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continued on the back inside cover

Cover: A digital art of water birds of Noyyal River and its wetlands in Coimbatore District by Megha A. Kashyap.



## First characterization of the bacteriological profile of the Mediterranean Pond Turtle *Mauremys leprosa* (Schweigger, 1812) in Reghaïa Lake, Algeria

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**Abstract:** The Mediterranean Pond Turtle *Mauremys leprosa*, a 'Near Threatened' species, is poorly studied in Algeria and no data exists on its bacteriological carriage. However, knowledge about the microbiota of wildlife species is essential to develop holistic conservation approaches that integrate microbial health, habitat preservation, and species-specific needs. Recent concerns regarding the potential transmission of zoonotic pathogens by turtles have been highlighted in several studies. In this context, the current study analyzed the aerobic cloacal/fecal bacteria associated with the Mediterranean Pond Turtles, which were collected from Reghaïa Lake. Samples collected from 24 turtles allowed the identification of 11 bacterial genera. *Salmonella* was the most frequent isolated genus with a percentage of 22 of the total isolates, followed by *Escherichia* and *Enterobacter*. The diversity of genera isolated from juveniles is relatively low compared with adults. Turtle-bacterial genera relationships were tested by logistic regressions and redundancy analysis (RDA). Results of RDA indicate a statistically significant association ( $p$ -value  $<0.01$ ) between morphological features and bacterial genera frequency. Our results confirm the reputation of freshwater turtles as a reservoir of several zoonotic bacterial pathogens. This microbiota analysis offers a non-invasive, multi-faceted approach to conserving endangered species by linking health, habitat, reproduction, and ecological dynamics. This highlights the importance of establishing an epidemiological surveillance system and an awareness program must be carried out to reduce the health risks associated with owning pet turtles.

**Keywords:** Chelonian, cloacal microbiota, freshwater ecosystems, health risk, wildlife conservation, zoonotic pathogens.

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**Author contributions:** Feriel Benhafid, Badis Bakhouché, and Abdenour Moussouni led the study design, software, data handling, and visualization. Methodology, analysis, and fieldwork involved all co-authors. Writing was led by Feriel Benhafid and Abdenour Moussouni, with contributions from all. Supervision: Abdenour Moussouni and Naouelle Azzag. All authors contributed equally to the article and approved the submitted version.

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## INTRODUCTION

Freshwater turtles are key elements of aquatic ecosystems and play various ecological roles as predators and herbivores. Their presence in, and interactions with, the environment are crucial for maintaining the ecological balance and biodiversity of freshwater ecosystems (Wilbur 1997; Garig et al. 2020).

Among the species of chelonians in limnic ecosystems, the Mediterranean Pond Turtle *Mauremys leprosa*, widely distributed in northern Africa, the Iberian Peninsula, and southern France, stands out from other turtles occupying the same range (European Pond Turtle *Emys orbicularis*) due to its high tolerance to salinity, environmental pollution, and its adaptation to anthropogenic changes (Maran 1996; Bertolero & Busack 2017; El hassani et al. 2019). It is listed as Near Threatened in the IUCN Red List of Threatened Species in 2023 (Luiselli 2024), considered Vulnerable in the European Red List of Reptiles (Cox & Temple 2009), and included in Appendix II of the Bern Convention.

However, compared to other species, the Mediterranean Pond Turtle remains one of the least studied species in ecological research (Nowakiewicz et al. 2015). Indeed, only three studies focus on the ecology of this species in Algeria (Bakhouché et al. 2019; Ramedani et al. 2024).

To the best of our knowledge, none have examined the bacterial carriage of the Mediterranean Pond Turtle. Recent concerns regarding the potential transmission of zoonotic pathogens by turtles and reptiles were highlighted in several studies demonstrating the presence of zoonotic fecal bacterial carriage in these animals (Kautman et al. 2016; Hernandez et al. 2021). These facts raise concerns about the potential risks of transmitting dangerous pathogens to humans in Algeria, due to the commercialization of wild species known to be reservoirs of pathogens in several provinces (Tiar et al. 2019).

On the other hand, the close association of hosts and their microbiomes and the functional role of the microbiota provide essential information for ecological aspects of conservation biology (Zhu et al. 2021). Knowledge of microbiota can help identify specific ecological needs of endangered species, such as dietary preferences or habitat types that support a particular microbial community (Redford et al. 2012).

Our study seeks to characterize the aerobic cloacal and fecal microbiota of the Mediterranean Pond Turtle, focusing on identifying potential zoonotic pathogens within this microbial community. By examining the

turtle–bacteria interactions, we aim to better understand the role of bacteria as pathogenic agents in wild turtles.

## METHODS

### Study site

The study was conducted in Réghaïa's Lake which was designated as a RAMSAR site during 2003. The site is located 30 km from the city of Algiers (36.785 °N, 3.342 °E). Bordered to the north by the Mediterranean Sea, this lake takes the shape of a basin with a depth of 7–9 m for which Oued de Réghaïa constitutes the main tributary (Image 1). The shores of the lake are slightly sloping. The immediate surroundings consist of fallow fields, wild olive scrub, and mastic trees, as well as few groves of Eucalyptus. It remains in the region the only witness and remnant of the various biogeographical characteristics of the former coastal wetland areas of the Mitidja Plain.

### Study population and data collection

All captured turtles were marked to avoid duplicate sampling of the same individuals' turtles during the same study period. The surveys were carried out between March and May 2023, at a rate of two monitoring sessions per week. This period is considered optimal for studying the Mediterranean Pond Turtle of Reghaïa Lake, according to our study already conducted on the site in 2019 (Bakhouché et al. 2019). As the site is classified as a Ramsar site and where many birds nest, we chose to sample only at one station to avoid any disturbance. Capture of individuals was carried out using a net and a fishing line. Once the individuals are captured, they are marked. The marking involves making an incision with a file on the marginal scales of the carapace. Recaptures allow tracking individuals over time through their marking. Biometric measurements allow, on one hand, to compare individuals with each other, and on the other hand, to test relationship between bacterial carriage and species characteristics.

The method used for age estimation is direct count of growth rings. Sexual maturity was assessed based on the external secondary sexual characteristics of the shell and the sexual behavior of individuals.

Comparison of different morphological features was carried out using the Mann-Whitney U test for independent samples. Statistical analysis was conducted using IBM SPSS software version 27.





Image 1. Map showing the location of the site study (Reghaïa Lake) where the turtles were captured.

### Analyses of bacterial communities

Samples were taken in situ for each individual captured turtle. Two cloacal swabs were taken per specimen. One was used for selective enrichment and the second for non-selective enrichment. Samples are transported in an isothermal bag at 4 °C, then frozen at (-20) °C until bacterial analysis.

Isolation and bacteriological characterization were carried out at the medical microbiology laboratory of the Higher National Veterinary School of Algiers, following the steps below:

- Pre-enrichment: Inoculation on salmonella-selective Rappaport Vassiliadis Soy (RSV) broth and non-selective Brain Heart Infusion (BHI) broth to amplify bacterial numbers and increase chances of isolation. The swabs are immersed in the liquid medium in a sterile manner, close to the Bunsen burner. The tubes are then incubated at 37 °C for 18–24 hours.

- Isolation of bacteria on selective media: The bacterial isolation was performed on selective media (Microbiology agar, Merck KGaA, Germany) following pre-enrichment and subsequent Gram staining. Inoculation of four prepared culture media and incubation at 37 °C for 24–48 hours (Salmonella-Shigella (SS) agar: for selective isolation of Salmonella. Eosin Methylene Blue (EMB) agar: for the selective isolation of pathogenic *Escherichia coli* and other Enterobacteriaceae. MacConkey agar: for the selective isolation of lactose-positive Enterobacteriaceae and other gram-positive

bacteria. Chapman agar: for the selective isolation of Staphylococci and other gram-positive bacteria.)

- Biochemical characterization of isolated bacteria: Biochemical testing was conducted using API E20 test strips (Biomérieux SA, Marcy l'Etoile, France). The table 1 summarizes the various tests that were used.

### Turtle-Bacterial genera relationships

To highlight the relationships between the turtles and bacterial genera, we first performed binary logistic regressions using the 'stepwise top-down' method, aiming to maximize the explanation of the dependent variable (bacterial genus) with the lowest number of independent variables (Turtle characteristics). The logistic model belongs to the family of generalized linear models and links, by a linear combination, the environmental variables to the variable to be predicted by means of a logistic link function. It is a statistical tool recommended for binary data (in our case presence/absence of bacterial genera) (Guisan & Zimmermann 2000). We used IBM SPSS version 27 software.

Secondly, we performed a redundancy analysis (RDA) to examine relationships between the bacterial genera frequency and characteristics of Mediterranean Pond Turtle. RDA can highlight associations between specific morphological characteristics (such as weight, shell length) and microbiota composition. It is particularly useful when the data are multivariate, which is our case (several microbial genera and several morphological

traits) and allows us to better understand the interactions between the species, its microbiota and the environment.

To test the relationship significance between mentioned variables, we performed a Monte Carlo permutation test. For the purposes of RDA, we constructed four groups of variables by combining age and sex. The groups are respectively adult male, adult female immature male and the finally immature female. We performed the RDA analysis in XLSTAT (version 2021.2.2, Addinsoft).

## RESULTS

### Mediterranean Pond Turtle population

A total of 24 individuals were captured, including six females and 18 males. 10 individuals were recaptured at least once. There were no juveniles.

Descriptive statistics for the morphological features of turtles are summarised in the table below. Average length of the carapace is 163 mm. The posterior width of the carapace varies 90.5–122.6 mm with an average of 111.6 mm. The weight shows a very high standard deviation (211.7).

While comparing morphological features between turtle groups the only significant difference ( $\alpha = 0.03$ ) was observed in the distance between the cloaca and

the carapace (immature males and immature females). Other comparison showed no significant difference (Table 3).

### Bacterial carriage

Bacterial growth was assessed on 74 bacterial cultures. Identification methods resulted in the identification of 70 strains. Four (4) strains could not be identified by the tests used in our study due to the absence of reaction after subculturing. After elimination of strains corresponding to contaminants, we obtained a pure 60 bacterial strains. A total of 11 bacterial genera were identified (Table 4, Image 1). *Salmonella* was the most frequent isolated genus with a percentage of 21.66 (13/60) of the total isolates, followed by *Escherichia* and *Enterobacter*. (Figure 1). These are in reality the most abundant bacterial genera in the cloacal flora of the studied population, with prevalences of 54% and 42%, respectively. The prevalence of *Salmonella* spp. and *Klebsiella* spp. is significantly higher in females than in males. *Vibrio*, *Listeria*, *Yersinia*, and *Lactobacillus* were only found in males, while *Proteus* is present only in females (Table 4).

### Turtle–Bacterial genera relationships

We obtained a logistic regression model for six bacterial genera, namely: *Lactobacillus*, *Pseudomonas*, *Enterobacter*, *Vibrio*, *Salmonella*, and *Escherichia*.

**Table 1. Appropriate biochemical tests for bacterial identification.**

Test	Test principle	Reading
Voges-Proskauer (VP)	Reveals the capacity of bacteria to produce acetone during glucose fermentation. The test is carried out on Clark and Lubs broth already seeded, then incubated at 37 °C for 18 to 24 hours, to which VP1 and VP2 reagents are added respectively, with 10-minute intervals in between (Varghese & Joy 2014).	- Red/pink Coloration (VP +) - Incolore (VP -)
Rouge de méthyle (RM)	Reveals the ability of bacteria to oxidize glucose with the production of acid derivatives. This test is carried out on Clark and Lubs broth, which has already been seeded and then steamed at 37°C for 18 to 24 hours, to which methyl red reagent is added (Varghese & Joy 2014).	- Red Coloration (RM +) - Yellow Coloration (RM -)
Triple sugar iron (TSI)	Highlights the bacteria's ability to ferment the three sugars present in the medium (Glucose, sucrose and lactose). Inoculation is performed by a central prick in the tube and slope inoculation, followed by incubation at 37°C for 24 hours (Varghese & Joy 2014).	- Acidification of the slope (fermentation of sucrose, lactose or both) - Pellet acidification (glucose fermentation) - Black precipitates (H <sub>2</sub> S production and glucose fermentation)
Citrate	Highlights the ability of bacteria to use citrate as their sole carbon source. This test is performed by plating a slant agar on Simmons citrate tube incubated at 37°C for 24–48 hours (Varghese & Joy 2014).	- Blue Coloration (Citrate +) - Green coloration (Citrate -)
Mannitol motility	Used to detect bacterial mobility and mannitol fermentation. It is performed by central pricking in Mannitol motility agar incubated at 37 °C for 24–48 hours (Varghese & Joy 2014).	- Yellow Coloration (Mannitol +) - Red Coloration (Mannitol -) - Growth by creating a disturbance from the central prickle (Mobility +) - Growth all along the central sting (Mobility -)
Urease	Demonstrates the ability of bacteria to degrade urea to ammonia using urease. Inoculation of urea indole medium and incubation at 37 °C for 24 hours (Varghese & Joy 2014).	- Pink coloration (Urease +) - No colour change (Urease -)
Indole production	Demonstrates the ability of bacteria to degrade tryptophan to indole. Test performed on urea indole medium after seeding and incubation for 24 hours with the addition of KOVAC's reagent (Varghese & Joy 2014).	- Formation of a red ring on the surface of the medium (Indole +) - No red ring formation (Indole -)

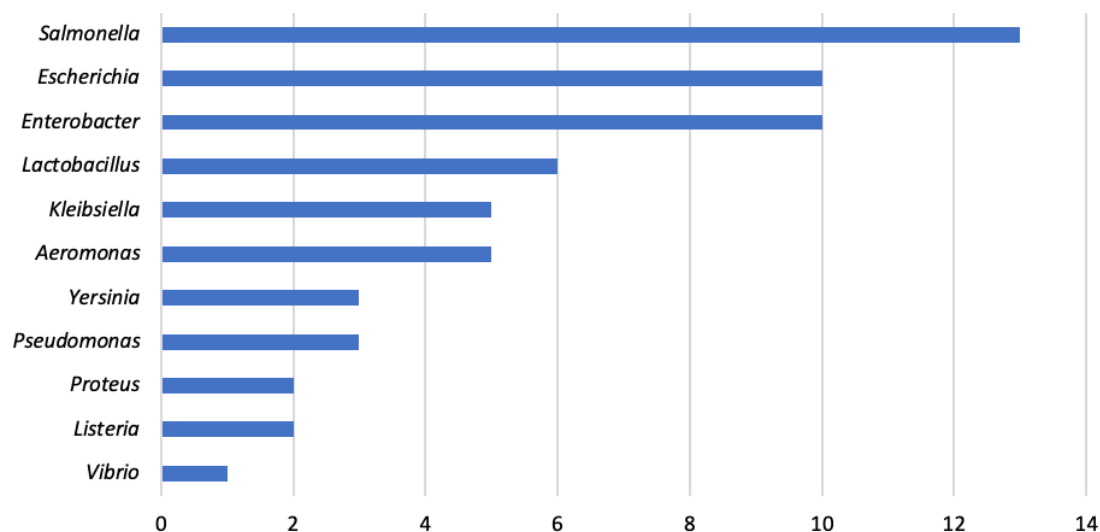


Figure 1. Graph showing frequency of isolation of bacterial genera the Mediterranean Pond Turtle captured in Reghaia Lake.

Table 2. Descriptive statistics of morphological features of the Mediterranean Pond Turtle.

Parameters (unit)	Code	Min.	Max.	Mean	Standard deviation
Carapace length (mm)	CL	126.75	185.00	163.69	26..22
Anterior width of carapace (mm)	AWC	79.25	109.33	97.98	13.34
Posterior width of carapace (mm)	PWC	90.50	122.57	111.58	14.91
Carapace height (mm)	H	41.75	58.71	52.71	7.76
Tail length (mm)	TL	75.25	87.67	82.07	5.14
Distance carapace–cloaque (mm)	CC	32.00	39.23	36.41	3.15
Weight (g)	W	268.50	727.46	567.65	211.69

Models were invalid for the other bacterial genera (Table 5).

The assessment of models' fit was based on Nagelkerke's  $R^2$ . Like the  $R^2$  for multiple regression, the higher the value of this coefficient, the better the model fit to data. Table 5 shows that models for *Pseudomonas* and *Vibrio* genera give a perfect prediction with a probability of one.

In order to identify variables that most predict presence probability of bacterial genera, we used the Wald statistic. For the *Enterobacter* genus model (Table 5) the 'Age' and 'H' parameters positively influenced ( $\text{Exp}(B) > 1$ ) the probability of presence of this genus at the threshold  $\alpha \leq 0.05$ , whereas there was a negative effect ( $\text{Exp}(B) < 1$ ) of the 'CL' ( $\alpha \leq 0.05$ ). The *Pseudomonas* and *Vibrio* models did not show any significant variables.

The 'CL' variable had a negative effect ( $\text{Exp}(B) < 1$ ;  $\alpha \leq 0.05$ ) on the presence of *Salmonella* and *Escherichia*. However, 'TL' had a positive effect ( $\text{Exp}(B) > 1$ ;  $\alpha \leq 0.05$ ) on the presence of *Escherichia*.

Results of RDA indicate a statistically significant association ( $p\text{-value} < 0.01$ ) between morphological features and bacterial genera frequency. The first two axes of the RDA together carry 83.36% of the constrained inertia. F1 axis carry 56% of the constrained inertia and F2 axis carry 27.36% (Figure 2).

MI and FA individuals contributed significantly to the construction of F1 axis and are well represented on this axis. Male individuals contributed to the construction of F2 axis and are also well represented on this axis (Table 6, Figure 2).

The bacterial genera *Lactobacillus*, *Klebsiella*, *Listeria*, and *Proteus* contributed significantly to the construction of F1 axis and are also well represented on it. The genera *Aeromonas*, *Enterobacter*, *Vibrio*, and *Yersinia* contributed significantly to the construction of F2 axis (Table 6, Figure 2).

Genera *Klebsiella* and *Proteus* are positively correlated with female individuals. Genera *Vibrio*, *Aeromonas*, *Yersinia*, and *Lactobacillus* are positively

Table 3. Summary of the Mann-Whitney U test.

Male Adult (MA) – Female Adult (FA)							
	CL	AWC	PWC	H	TL	CC	W
Mann-Whitney	25.5	14.5	21.5	22.00	20.5	27.0	22.0
Wilcoxon	130.5	119.5	126.5	127.0	125.5	132.0	127.0
Test statistics	25.50	14.50	21.50	22.00	20.50	27.00	22.0
SD	7.93	7.90	7.89	7.90	7.86	7.88	7.94
Standardised test statistics	0.57	-0.82	0.06	0.13	-0.06	0.76	0.13
Bilateral test	0.57	0.41	0.95	0.90	0.95	0.45	0.90
Male Immature (MI) – Female Immature (FI)							
	CL	AWC	PWC	H	TL	CC	W
Mann-Whitney	7,000	6.00	7.50	5.00	9.00	12.00	6.00
Wilcoxon	17,000	16.00	17.50	15.00	19.00	22.00	16.00
Test statistics	7,000	6.00	7.50	5.00	9.00	12.00	6.00
SD	2,828	2.83	2.78	2.83	2.80	2.83	2.83
Standardised test statistics	,354	0.00	0.54	-0.35	1.07	2.12	0.00
Bilateral test	,724	1.00	0.59	0.72	0.28	0.03	1.00

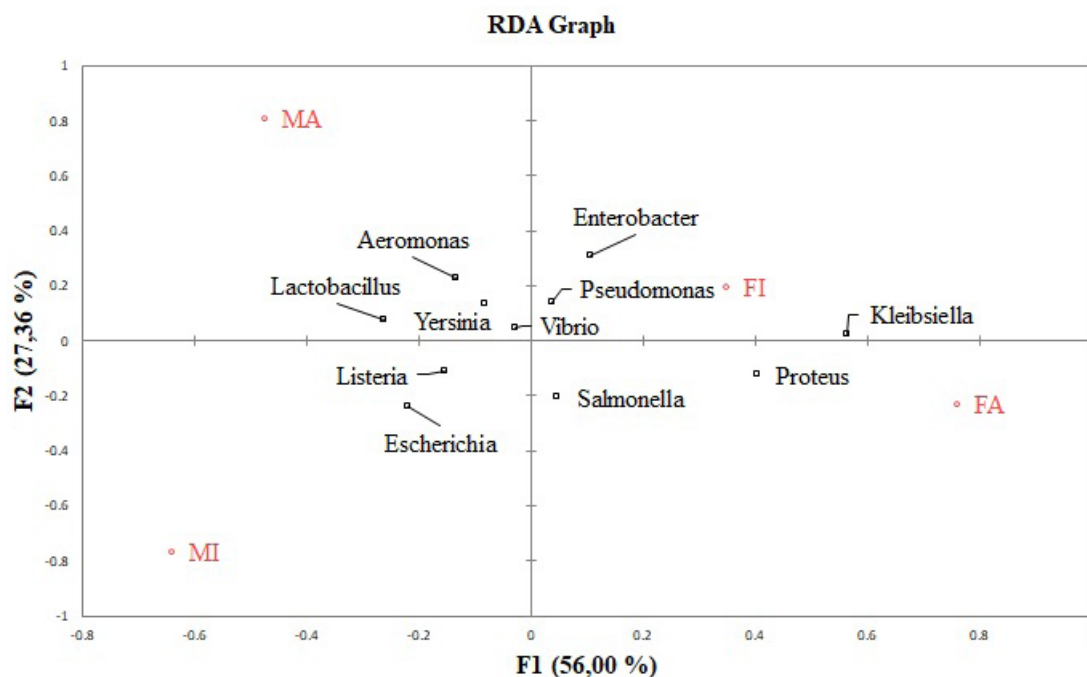


Figure 2. Redundancy analysis (RDA) ordination plot showing the relationship between the bacterial genera frequency and characteristics the individuals of the Mediterranean Pond Turtle at the site of Reghaïa Lake along RDA axes 1 and 2 (explaining 83.36% on the total variation).

correlated with adult males, while genera *Listeria* and *Escherichia* are more associated with immature males (Figure 2).

## DISCUSSION

The duration (March–May 2023) and the main objective of the study, which required laboratory work to isolate and characterise bacterial carriage, were the main cause for sampling effort reduction at one site.



Table 4. Bacterial genera identified and their prevalence in the Mediterranean Pond Turtle.

Turtles			Identified bacterial genus										
ID	Sex	Maturity	<i>Lactobacillus</i>	<i>Listeria</i>	<i>Aeromonas</i>	<i>Pseudomonas</i>	<i>Vibrio</i>	<i>Klebsiella</i>	<i>Enterobacter</i>	<i>Salmonella</i>	<i>Escherichia</i>	<i>Proteus</i>	<i>Yersinia</i>
1	F	A	0	0	0	0	0	1	0	0	0	1	0
2	F	A	0	0	0	0	0	0	0	1	1	1	0
3	F	A	0	0	0	0	0	1	1	1	0	0	0
4	F	I	0	0	0	1	0	1	0	0	0	0	0
5	F	I	0	0	0	0	0	0	0	1	1	0	0
6	F	I	0	0	0	0	0	1	1	1	0	0	0
7	M	A	1	0	1	0	0	1	1	0	0	0	0
8	M	A	0	0	0	0	0	0	0	0	0	0	1
9	M	A	1	0	0	0	0	0	0	0	1	0	1
10	M	A	0	1	0	1	0	0	0	0	0	0	1
11	M	A	0	0	1	0	0	0	0	1	1	0	0
12	M	A	0	0	1	1	0	0	1	0	1	0	0
13	M	A	0	0	1	0	0	0	0	0	0	0	0
14	M	A	0	0	0	0	0	0	0	0	0	0	0
15	M	A	0	0	1	0	0	0	1	0	0	0	0
16	M	A	1	0	0	0	0	0	1	1	0	0	0
17	M	A	0	0	0	0	0	0	1	1	1	0	0
18	M	A	1	0	0	0	0	0	1	1	0	0	0
19	M	A	0	0	0	0	0	0	0	1	1	0	0
20	M	A	1	0	0	0	1	0	1	1	0	0	0
21	M	I	0	0	0	0	0	0	0	1	1	0	0
22	M	I	0	1	0	0	0	0	1	0	1	0	0
23	M	I	0	0	0	0	0	0	0	1	1	0	0
24	M	I	1	0	0	0	0	0	0	1	0	0	0
Prevalence (%)			25	8.33	20.83	12.5	4.17	20.83	41.67	54.17	41.67	8.33	12.5

It is interesting to note, however, that sex ratio favour males, supporting that described in a study carried out on the same population by Bakhouch et al. (2019).

The Mediterranean Pond Turtle population of lake Reghaïa exhibits high variability in individual weight. This may influence microbiota diversity, particularly due to variation in body spaces, diets, physiological conditions, and social behaviors. Heavier individuals, often having more resources and space for their microbes, may support a more diverse microbiota than smaller individuals (Youngblut et al. 2019; Budd et al. 2020).

Bacteriological analysis led to the identification of 11 bacterial genera (*Lactobacillus*, *Listeria*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Klebsiella*, *Enterobacter*, *Salmonella*, *Escherichia*, *Proteus*, *Yersinia*). These results

correspond to a basic reptilian microbiota (Colston 2017). The predominance of *Pseudomonadota* could provide information on the diet of Mediterranean Pond Turtle population of lake Réghaïa, as they are associated with a predominantly carnivorous diet.

The growth of two gram+ bacterial genera (*Lactobacillus* and *Listeria*) on Chapman culture media (Salt Mannitol Agar) was made possible by their high tolerance of the medium's salinity. In fact, these genera tolerate concentrations of 12% and 20%, respectively (Cole et al. 1990; Osek et al. 2022), far exceeding the concentration of salt (selective agent) present in the culture media used (7.5%), which enabled these strains to be isolated. The mobility of the *Yersinia* strains isolated excludes the possibility that they belong to the

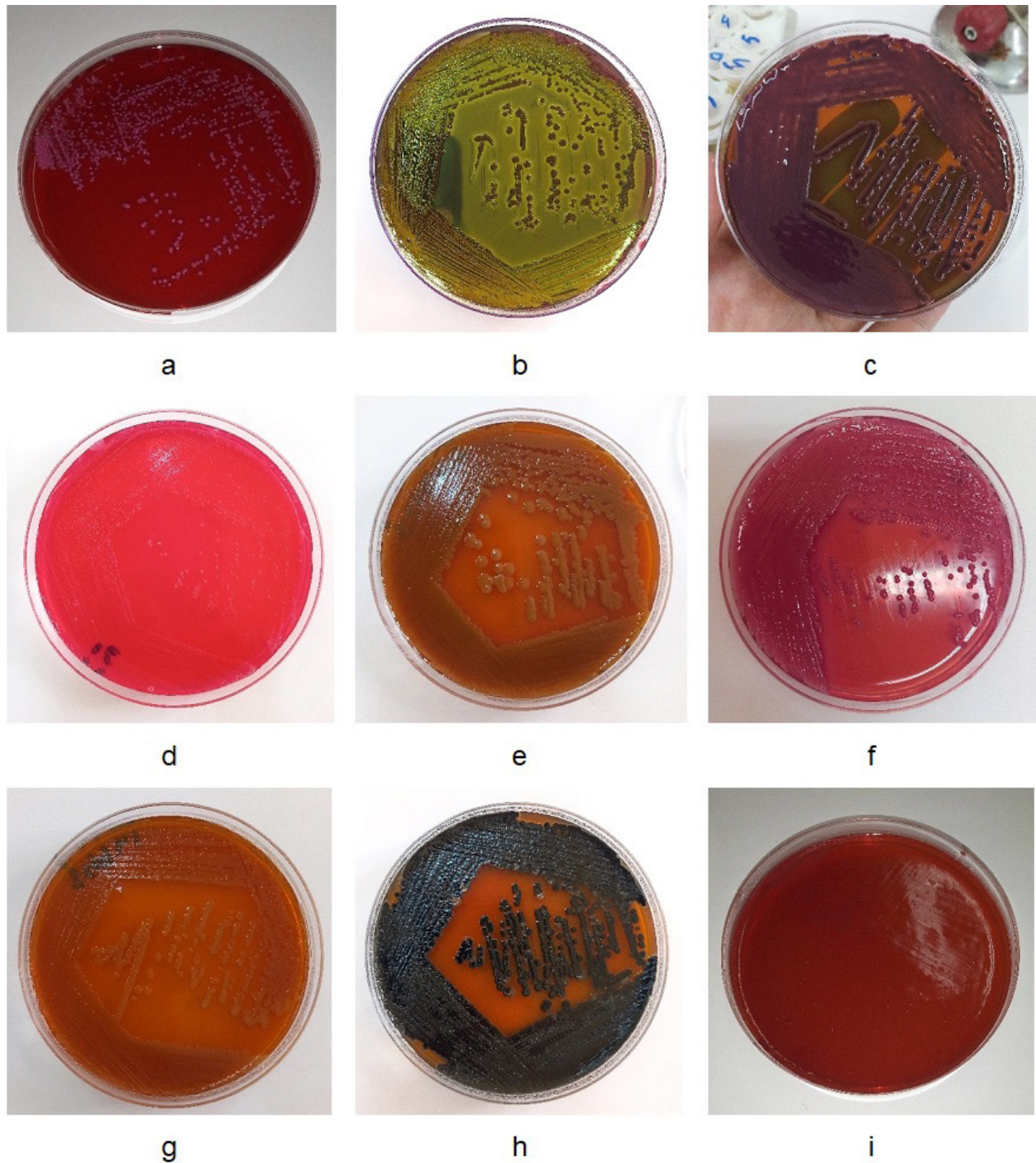


Image 2. Illustrations on some bacterial genera identified by biochemical tests. a—*Aeromonas* on MAC | b—*Escherichia* on EMB agar | c—*Klebsiella* on EMB agar | d—*Listeria* on MSA | e—*Proteus* on MAC | f—*Pseudomonas* on MAC | g—*Salmonella* (H2S-) on SS | h—*Salmonella* (H2S+) on SS | i—*Yersinia* on MAC. © Rayane Ahcene Reda BELAIDI.

species *Yersinia pestis*, which is an immobile bacterium (Jorgensen et al. 2015). This implies that our strains would belong to the species *Yersinia enterocolitica* or *Yersinia pseudotuberculosis*.

The cloacal bacterial carriage corresponds qualitatively and in proportions to those highlighted

in several previous studies of freshwater turtles, in particular *Emys orbicularis*, which is commonly recognised as syntopic for Mediterranean Pond Turtle (Hacioglu et al. 2012; Marin et al. 2013; Nowakiewicz et al. 2015; Ruzauskas et al. 2016).

The high prevalence of *Salmonella* and *Klebsiella*

Table 5. Logistic regression models of bacterial genera in the Mediterranean Pond Turtle.

		B	E.S	Wald	ddl	Sig.	Exp(B)	R <sup>2</sup> de Nagelkerke
<i>Lactobacillus</i>	Sex	-20.51	16408.71	0.00	1	0.999	0.000	0.231
	Constant	-0.69	0.50	1.92	1	0.166	0.500	
<i>Pseudomonas</i>	Age	4225.05	87867.62	0.00	1	0.962		1.000
	CL	-112.58	2356.29	0.00	1	0.962	0.000	
	AWC	161.06	3308.13	0.00	1	0.961	8.87E+69	
	PWC	-130.33	2688.50	0.00	1	0.961	0.000	
	H	84.24	1814.76	0.00	1	0.963	3.86E+36	
	TL	-100.06	2066.50	0.00	1	0.961	0.000	
	CC	135.82	2822.41	0.00	1	0.962	9.70E+58	
	Constant	12401.99	257536.43	0.00	1	0.962		
<i>Enterobacter</i>	Sex	-2.25	1.48	2.32	1	0.128	0.105	0.392
	Age	7.58	3.67	4.27	1	<b>0.039</b>	1956.076	
	CL	-0.29	0.13	5.07	1	<b>0.024</b>	0.748	
	H	0.66	0.32	4.29	1	<b>0.038</b>	1.939	
	Constant	7.76	5.95	1.70	1	0.192	2343.684	
<i>Vibrio</i>	Age	227.97	42188.59	0.00	1	0.996	1.01E+99	1.000
	CL	-4.96	1330.69	0.00	1	0.997	0.007	
	PWC	-2.13	1513.71	0.00	1	0.999	0.118	
	H	10.85	2657.93	0.00	1	0.997	51704.704	
	Constant	269.77	94378.88	0.00	1	0.998	1.44E+117	
<i>Salmonella</i>	CL	-0.20	0.10	3.97	1	<b>0.046</b>	0.820	0.348
	W	0.02	0.01	3.59	1	0.058	1.025	
	Constant	18.98	9.29	4.18	1	0.041	175005394.480	
<i>Escherichia</i>	Sex	-2.99	1.66	3.27	1	0.071	<b>0.050</b>	0.495
	CL	-0.30	0.15	3.88	1	<b>0.049</b>	0.740	
	PWC	0.46	0.25	3.51	1	0.061	1.585	
	TL	0.37	0.18	4.41	1	<b>0.036</b>	1.446	
	CC	-0.35	0.19	3.42	1	0.064	0.703	
	Constant	-19.51	10.40	3.52	1	0.061	0.000	

genera, along with the detection of *Listeria*, *Vibrio*, and *Yersinia* in our study, combined with the known role of freshwater turtles and reptiles as reservoirs of various zoonotic bacterial pathogens (Gaertner et al. 2008; Back et al. 2016; Hernandez et al. 2021), underscores the need to implement a surveillance system at the Reghaïa Lake, which is frequently visited by the public.

The presence of *Pseudomonas* spp. which are bacteria that inhabit soil and water, can also cause disease in plants, animals as well as immunocompromised humans (Wu et al. 2015). It is commonly found in the oral cavity and intestinal tract of reptiles and can cause a number of diseases such as ulcerative stomatitis, pneumonia, dermatitis and septicaemia (Campa et al. 1993; Warwick et al. 2013). In the Silene Nature Park (Latvia), it was

the most common bacterium found in all skin areas (cloaca, mouth, and feet) of *Emys orbicularis* individuals (Umbrasko et al. 2020).

Presence of *Enterobacter* genus in Mediterranean Pond Turtle was positively influenced by age and carapace height parameters, which are associated with larger individuals. In *Emys orbicularis*, this genus was more abundant in juveniles in the study conducted by Nowakiewicz et al. (2015).

The presence of *Salmonella* in Mediterranean Pond Turtle is negatively influenced by carapace size. It is therefore associated with small turtles. This poses an even greater risk to children who acquire them as pets. In fact, children run a high risk of contracting salmonellosis associated with small turtles, as they can



Table 6. Results of the redundancy analysis (RDA).

Observations						
	Scores		Contributions		Square cosines	
	F1	F2	F1	F2	F1	F2
Obs1 (MA)	-0,446	0,759	0,168	0,488	0,224	0,650
Obs2 (MI)	-0,601	-0,724	0,305	0,443	0,407	0,591
Obs3 (FA)	0,717	-0,219	0,435	0,041	0,580	0,054
Obs4 (FI)	0,330	0,183	0,092	0,028	0,122	0,038
Response variables						
	Scores		Contributions		Square cosines	
	F1	F2	F1	F2	F1	F2
<i>Lactobacillus</i>	-0,262	0,076	0,103	0,018	0,827	0,070
<i>Listeria</i>	-0,154	-0,107	0,036	0,036	0,671	0,325
<i>Aeromonas</i>	-0,135	0,229	0,027	0,163	0,224	0,650
<i>Pseudomonas</i>	0,039	0,143	0,002	0,064	0,024	0,325
<i>Vibrio</i>	-0,027	0,046	0,001	0,007	0,224	0,650
<i>Klebsiella</i>	0,563	0,026	0,479	0,002	0,936	0,002
<i>Enterobacter</i>	0,107	0,311	0,017	0,299	0,102	0,868
<i>Salmonella</i>	0,047	-0,204	0,003	0,129	0,046	0,853
<i>Escherichia</i>	-0,221	-0,240	0,074	0,178	0,458	0,541
<i>Proteus</i>	0,404	-0,123	0,247	0,047	0,580	0,054
<i>Yersinia</i>	-0,081	0,138	0,010	0,059	0,224	0,650

be easily handled and placed in the mouth (CDC 2008). Two cases of turtle-associated salmonellosis in children were reported in Japan in 2007 and 2008 (Kuroki et al. 2015).

RDA is a proven multivariate analysis technique for processing species-environment data (Legendre & Legendre 1998). It was used to analyse the determinism of biometric parameters on the presence of bacterial genera. The first group identified comprises the genera isolated only from adult male Mediterranean Pond Turtle, which are generally isolated from freshwater (*Vibrio* and *Aeromonas*), plants and soil (*Yersinia*). Cloacal transmission during copulation could also be a means of acquiring new microbial species (Hidalgo-Vila et al. 2007; Nowakiewicz et al. 2015).

The second group includes the genus *Proteus* and *Klebsiella* specific to adult females in captured Mediterranean Pond Turtle. These bacteria of *Proteus* genus are part of pathogenic or normal microflora and can be symbiotic or change from neutral/commensal to parasitic (Drzewiecka 2016). They can also be interpreted as an indicator of pollution (Al-Bahry et al. 2012). In our case, the individuals captured were apparently in very good health, which suggests that the presence of the *Proteus* genus is due more to environmental pollution,

which is well established in the lake. However, the risk remains high, as studies (Oros et al. 2005; Awong-Taylor et al. 2008) have reported a low hatching success rate in Loggerhead Turtles linked to *Proteus* spp. and mortality in marine turtles in the Canary Islands.

The diversity of genera isolated from juveniles is relatively low compared with adults. The same observation has been made in *Emys orbicularis* in Poland (Nowakiewicz et al. 2015). This phenomenon may be linked to a number of nutritional and/or behavioural factors.

These findings highlight the presence of bacterial genera that include species known to be potentially pathogenic to humans, suggesting the need for further investigation into their pathogenicity and potential implications for public health. People living near the Réghaia wetland, visitors and traders who sell turtles should be more careful when handling them.

Despite its status as a protected species under national regulations (Executive Decree No. 12-235 of 24 May 2012 establishing the list of protected non-domestic animal species in Algeria), Mediterranean Pond Turtle is still one of the illegally traded species (personal unpublished data). Tighter controls should minimise the risk of diseases being transmitted by turtles. A ban

on small turtles' sale (shells less than four inches long) prevented around 100,000 cases of turtle-associated salmonellosis in children in 1980 which constitutes a good example of biodiversity health related conservation (CDC 2008).

This data highlights the importance of setting up an epidemiological and microbiological surveillance system, and strengthens the need to implement environmental protection programmes. In addition, an awareness-raising programme needs to be carried out to raise awareness of significant health risks associated with pet turtle ownership.

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