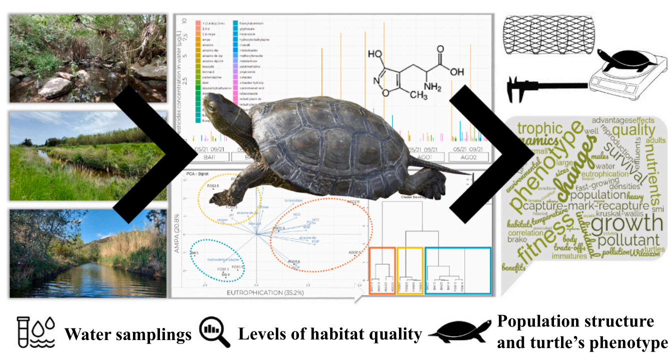
HIGHLIGHTS

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

GRAPHICAL ABSTRACT



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ABSTRACT

Human activities affect terrestrial and aquatic habitats leading to changes at both individual and population levels in wild animal species. In this study, we investigated the phenotype and demographics of the Mediterranean pond turtle *Mauremys leprosa* (Schweigger, 1812) in contrasted environments of Southern France: two peri-urban rivers receiving effluents from wastewater treatment plants (WWTP), and another one without sewage treatment plant. Our findings revealed the presence of pesticides and pharmaceuticals in the three rivers of investigation, the highest diversities and concentrations of pollutants being found in the river subsections impacted by WWTP effluents. Principal component analysis and hierarchical clustering identified three levels of habitat quality, with different pollutant concentrations, thermal conditions, nutrient, and organic matter levels. The highest turtle densities, growth rates, and body sizes were estimated in the most disturbed habitats, suggesting potential adult benefits derived from harsh environmental conditions induced by pollution and eutrophication. Conversely, juveniles were the most abundant in the least polluted habitats, suggesting adverse effects

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of pollution on juvenile survival or adult reproduction. This study suggests that turtles living in polluted habitats may benefit from enhanced growth and body size, at the expense of reproductive success.

Abbreviations

AGO	Agouille de la Mar River
AMPA	Aminomethylphosphonic acid
BAI	Baillaury River
BM	Body mass
FOS	Fosseille River
OSR	Operational sex ratio
PCA	Principal component analysis
SCL	Straight carapace length
SMI	Scaled mass index
WWTP	Wastewater treatment plant

1. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1. Study sites

2.1.1. The Baillaury environment

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

2.1.2. The Fosseille environment

The Fosseille River is a lentic river, flowing from the peri-urban south of Perpignan to the Saint Nazaire lagoon, which is connected to the Mediterranean Sea (Fig. 1). The river section of investigation flows from an altitude of 16 to three meters, is 2.6 km long, one to four meters wide, and two meters deep. It receives effluents from the WWTP of Cabestany, which contributes to maintain the continuous water flow of the river year-round. It consists of three subsections according to the position of the WWTP: the upstream, outfall, and downstream subsections (Fig. 1). Its watershed is highly anthropized by urban and agricultural activities such as vine growing. The riparian zone is homogeneous, with sparse border vegetation dominated by *A. donax*, the common reed *Phragmites australis* (Cavanilles) Trinius ex Steudel, 1840, the lakeshore bulrush *Schoenoplectus lacustris* (Linnaeus) Palla, 1888, and the yellow flag *Iris pseudacorus* (Linnaeus, 1753). The frequently encountered aquatic species were *M. leprosa*, the European roach *Rutilus rutilus* (Linnaeus, 1758), *Squalius* spp., *A. anguilla*, and *N. maura*. Alien species were also encountered, among which the red-eared slider *Trachemys scripta elegans* (Wied, 1838) and the red swamp crayfish *Procambarus clarkii* (Girard, 1852).

2.1.3. The Agouille de la Mar environment

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

2.2. Habitat quality

2.2.1. Environmental parameters of investigation

A total of 14 water samples were collected to assess water quality of the three rivers, among which seven were collected on 2021/05/07, and seven on 2021/09/27 (Fig. 1). On the Baillaury River, point sampling was done on the upper (BAI_1: N 42°27′25″ E 3°4′57″) and lower (BAI_2: N 42°27′45″ E 3°5′26″) reaches. On the Fosseille River, point sampling was done on the upstream (FOS_1: N 42°40′2″ E 2°56′53″), outfall (FOS_2: N 42°40′11″ E 2°57′26″) and downstream (FOS_3: N 42°40′1″ E 2°58′1″) subsections. Finally, on the Agouille de la Mar River, point sampling was conducted on the upstream (AGO_1: N 42°36′57.5″ E 2°53′41″) and downstream (AGO_2: N 42°36′53.6″ E 2°54′24″) subsections. Environmental parameters, such as temperature (T, in °C), pH, dissolved oxygen (DO, in $mg{\cdot}L^{-1}$), and conductivity (σ, in $\mu S{\cdot}cm^{-1}$), were measured *in natura* with a HQ40D Portable Meter (Hach).

Water samples were filtered on 0.45 μm (47 mm diameter) MF-Millipore membrane filters to quantify suspended particulate matter (SPM, in $mg{\cdot}L^{-1}$) at the Centre de Formation et de Recherche sur les Environnements Méditerranéens, and on 0.7 μm (47 mm diameter)

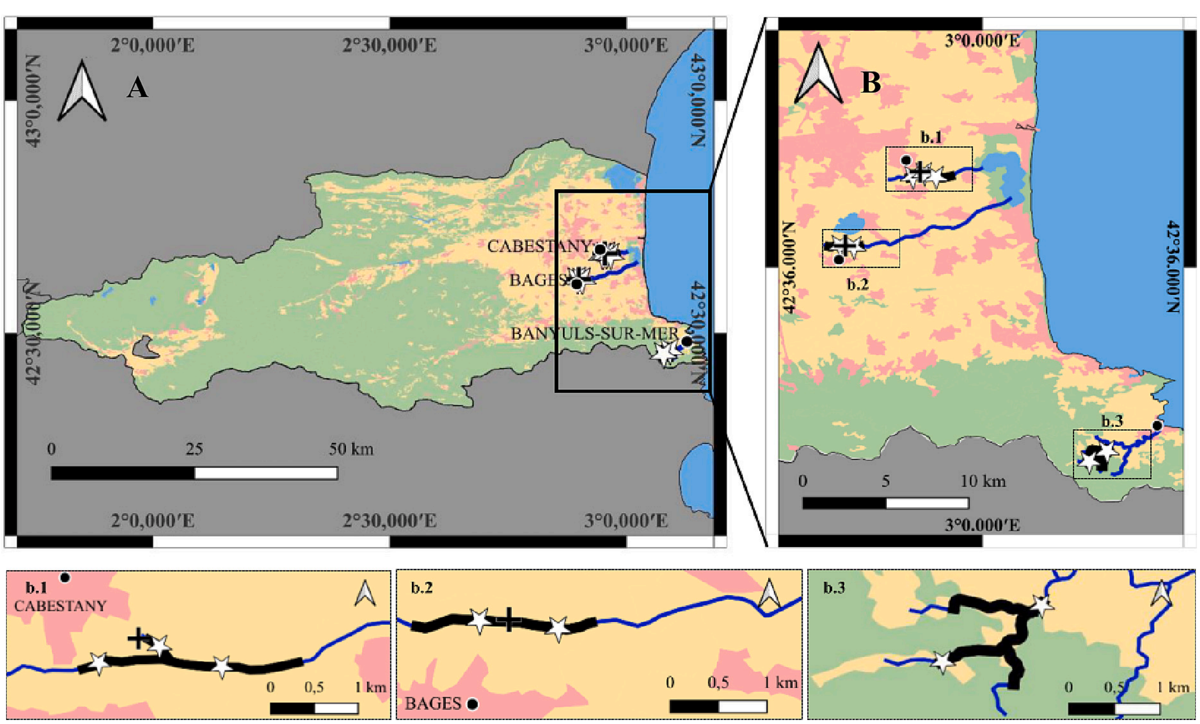

Fig. 1. (A) Region of Pyrénées-Orientales in Southern France, with aquatic areas in blue, pristine areas in green, bare soil in yellow, cropland in orange and urbanized areas in red. (B) The studied rivers are in navy blue, and their configuration is shown at the bottom of the main map, with the river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

Whatman GF/F glass fiber filters (precombusted at 500 °C for 4 h) to quantify particulate organic matter, i.e., particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP) (in mg·L^{-1}), at the Mediterranean Institute of Oceanography (MIO; Marseille, France). Glass fiber filters were dried at 60 °C and processed by the analytical platform for the chemistry of marine environments at the MIO, with a SEAL analytical chain, using the wet-oxidation technic (Raimbault et al., 1999). The detection limits were 120 ng·L^{-1}, 1 ng·L^{-1}, and 2 ng·L^{-1} for POC, PON, and POP, respectively.

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

2.2.2. Statistical analyses of water quality

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

2.3. Turtles monitoring

2.3.1. Animals handling

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

2.3.2. Population structure

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

Differences in BM and SCL along the river subsections were assessed using Kruskal-Wallis rank sum comparison tests, followed by Dunn post-hoc tests. Then, body condition, and growth rate were studied, with only the first annual capture being considered to account for pseudo replication. For body condition, the scaled mass index (SMI) was calculated following the procedure developed by Peig and Green (2009). The SMI is a hypothetical mass that allows for consideration of the relationship between body mass and body length within the group of interest, and the impact of individual variability and measurement errors on BM and SCL. The growth rate (GR) was calculated following the following equation designed by us: $GR_i = (SCL_{2021} - SCL_y) / [(2021 - y) * SCL_y]$, with i the turtle, and y the first year of capture. Following a model selection process using AIC in a stepwise algorithm, ANOVA was used to search for mixed effects of SCL and river subsection on SMI and growth rate, respectively. Durbin-Watson and Shapiro-Wilk tests were used to verify the assumptions of the ANOVA. Tukey HSD tests were then used to determine the groups that exhibited statistically significant differences. In view of the variations in body composition specific to age and sex, separate analyses were performed for juveniles, males, and females. All analyses were performed on RStudio 3.6.2 (RStudio Team, 2020) with significance levels set at 5 %; the MASS package was used for model selection (Venables and Ripley, 2002); and the ggplot2 package was used for data visualization (Wickham, 2016).

3. Results

3.1. Diversity and concentration of contaminants

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

In the Baillaury River, 11 pesticides and one pharmaceutical were detected in the water. Among the pesticides, nine were detected in BAI_1, and eight in BAI_2. The pesticides with the highest concentrations were the hydroxy simazine (0.10 μg·L^{-1}) and the AMPA (0.06 μg·L^{-1}). The other pesticides included three derivatives of atrazine, one derivative of norflurazon, two derivatives of terbuthylazine, fenpyroximate, simazine

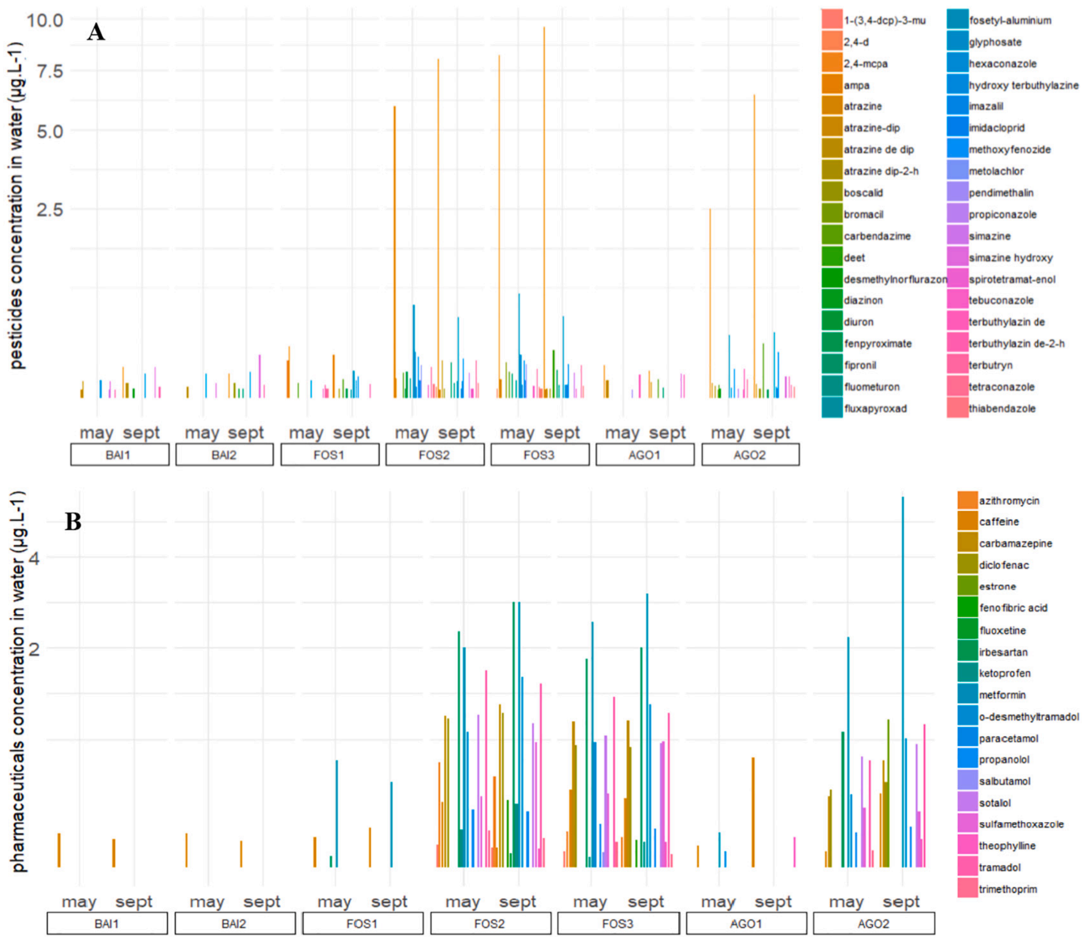

Fig. 2. Concentration of (A) pesticides and (B) pharmaceuticals detected in the water. BAI_1 and BAI_2 represent the upper and lower water sampling points on the Baillaury River section; FOS_1, FOS_2, and FOS_3 represent respectively the upstream, outfall, and downstream subsections of the Fosseille River; AGO_1 and AGO_2 represent respectively the upstream and downstream subsections of the Agouille de la Mar River. "may" is displayed for May 2021, and "sept" for September 2021.

and one of its derivatives. Caffeine was the only pharmaceutical detected, with a concentration ranging from 0.03 to 0.05 $\mu g \cdot L^{-1}$. The concentrations in contaminants did not differ between the two sampling sites (Kruskal-Wallis rank sum test, $\chi^2 = 1.78$, df = 3, $p = 0.62$).

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

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

3.2. Levels of water quality

The hierarchical clustering revealed three levels of habitat quality: (i) the Baillaury River section (BAI_1 and BAI_2) and the upstream subsection of the Fosseille River (FOS_1) present a low level of pollution and are classified as the first level; (ii) the outfall (FOS_2) and the downstream (FOS_3) subsections of the Fosseille River present a moderate level of pollution and are classified as the second level; (iii) the Agouille de la Mar River section (AGO_1 and AGO_2) presents the highest level of pollution and is classified as the third level (Fig. 3; Supplementary Material A).

The first dimension of the PCA distinguished the downstream subsection of the Agouille de la Mar River (AGO_2) from the Baillaury River section (BAI_1 and BAI_2) (Fig. 3). The latter was characterized by low concentrations of contaminants to the exception of hydroxy-terbuthylazine (0.040 ± 0.006 $\mu g \cdot L^{-1}$; versus AGO_2: 0.009 ± 0.001 $\mu g \cdot L^{-1}$), low conductivity (488.00 ± 51.56 $\mu S \cdot cm^{-1}$; versus AGO_2: 990.00 ± 22.00 $\mu S \cdot cm^{-1}$), and low temperature (17.78 ± 0.48 °C; versus AGO_2: 23.15 ± 1.15 °C) (Fig. 3). AGO_2 was characterized by high concentrations of particulate organic matter such as POP (0.090 ± 0.010 $\mu g \cdot L^{-1}$; versus BAI: 0.010 ± 0.010 $\mu g \cdot L^{-1}$), high nutrients levels such as NO_2^- (0.49 ± 0.09 $\mu g \cdot L^{-1}$; versus BAI: <0.01 $\mu g \cdot L^{-1}$), atrazine-dip (0.016 ± 0.001 $\mu g \cdot L^{-1}$; versus BAI: 0.001 ± 0.001 $\mu g \cdot L^{-1}$), and high water temperature (23.15 ± 1.15 °C; versus BAI: 17.78 ± 0.48 °C) (Fig. 3). The second dimension of the PCA distinguished the outfall

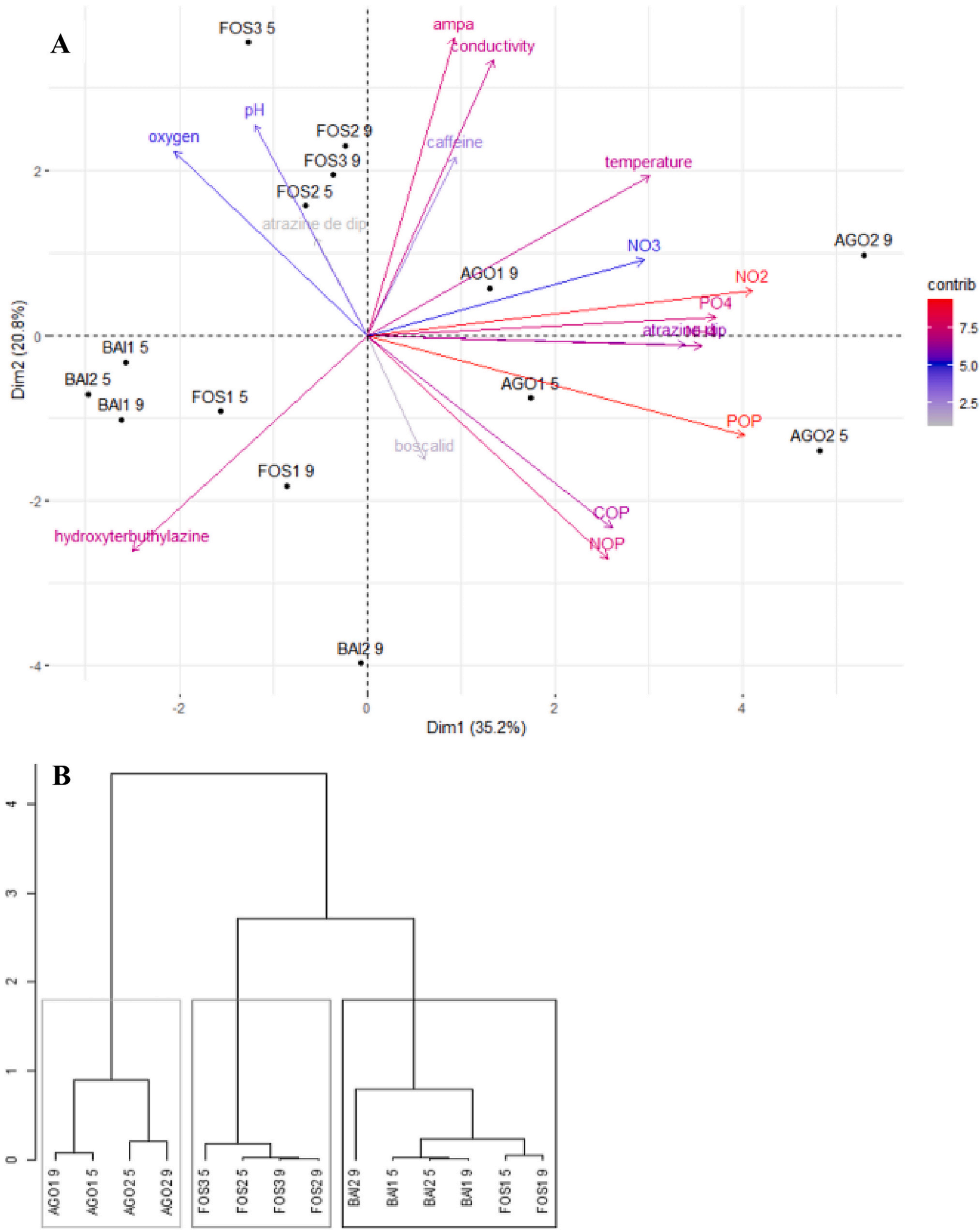

Fig. 3. (A) Biplot of the principal component analysis: the color gradient corresponds to the contribution of each variable to the construction of the plan (Dim1, Dim2). Each water sample is associated to a river (BAI for the Baillaury River section, FOS for the Fosseille River section, and AGO for the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

(FOS_2) and the downstream (FOS_3) subsections of the Fosseille River from the Baillaury River section (BAI_1 and BAI_2) (Fig. 3). FOS_2 and FOS_3 were characterized by high conductivity (1412.00 ± 78.33 μS·cm^{-1}; versus BAI: 488.00 ± 51.56 μS·cm^{-1}), high concentrations of AMPA (7.93 ± 0.76 μg·L^{-1}; versus BAI: 0.028 ± 0.017 μg·L^{-1}), and high concentrations of caffeine (0.17 ± 0.05 μg·L^{-1}; versus BAI: 0.040 ± 0.005 μg·L^{-1}).

Because samples collected in BAI_1 and BAI_2 did not differ in pollution levels, a single river subsection (BAI) was considered for the Baillaury River when analyzing turtle population structure. Similarly, as FOS_2 and FOS_3 did not show significant differences in pollution levels, they were considered as a single subsection named hereafter FOS_{2+3}. Five subsections were therefore considered for population structure analyses, namely BAI, FOS_1, FOS_{2+3}, AGO_1, and AGO_2.

3.3. Population structure

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

The number of individuals ranged from 43 turtles on BAI to 135 turtles on FOS_{2+3} (Table 1). The OSR was male-biased in most of the river subsections and ranged from 0.56 to 0.76, with the exception of FOS_1, where it was balanced at 0.48 (Table 1). The adult population of the Baillaury River consisted of 45 ± 5 turtles (95 % CI = [40;64]) and had a density of 27 turtles·km^{-1}. On the Fosseille River, the adult population consisted of 157 ± 24 turtles (95 % CI = [121;223]) and density ranged from 63 to 68 turtles·km^{-1}, upstream to downstream of the WWTP respectively. On the Agouille de la Mar River, the adult population consisted of 138 ± 18 turtles (95 % CI = [112;186]), and density ranged from 44 to 50 turtles·km^{-1}, upstream to downstream of the WWTP respectively. Juveniles accounted for 16–45 % of the total captures in habitats of level 1 (BAI and FOS_1), 12 % in habitat of level 2 (FOS_{2+3}), and 7–9 % in habitats of level 3 (AGO_1 and AGO_2).

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

		MAL	FEM	JUV	Total captures	Adults ratio	OSR
BAI		45 (23)	21 (13)	7 (7)	73 (43)	0.84	0.64
FOS	Upstream (FOS_1)	29 (10)	26 (11)	37 (17)	92 (38)	0.55	0.48
	Downstream (FOS_{2+3})	172 (91)	39 (28)	28 (16)	239 (135)	0.88	0.76
	Full river	201 (101)	65 (39)	65 (33)	331 (173)	0.81	0.72
AGO	Upstream (AGO_1)	39 (25)	22 (16)	4 (3)	65 (44)	0.93	0.61
	Downstream (AGO_2)	32 (23)	31 (18)	4 (4)	67 (45)	0.91	0.56
	Full river	71 (48)	53 (34)	8 (7)	132 (89)	0.92	0.59

3.4. Turtles body metrics

Body size (SCL) and mass (BM) of adults were different across the five river subsections, i.e., BAI, FOS_1, FOS_{2+3}, AGO_1, and AGO_2 (Fig. 4; Supplementary Material B). Differences were significant in males ($n = 172$; Kruskal-Wallis rank sum test; SCL: $\chi^2 = 11.00$, df = 4, $p = 0.03$; BM: $\chi^2 = 11.62$, df = 4, $p = 0.02$), and near so in females ($n = 86$; Kruskal-Wallis rank sum test; SCL: $\chi^2 = 8.52$, df = 4, $p = 0.07$; BM: $\chi^2 = 9.71$, df = 4, $p = 0.05$). The largest and heaviest turtles were located in the most degraded habitats, i.e., in FOS_{2+3} and in both subsections of the Agouille de la Mar River (Fig. 4; Supplementary Material B). However, the SMI of males did not differ between the five river subsections (n = 172; ANOVA; SMI: $F_{4,167} = 0.72$, $p = 0.58$; Shapiro-Wilk: W = 0.99, $p = 0.08$), nor in females (n = 86; ANOVA; SMI: $F_{4,81} = 1.77$, $p = 0.14$; Shapiro-Wilk: W = 0.98, $p = 0.11$) (Fig. 5). The body size and SMI of juveniles were not different across the five river subsections ($n = 47$; 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) (Figs. 4–5). However, body mass tended to increase along the pollution gradient, from level 1 to level 3 (n = 47; Kruskal-Wallis rank sum test; BM: $\chi^2 = 8.99$, df = 4, $p = 0.06$) (Supplementary Material B).

Growth rates were different across the five river subsections (Fig. 6) and also differed according to turtle body size. Differences were significant in males (n = 172; ANOVA; SCL: $F_{1,\ 77} = 12.98$, $p = 5.55e{-}04$; River subsection: $F_{4,\ 77} = 4.27$, $p = 3.58e{-}03$; Shapiro-Wilk: W = 0.98; $p = 0.41$) and in females (n = 86; SCL: $F_{1,\ 32} = 36.41$, $p = 9.84e{-}07$; River subsection: $F_{4,\ 32} = 7.28$, $p = 2.75e{-}04$; Shapiro-Wilk: W = 0.95; p = 0.08). Adults grew faster in the habitats of level 3 (AGO_1 and AGO_2) than in other habitats. However, females grew slower in the habitat of level 2 (FOS_{2+3}) than in other habitats (Fig. 6). The inflexion point of male growth rate was reached for a body size of 125 mm, whereas no such point was detected in females (Supplementary Material C). The growth rate was close to zero for males with a body size above 125 mm, and for females with a body size above 200 mm (Supplementary Material C).

4. Discussion

4.1. Aquatic habitat quality

The least polluted habitats (level 1) encompassed the Baillaury River (BAI_1 and BAI_2) and the upstream subsection of the Fosseille River (FOS_1), which exhibited the lowest diversity and concentrations of contaminants, the lowest concentrations of nutrients, the lowest conductivity, and the lowest water temperatures. Whereas pesticides and caffeine detected in these subsections might have been drained by rain, they likely resulted from illegal discharge as they were assessed during low flow periods. Caffeine is indeed a marker of anthropization and is the most detected emerging contaminant in surface water (Korekar et al., 2020). In the absence of WWTP, such discharge could occur via semi-rigid pipes connecting private plots to the river, which were observed along the Baillaury River. The contaminants with the highest concentrations in these habitats were caffeine, glyphosate, and AMPA. AMPA, which is the main metabolite of glyphosate and persistent in water, can have significant negative effects on aquatic organisms (Daouk et al., 2013; Guilherme et al., 2014; Moore et al., 2014; Tresnakova et al., 2021). While the low DO concentration measured in the Baillaury River in September indicated fairly poor water quality and anoxic conditions during drought periods (Supplementary Material A; Légifrance, 2010), the presence of bio-indicators such as the amphibians *P. perezi* and *B. spinosus* indicated good aquatic and terrestrial habitats (see Marques et al., 2008; Napoletano et al., 2023). On the opposite, the diversity of pollutants reported in FOS_1, which was higher than in the Baillaury section, and the absence of amphibians suggested that the

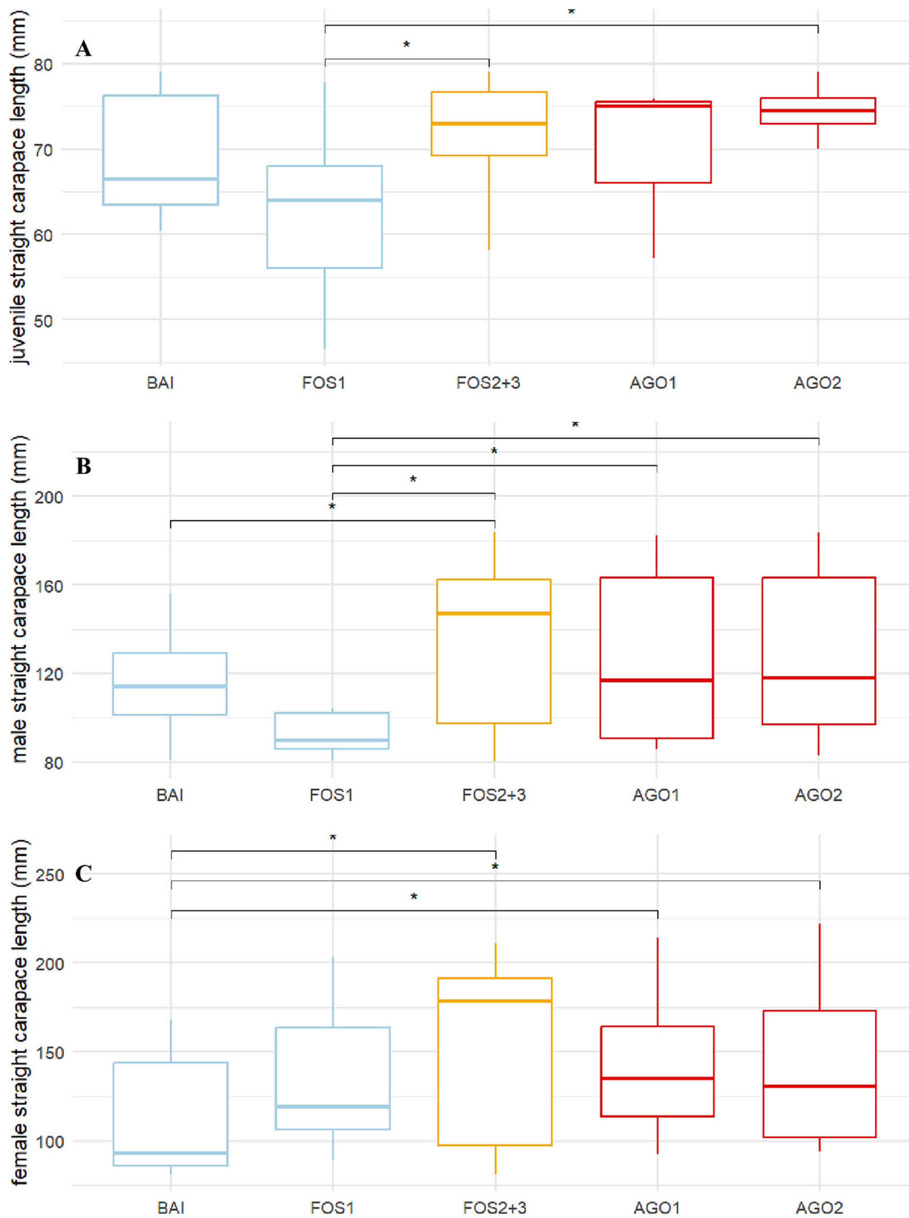

Fig. 4. Body size of juveniles (A), males (B), and females (C) along the investigated rivers: Baillaury River (BAI); upstream (FOS_1) and downstream (FOS_{2+3}) wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO_1) and downstream (AGO_2) WWTP subsections of the Agouille de la Mar River. Each color represents a quality level: blue (level 1), yellow (level 2), and red (level 3). Pairwise Wilcoxon tests *p*-values are indicated (alpha = 5 % *; 1 % **). The median of each group is displayed as a bold vertical line.

quality of FOS_1 was lower than that of the Baillaury River. FOS_1 also contained metformin which is detrimental to aquatic fauna with notorious effects on the endocrine function and fecundity of fish (Niemuth and Klaper, 2015; Lee et al., 2019). Traditional agricultural practices, such as those used along the Baillaury environment, may be thereby more beneficial to species richness and diversity than the conventional agricultural practices used along the Fosseille environment (see Kazes et al., 2020). However, the presence of alien species such as the crayfish *P. clarkii* in the Fosseille River could also explain the absence of amphibians due to predation (Gherardi et al., 2001).

The habitats of medium quality (level 2) encompassed both the outfall (FOS_2) and the downstream (FOS_3) subsections of the Fosseille River. They exhibited high diversity and concentrations of pesticides and pharmaceuticals, high concentrations of suspended particles, and high water temperatures compared to habitats of level 1. Alterations in water quality were probably due to the presence of the Cabestany WWTP, which receives wastewater from the urban community, but also from agricultural plots. Because not all pollutants are removed by WWTPs (Bruton et al., 2010; Deo, 2014), pesticides and pharmaceuticals are commonly found in water. The most concentrated pollutants were indeed the AMPA and the metformin, which are detrimental to wildlife, as discussed previously. Among the detected pharmaceuticals (Fig. 2), carbamazepine was reported to negatively affect eggs viability, female gonads, and metabolism in the zebrafish *Danio rerio* (Hamilton, 1822) (see Da Silva Santos et al., 2018), but also steroid production and reproductive output (Fraz et al., 2019). Fluoxetine, which was detected in these habitats, was reported to alter male mating strategy in the guppy fish *Poecilia reticulata* (Peters, 1859) (see Fursdon et al., 2019). As in FOS_1, no amphibians were detected in FOS_2 and FOS_3, which could be explained by the high diversity and concentrations of pollutants in the water, but also by the presence of alien animal species such as *P. clarkii*.

The most disturbed habitats (level 3) encompassed both subsections

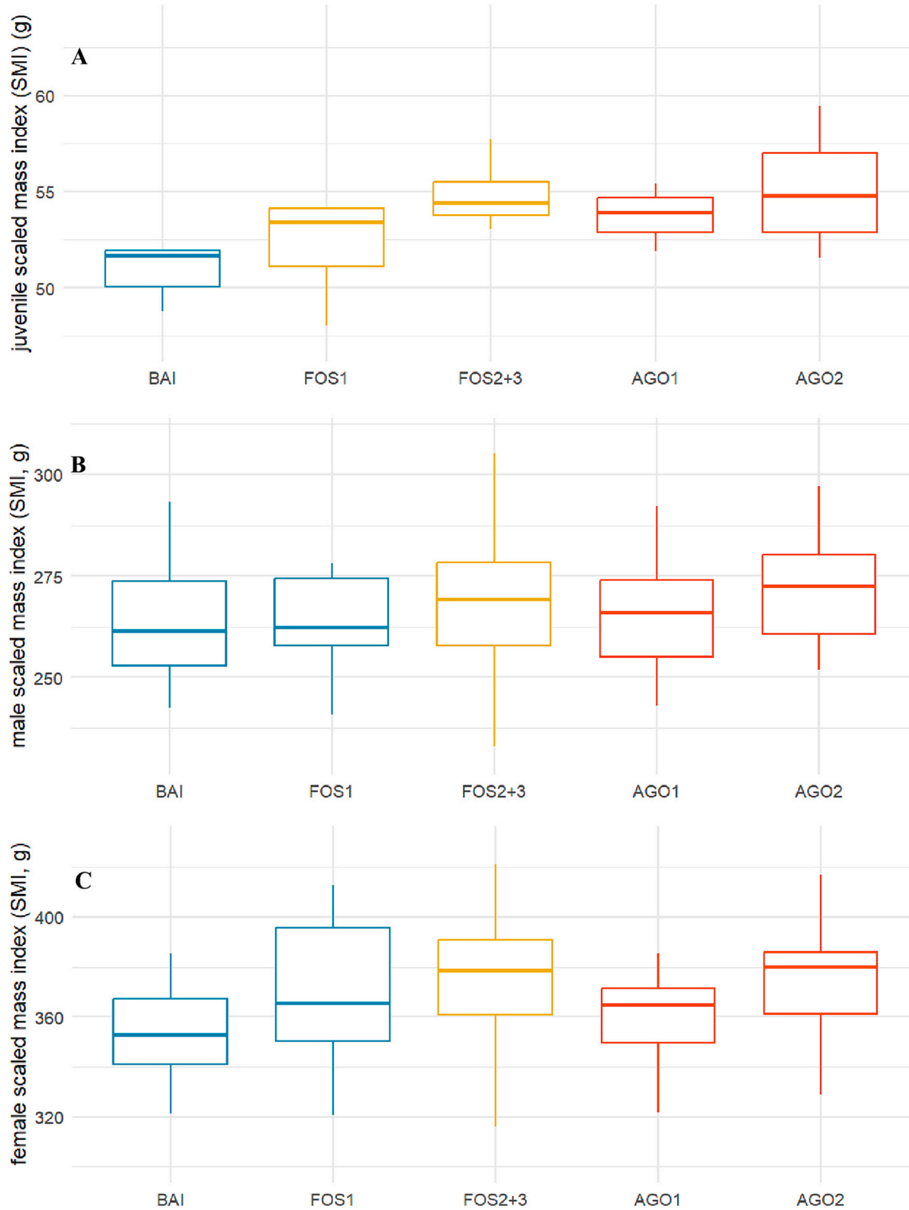

Fig. 5. Scaled mass indices in juveniles (A), males (B), and females (C) along the investigated rivers: Baillaury River (BAI); upstream (FOS_1) and downstream (FOS_{2+3}) wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO_1) and downstream (AGO_2) WWTP subsections of the Agouille de la Mar River. Each color represents a quality level: blue (level 1), yellow (level 2), and red (level 3).

of the Agouille de la Mar River (AGO_1 and AGO_2). They were characterized by high concentrations of nutrients and organic matter, resulting in eutrophication of the watercourse. Pesticides and pharmaceuticals (caffeine, metformin, paracetamol, and theophylline) that were detected in AGO_1 could also be the result of illegal discharges. Conversely, the high diversity and concentrations of contaminants detected in AGO_2 most likely originated from wastewater effluents. Similar as in FOS_{2+3}, the most concentrated pollutants in AGO_2 were the AMPA and metformin, both known for their harmful effects on aquatic organisms. Carbamazepine, which can have detrimental effects on the physiology and reproduction of *D. rerio* as mentioned previously (see Da Silva Santos et al., 2018), was also detected in that river (Fig. 2). In addition to pollutant discharges, effluents may have dumped organic matter in the downstream subsection, as the Bages WWTP has been exceeding its treatment limits for several years and is no longer able to adequately treat incoming wastewater (Sandre, 2023). High organic matter pollution, which consumes oxygen for oxidation processes, may induce low DO levels (Navarrete-Salgado et al., 2004) and high nitrite concentrations (Roberts and Palmeiro, 2008), resulting in very poor water quality (Supplementary Material A; Légifrance, 2010). As in the Fosseille River, no amphibians were detected, while the alien species *P. clarkii* was still observed.

4.1.1. *Correlation between turtle demographics and habitat quality*

Turtle densities in the Fosseille (63 to 68 adults·km^{-1}) and Agouille de la Mar (44 to 50 adults·km^{-1}) rivers were higher than in the Baillaury River (22 adults·km^{-1}), which was unexpected because of the poor water quality reported downstream of the Fosseille River (FOS_{2+3}) and in both subsections of the Agouille de la Mar River (AGO_1 and AGO_2). These contrasting results were not due to differences in capture rates of turtles across the three rivers, as capture rates in the Baillaury River were similar, even higher ($p = 0.26$ in May, 0.56 in June, and 0.49 in July 2021) than in the Fosseille ($p = 0.22$) and Agouille de la Mar ($p = 0.26$) rivers. Moreover, these rates were consistent yet slightly lower

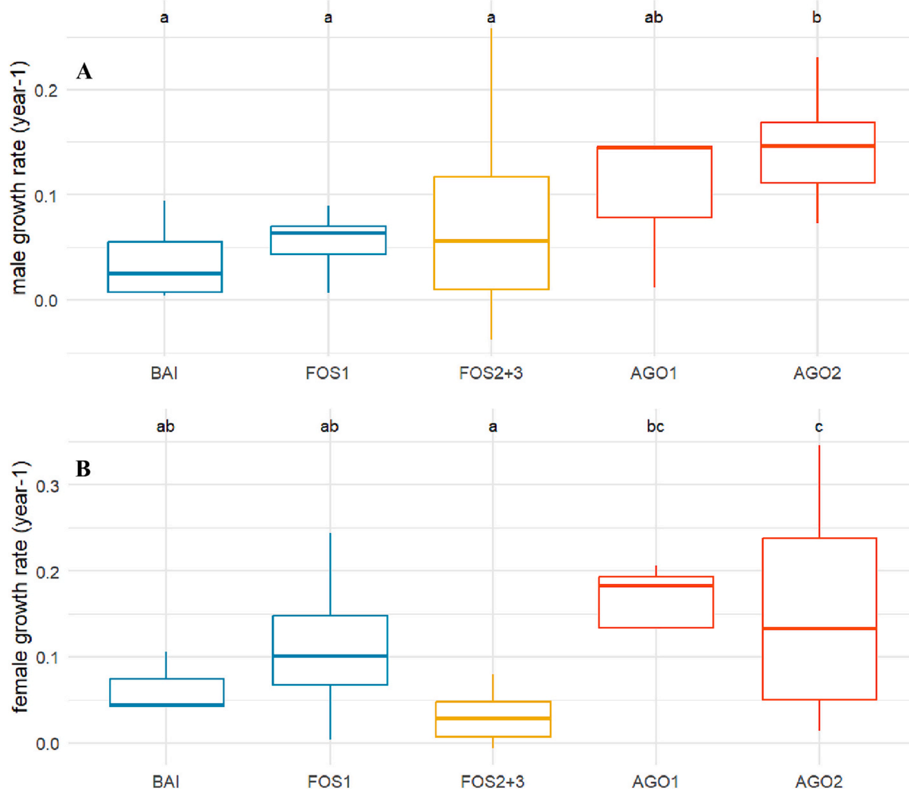

Fig. 6. Growth rates in males (A) and females (B) along the investigated rivers: Baillaury River (BAI); upstream (FOS_1) and downstream (FOS_{2+3}) wastewater treatment plant (WWTP) subsections of the Fosseille River; upstream (AGO_1) and downstream (AGO_2) WWTP subsections of the Agouille de la Mar River. Each color represents a quality level: blue (level 1), yellow (level 2), and red (level 3). Following ANOVA, Tukey test comparisons results are displayed as letters.

than those reported for the European pond turtle *Emys orbicularis* (Linnaeus, 1758) (see Olivier et al., 2010; Owen-Jones et al., 2016; Escoriza et al., 2021). It should be mentioned that the Baillaury River consists of several river sections, which are hydrologically interconnected. Therefore, the low turtle density in the investigated section could be explained by temporary transitions between sections.

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

4.1.2. Correlation between phenotypic changes and habitat quality

Our results showed that turtle growth rate and body size increased with habitat pollution. In the least polluted habitats (BAI and FOS_1), the low growth rates could be related to chilly waters, as observed in the freshwater turtle *A. marmorata* (Snover et al., 2015). The partial dryness of the Baillaury River during drought could also adversely affect turtle growth, because of limited food resources. Consistent with this, Powell et al. (2023) showed that drought could have slow down growth of the freshwater turtle *C. picta*. The Baillaury River and the upstream subsection of the Fosseille River appeared to be the least supportive habitats for turtle growth and body size, due to chilly waters and altered flows. On the opposite, the high nutrient levels reported in polluted and eutrophic habitats may have enhanced growth rate and body size (Figs. 4 and 6). Consistent with this, it has been shown that *C. picta* exhibited higher growth rates in polluted lentic habitats (Knight and Gibbons, 1968) as well as in wastewater lagoons (Lindeman, 1996), than in pristine ones. A similar result was also reported by Germano (2010) for *A. marmorata*. While Knight and Gibbons (1968) already pointed out that high growth rates in polluted environments could be the result of diet changes in *C. picta*, Sidis and Gasith (1985) illustrated shifts from plant to detrital organic matter in the diet of the Caspian turtle *Mauremys caspica* (Gmelin, 1774) when present in polluted habitats. The low growth rate of females observed in the habitat of medium quality (FOS_{2+3}) may be related to turtles age and reproductive status, as growth rate usually decreases after females become reproductive (Bury, 1979; Galbraith et al., 1989) (Fig. 6). Females may have stored more reserves to compensate for low growth rates, resulting in higher SMI (Fig. 5) (Ashton et al., 2015). Finally, the high SMI of juveniles reported in the most disturbed habitats (Fig. 5) could reflect a poor health status induced by pollution, as reported earlier for the Central American river turtle *Dermatemys mawii* (Gray, 1847) (see Rangel-Mendoza et al., 2014).

5. Conclusion

Our results highlighted different morphologies and population structures in *M. leprosa* in relation to increased levels of pollution and eutrophication of aquatic habitats. Growth rate as well as adult body size were the highest in the most polluted and eutrophic habitats. However, juveniles were under-represented in such habitats, suggesting an issue with their survival or the adult reproduction. Although differences in population structure may result from alterations in water quality, additional factors such as predation and competition with invasive species, not investigated here, could also have impacted the demography of *M. leprosa*.

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CRediT authorship contribution statement

Anne-Sophie Le Gal: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jean-Yves Georges:** Writing – review & editing, Supervision, Conceptualization. **Christine Sotin:** Investigation. **Bruno Charrière:** Investigation. **Olivier Verneau:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

The authors do not have permission to share data.

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