Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Blood cell morphology of the Mediterranean pond turtle (*Mauremys leprosa* Schweigger, 1812) from contrasting habitats in northeastern Algeria

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ABSTRACT. We examined the blood cell profiles of the Iberian pond turtle, also known as the Mediterranean pond turtle (*Mauremys leprosa*) in two habitats in northeastern Algeria with markedly contrasting water quality. Blood smears were prepared from 38 adult turtles collected at a polluted reference site (Bouhamra) and an unpolluted site (Brabtia). Differential leukocyte counts and erythrocyte morphometrics (length, width, and surface area) were quantified. At Brabtia Reserve, heterophils were the most abundant leukocyte type (41.35%), followed by lymphocytes (27.54%) and monocytes (10.16%). At the Bouhamra site, heterophils are also dominant (57.86%), followed by monocytes (21.40%) and lymphocytes (12.80%). The average ratio of heterophils to lymphocytes (H/L) was 4.39 in the polluted site, Bouhamra, which is about three times higher than the ratio of 1.60 in the pristine, indicating elevated physiological stress. Erythrocyte dimensions differed significantly between sites. In turtles from Brabtia, mean erythrocyte length, width, and surface were 24.10 μm, 13.68 μm, and 260.97 μm^2, respectively. Corresponding values in turtles from the degraded habitat (Bouhamra) were larger (25.07 μm, 13.96 μm, and 274.95 μm^2). Nuclear length, nuclear width, nuclear surface, and nuclear shape index (NL/NW) did not vary between habitats. Our results show that contamination mainly affects the size of red blood cells, while the morphological parameters of the nucleus remain stable. The higher H/L ratio at the polluted site (Bouhamra) also supports the use of leukocyte profiles as solid indicators of chronic environmental stress in freshwater turtles.

Keywords: *Mauremys leprosa*, blood cell morphology, H/L ratio, environmental stress, Algeria

For citation: Frih A., Sahraoui L., Hadiby R., Ziane N., Frih H., Rouag R. Blood cell morphology of the Mediterranean pond turtle (*Mauremys leprosa* Schweigger, 1812) from contrasting habitats in northeastern Algeria // Limnology and Freshwater Biology. 2025. - № 6. - P. 1281-1288. DOI: 10.31951/2658-3518-2025-A-6-1281

1. Introduction

Mauremys leprosa (Schweigger, 1812), also known as the Mediterranean pond turtle, is a freshwater turtle species endemic to southwestern Europe and northwestern Africa (Palacios et al., 2015; Laghzaoui et al., 2020). Two subspecies are present in the northwest of Africa: *M. leprosa leprosa* and *M. leprosa saharica* (Fritz et al., 2006; Veríssimo et al., 2016). The subspecies *M. l. saharica* is widely distributed in Algeria, from the coastal zones to the Sahara (Rouag et al., 2024). The population of this species is decreasing for several reasons, and the reasons for this decline are numerous, including habitat loss, urbanisation, intensified agriculture, and habitat fragmentation (Mateo et al., 2003; Rouag et al., 2024; Díaz-Paniagua et al., 2015).

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Received: July 31, 2025;
Accepted after revised: November 07, 2025;
Available online: December 25, 2025

In the present study, we evaluate the morphology and size of blood cells of *M. leprosa* in contrasted habitats to measure the impact of habitat quality on turtle physiology. The haematological profile is a commonly used practical method for assessing environmental effects. While many studies have described the blood profiles of reptiles (Sypek and Borysenko, 1988; Campbell, 1998; Stein, 1996; Mader, 2000; Sykes and Klaphake, 2008), reference values for many species are unavailable, particularly for freshwater turtles, where haematological reference values are still poorly understood. Haematological values can change due to factors such as gender, age, season, and location, and without reference values, it becomes difficult to compare and understand the results. This study aims to establish haematological values for *Mauremys leprosa* and provide a detailed description of blood cell morphology by comparing individuals from contrasting environments. These results will help improve the assessment of health status in wild populations of *Mauremys leprosa* and contribute valuable reference data for future ecological and conservation studies.

2. Material and Methods

The first site, Oued Bouhamra (36°52′25.0″N 7°43′23.0″E), is a polluted watercourse with low levels of dissolved oxygen (5.76-13.9 mg/L), high turbidity (28.3-31.2 NTU), high conductivity (2,731-1,950 μS/cm) and high total dissolved solids (1,200-1,365 mg/L). This site has the characteristics of a degraded habitat. Several metallic elements also exceed or approach internationally established reference thresholds, including manganese (60.1 mg/L), nickel (17.07 mg/L), iron (1.48 mg/L), arsenic (0.063 mg/L), and lead (0.049 mg/L). These results suggest that the site is subject to significant anthropogenic inputs, reflecting a state of contamination that could potentially affect the ecological functioning of the aquatic ecosystem. The vegetation consists mainly of reeds (*Phragmites australis*) and rushes (*Juncus acutus*) on the banks and patches of ribbon grass (*Typha angustifolia*) in the middle of the stream. The pristine site is a pond situated in El Kala (36°51′09.0″ N and 8°19′52.0″ E), located in the Brabtia Zoological Park. The site is a spring-fed pond of 0.5 ha, surrounded by a belt of woody vegetation composed of *Fraxinus communis*, *Populus alba*, and *Alnus glutinosa*, with emergent vegetation composed of *Nuphar luteum*, *Juncus acutus*, and *Phragmites communis*. The study was conducted from March to August 2024. Sampling was conducted manually and with the aid of a dip net. Individual variables were measured for all specimens, such as body size and weight. Carapace length was measured using a digital caliper (precision ± 0.1 mm). The body weight of the turtles was measured using a digital balance (precision ± 1 g). Sex was determined by secondary sexual characteristics (e.g., plastral concavity, tail length) (Servan et al., 1989). We prepared a blood smear on a microscope slide using a 1 mL syringe with a 27 G, 0.5 inch, and 0.413 mm diameter needle taken from the caudal sinus at the base of the tail. The blood smears were then air-dried, fixed in absolute methanol for 10 minutes and stained with Giemsa diluted 1:9 with phosphate buffer solution (pH 7.2) for 40 minutes. The blood smears were then used to identify and quantify leukocytes, as well as to measure the size of erythrocytes. The proportion of different types of leukocytes was assessed on the basis of an examination of 100 leukocytes under 1000x magnification under oil immersion. On each blood smear, the length (EL) and width (EW) of randomly selected erythrocytes, as well as the length (NL) and width (NW) of their nuclei, were measured. The size of the erythrocytes and their nuclei were calculated using the formulas: $S = \pi \times (L/2) \times (W/2)$, where S is the surface area (μm^2); L is the length (μm); and W is the width (μm) of the cell or nucleus (Petrov and Stepanyan, 2016). Cell and nuclear shapes were compared using the EL/EW and NL/NW ratios, while the nucleus/cytoplasm ratio was compared using the N/C ratio.

Photographs of blood cells were taken with the Olympus CX21 imaging system. SPSS software (version 29.0) was used to perform the statistical analyses. After performing Levene's and Shapiro-Wilk tests, the means were compared using a t-test and a U-test, presented with standard deviations (SD).

3. Results

3.1. Body size

In the pond at the Brabtia reserve, the females are larger and heavier than the males, but this difference is not statistically significant: Weight (males: 437.82 ± 193.84 g, range: 130-698 g; female: 584.00 ± 225.49 g, range: 230-799 g; $p > 0.05$), straight carapace length (male: 157.41 ± 32.10 mm, range: 102.5-197 mm; female: 164.33 ± 24.88 mm, range: 123-188 mm; $p > 0.05$). In the Bouhamra site, males and females do not differ significantly in terms of straight carapace length (males: 179.52 ± 31.18 mm, range 109.58-218.12; females: 174.49 ± 29.78 mm, range 129-220 mm; $P > 0.05$) and body weight (males: 699 ± 230.25 g, range: 195-980 g; females: 668.05 ± 260.48 g, range 258-1295 g; $P > 0.05$).

3.2. Differential white blood cell counts

In the pond at the Brabatia Reserve, heterophils are the most abundant leukocytes, with an average of 41.35% in both males and females. Lymphocytes are more abundant than monocytes, with an average of 27.54% and 10.16%, respectively. There are no significant differences between males and females except for basophils, which are more elevated in females. This difference was confirmed by a Student's t-test ($t = -2.35$; $p = 0.03$), as shown in (Table 1).

At the Bouhamra site, heterophils are the most abundant granulocytes, making up 53.67% of the leukocytes in males and 61.53% in females. They are followed by monocytes, which make up 19.47% in males and 23.09% in females. Lymphocytes account for 13.44% of males and 12.25% of females. All leukocyte subtypes except lymphocytes show significantly higher percentages in females than in males ($p < 0.001$). No

Table 1. Differential white blood cell counts in peripheral blood of male and female *M. leprosa* at the Brabtia site. For each leukocyte cell type, mean ± SD and range are shown.

	Male (N = 9)		Female (N = 9)		t-Test	
	X ± SD	Min-Max	X ± SD	Min-Max	*t*	*p*
Lymphocyte (%)	28.11 ± 9.38	18.27 - 50.43	26.97 ± 7.01	19.99 - 42.27	-0.29	0.77
Heterophil (%)	39.16 ± 9.56	22.70 - 54.59	43.54 ± 8.03	27.31 - 52.87	1.05	0.30
Eosinophil (%)	13.53 ± 5.13	7.00 - 21.64	11.84 ± 4.75	6.60 - 20.53	-0.72	0.47
Basophil (%)	9.17 ± 4.64	3.23 - 17.58	5.08 ± 2.36	2.94 - 10.26	-2.35	**0.03**
Monocyte (%)	10.02 ± 4.26	2.30 - 16.21	10.29 ± 3.91	4.69 - 16.58	0.13	0.89
H/L	1.52 ± 0.53	0.45 - 2.40	1.68 ± 0.41	0.96 - 2.24	0.67	0.50

significant difference exists between males and females for the H/L ratio ($t = -0.77$, $p = 0.446$). The strong predominance of heterophils and the low proportion of lymphocytes indicate a stress-related leukocyte shift in both sexes. However, sex does not influence the H/L ratio at this polluted site, suggesting a generalized physiological stress response (Table 2).

A comparative analysis of leukocyte parameters in the *Mauremys leprosa* population from unpolluted and polluted sites revealed significant differences in all examined variables (Student's t-test, $p < 0.001$ for each parameter). More specifically, the proportion of lymphocytes was significantly higher at the unpolluted site (27.54%), while the proportions of heterophils, basophils, monocytes, and the H/L ratio were significantly higher in the polluted site population.

The percentage of eosinophils was significantly lower in individuals from the polluted site (2.89%) than in turtles from the unpolluted site (12.68%). These results demonstrate the significant impact of pollution on leukocyte composition, leading to a reduction in lymphocytes and an increase in leukocytes associated with the immunity response in populations exposed to pollutants. The H/L ratio indicates a state of physiological stress caused by a degraded environment; the average value is 4.39 for both males and females, which is almost three times higher than at the unpolluted site (1.60). This suggests the presence of stress or activation of the innate immune response (Aguirre et al., 1995; Davis et al., 2008). These results highlight the profound influence of environmental quality on the immune status of *M. leprosa*, supporting the use of leukocyte profiles as effective biomarkers of ecological health and pollution exposure in freshwater turtles (Fig. 1).

3.3. Erythrocyte measurement

At the Brabtia site, a morphometric analysis of *Mauremys leprosa* erythrocytes revealed no significant differences between males and females for any measured parameter. The average values of cell dimensions (length, width, and surface area) and nuclear dimensions, as well as morphometric ratios, were similar between sexes. All statistical tests (Mann-Whitney) showed non-significant differences ($p > 0.05$), including erythrocyte length ($U = 45.0$, $p = 0.72$); erythrocytes width ($U = 53.5$, $p = 0.26$); erythrocytes surface ($U = 37.0$, $p = 0.79$); nucleus length ($U = 43.0$, $p = 0.85$); nucleus width ($U = 38.5$, $p = 0.89$); and nucleus surface ($U = 35.0$, $p = 0.20$), as well as EL/EW ($U = 31.0$, $p = 0.421$), NL/NW ($U = 52.5$, $p = 0.308$), N/C ratio ($U = 27.5$, $p = 0.209$). This further confirms that blood cell size and shape in *Mauremys leprosa* are not sexually dimorphic at this site (Table 3).

At the Bouhamra site, the average length of erythrocytes in males was established as 25.12 ± 0.21 µm, and the width–13.95 ± 0.18 µm. The size was calculated as 276.66 ± 6.28 µm². The EL/EW ratio was identified as 1.8 ± 0.02. The N/C ratio was 1.4. The average length of the nucleus was 9.76 ± 0.11 µm and its width was 5.02 ± 0.10 µm. The average size was near 38.49 ± 1.17 µm². The NL/NW ratio was 1.93 ± 0.03 (Table 4). These values are close to those of females, where the average length of erythrocytes was 25.03 ± 0.21 µm, the width 14.00 ± 0.20 µm, and the size was 275.64 ± 6.78 µm². The EL/EW ratio was calculated as 1.79 ± 0.02. The average length of the nucleus was calculated as 9.73 ± 0.12 µm and the width–as 5.01 ± 0.12 µm, and the size as 38.40 ± 1.130 µm². The NL/NW ratio of

Table 2. Differential white blood cell counts in peripheral blood of male and female *M. leprosa* at the Bouhamra site. For each leukocyte cell type, mean ± SD and range are shown.

	Male (N = 14)		Female (N = 16)		t-Test	
	X ± SD	Min-Max	X ± SD	Min-Max	*t*	*p*
Lymphocyte (%)	13.44 ± 3.53	9.64-19.38	12.25 ± 1.95	8,94-15.18	1.17	0.251
Heterophil (%)	53.67 ± 2.25	46.92-56.08	61.53 ± 1.72	57.35-63.62	-10.60	**0.000**
Eosinophil (%)	2.68 ± 0.11	2.35-2.80	3.08 ± 0.09	2.87-3.18	-10.60	**0.000**
Basophil (%)	10.73 ± 0.45	9.38-11.22	12.31 ± 0.34	11.47-12.72	-10.60	**0.000**
Monocyte (%)	19.47 ± 2.76	13.70-22.73	23.09 ± 2.15	20.48-28.32	-3.98	**0.000**
H/L	4.26 ± 1.10	2.51-5.72	4.55 ± 0.80	3.52-6.07	-0.77	0.446

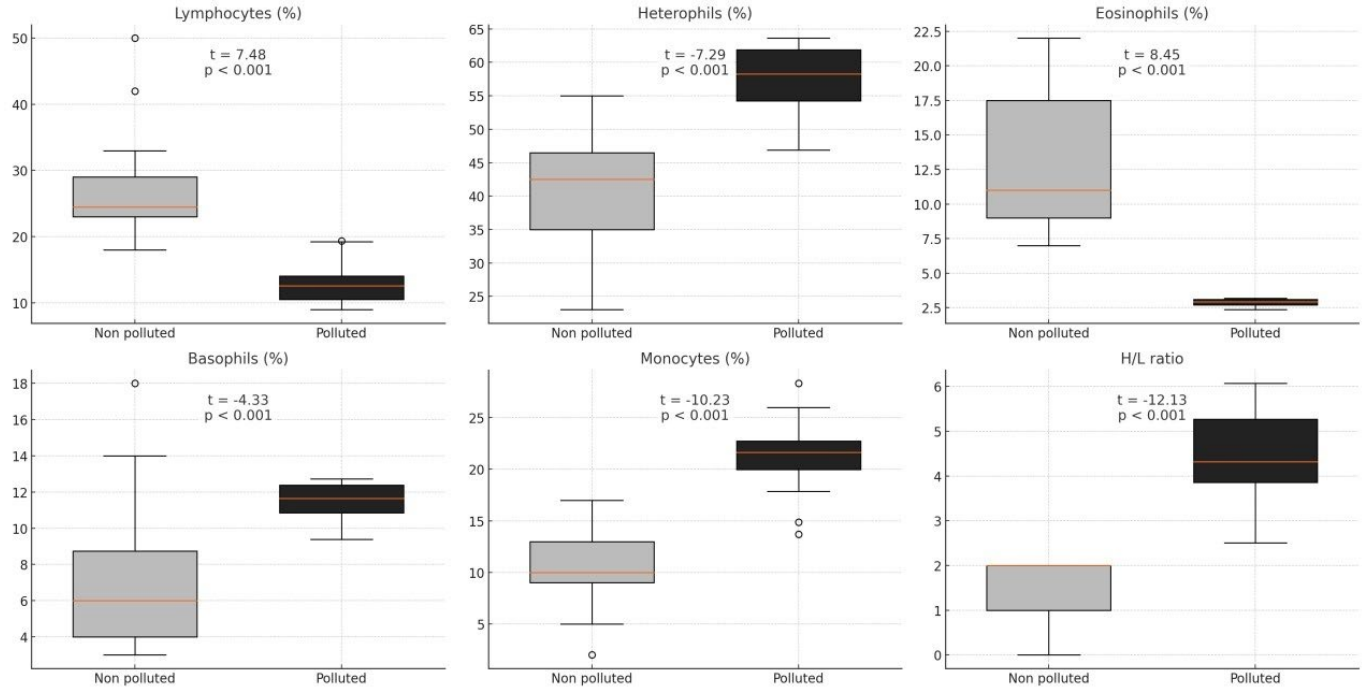

Fig.1. Comparison of leukocyte parameters between non-polluted and polluted sites in *Mauremys leprosa*.

1.93 ± 0.03 is also close to the male ratio. The Mann-Whitney tests of the morphometric descriptors of leukocytes carried out between males and females revealed no significant difference in all parameters, including erythrocyte length (U = 34.0, p = 0.38;); erythrocyte width (U = 46.5, p = 0.057); erythrocyte surface (U = 38.0, p = 0.59); nucleus length (U = 30.0, p = 0.21); nucleus width (U = 42.5, p = 0.85); and nucleus surface (U = 37.0, p = 0.60) (Table 4).

The morphometric comparison of erythrocytes and their nuclei in individuals of *Mauremys leprosa* from unpolluted and polluted habitats reveals significant differences in erythrocyte morphology, particularly in length (EL), width (EW), surface (ES), and the EL/EW ratio. Erythrocyte length was significantly higher in turtles from the polluted site (25.07 ± 0.21 µm) than in those from the unpolluted site (24.10 ± 1.20 µm; p = 0.003), as was erythrocyte width (25.12 ± 0.21 µm vs. 24.16 ± 1.20 µm; p = 0.043). Similarly, the erythrocyte surface (ES) was significantly higher in turtles from the polluted site (274.95 ± 5.72 µm²) than in turtles from the unpolluted site (260.97 ± 19.74 µm²; p = 0.009), and a significant increase in the EL/EW ratio was observed in turtles from the polluted site (p = 0.027). The nucleocytoplasmic ratio (N/C) was higher in turtles from the unpolluted site (0.15 ± 0.01) than in those from the polluted site (0.14 ± 0.00; p = 0.028). However, nuclear dimensions (NL and NW), nuclear surface (NS), and nuclear shape (NL/NW) did not differ significantly between habitats (Fig. 2).

4. Discussion

This study highlights the impact of habitat quality on blood cell morphology in *Mauremys leprosa*, reflecting the physiological effects of environmental pollution. Research conducted over the past indicates that the quantification of haematological parameters

Table 3. Measurements of erythrocytes and their nuclei in male and female Brabtia turtles and the Mann-Whitney U test statistics.

	Population (N = 18)			Male (N = 09)			Female (N = 0 9)			U-Test	
	X ± SD	Min	Max	X ± SD	Min	Max	X ± SD	Min	Max	*U*	*P*
EL (µm)	24.10 ± 1.20	21.90	25.30	24.16 ± 1.20	22.00	25.30	24.05 ± 1.27	21.90	25.30	45.0	0.724
EW (µm)	13.68 ± 0.46	12.78	14.40	13.80 ± 0.35	13.10	14.17	13.55 ± 0.54	12.78	14.40	53.5	0.268
EL/EW	1.76 ± 0.06	1.58	1.83	1.75 ± 0.07	1.58	1.80	1.77 ± 0.05	1.69	1.83	31.0	0.421
ES (µm²)	260.97 ± 19.74	219.75	281.50	261.88 ± 17.97	231.00	281.10	260.06 ± 22.43	219.75	281.50	37.0	0.791
NL (µm)	9.68 ± 0.41	8.82	10.40	9.70 ± 0.48	8.82	10.40	9.67 ± 0.35	9.00	10.20	43.0	0.859
NW (µm)	5.04 ± 0.27	4.60	5.60	5.02 ± 0.32	4.60	5.60	5.07 ± 0.22	4.80	5.50	38.5	0.894
NL/NW	1.92 ± 0.05	1.81	2.02	1.94 ± 0.05	1.86	2.02	1.91 ± 0.05	1.81	1.96	52.5	0.308
NS (µm²)	39.19 ± 4.06	33.20	48.50	38.68 ± 4.75	33.20	48.50	39.70 ± 3.44	35.40	45.00	35.0	0.659
N/C ratio	0.15 ± 0.02	0.14	0.20	0.15 ± 0.02	0.14	0.20	0.15 ± 0.02	0.14	0.18	27.5	0.209

Note: EL: Erythrocyte length; EW: Erythrocyte width; ES: Erythrocyte size; NL: Nucleus length; NW: Nucleus width; NS: Nucleus size; and NS/ES: Nucleocytoplasmic ratio.

Table 4. Measurements of erythrocytes and their nuclei in male and female Bouhamra turtles and the Mann-Whitney U test statistics.

	Population (N = 19)			Male (N = 09)			Female (N = 10)			U-Test	
	X ±SD	Min	Max	X ±SD	Min	Max	X ±SD	Min	Max	*U*	*P*
EL (µm)	25.07±0.21	24.7	25.4	25.12±0.21	24.7	25.4	25.03 ± 0.21	24.6	25.3	34.0	0.38
EW (µm)	13.96±0.2	13.6	14.2	13.95±0.18	13.6	14.2	14.00 ± 0.20	13.6	14.2	46.5	0.93
EL/EW	1.8±0.02	1.77	1.83	1.8±0.02	1.77	1.83	1.79 ± 0.02	1.77	1.83	36.5	0.50
ES (µm²)	274. 95±5.72	263.6	282.3	276.66±6.28	269.1	282.3	275.64 ± 6.78	263.6	282.3	38.0	0.59
NL (µm)	9.72±0.1	9.5	9.9	9.76±0.11	9.6	9.9	9.73 ± 0.12	9.5	9.9	30.0	0.21
NW (µm)	5.04±0.11	4.8	5.2	5.02±0.10	4.8	5.2	5.01±0.12	4.8	5.2	42.5	0.85
NL/NW	1.93±0.03	1.9	2.0	1.93±0.04	1.9	2.0	1.93±0.03	1.9	1.98	36.5	0.49
NS (µm²)	38.44±1.21	36.0	40.9	38.49±1.17	36.2	39.6	38.40±1.130	36.0	40.9	37.0	0.53
N/C ratio	0.14	0.14	0.15	0.14	0.14	0.15	0.14	0.13	0.15	40.5	0.60

Note: EL: Erythrocyte length; EW: Erythrocyte width; ES: Erythrocyte size; NL: Nucleus length; NW: Nucleus width; NS: Nucleus size; and NS/ES: Nucleocytoplasmic ratio.

may be measured as a complement to the measurement of hormones in the study of vertebrate stress responses. Leukocyte profiles are particularly important in the study of physiology because they are directly linked to hormone levels. Considerable research has demonstrated that the adrenal and leukocyte responses to stress are closely related and similar across vertebrate taxa. (Gross and Siegel, 1983; McFarlane and Curtis, 1989; Romero and Reed, 2005; Davis et al., 2008). This hematological approach offers key advantages. Compared to adrenal hormone levels, which can vary quickly and need to be checked right away, white blood cell counts stay stable for a longer time after being checked, especially in ectotherms such as turtles (Romero and Reed, 2005; Davis et al., 2008). As shown in other studies, in contrast to many reptiles, the most common leukocytes in *M. leprosa* are not lymphocytes, but rather heterophils (Heatley and Russell, 2019).

In our study, heterophils were the most prevalent type of leukocyte in turtles living in pristine habitats, with mean values of 41.35%; lymphocytes followed with 28.11%. Heterophils were the most common leukocytes and were two times more prevalent than eosinophils. Similar high frequencies have been described for *Trachemys scripta* (Isis, 2002; Novoveský and Halán, 2019), *Mauremys leprosa* (Hidalgo-Vila et al., 2007; Marques et al., 2025), and Arrau turtle *Podocnemis expansa* (Rossini et al., 2012). However, the results of this research only partially corresponded with ours; the values of the other parameters differed greatly and only partially aligned with ours. It is important to note that the blood parameters of chelonians are influenced by many factors, such as season, age, sex, health status, geographic location, physiological state, and reproductive status (Christopher et al., 1999; Jacobson, 2007). Marques et al. (2025) found the

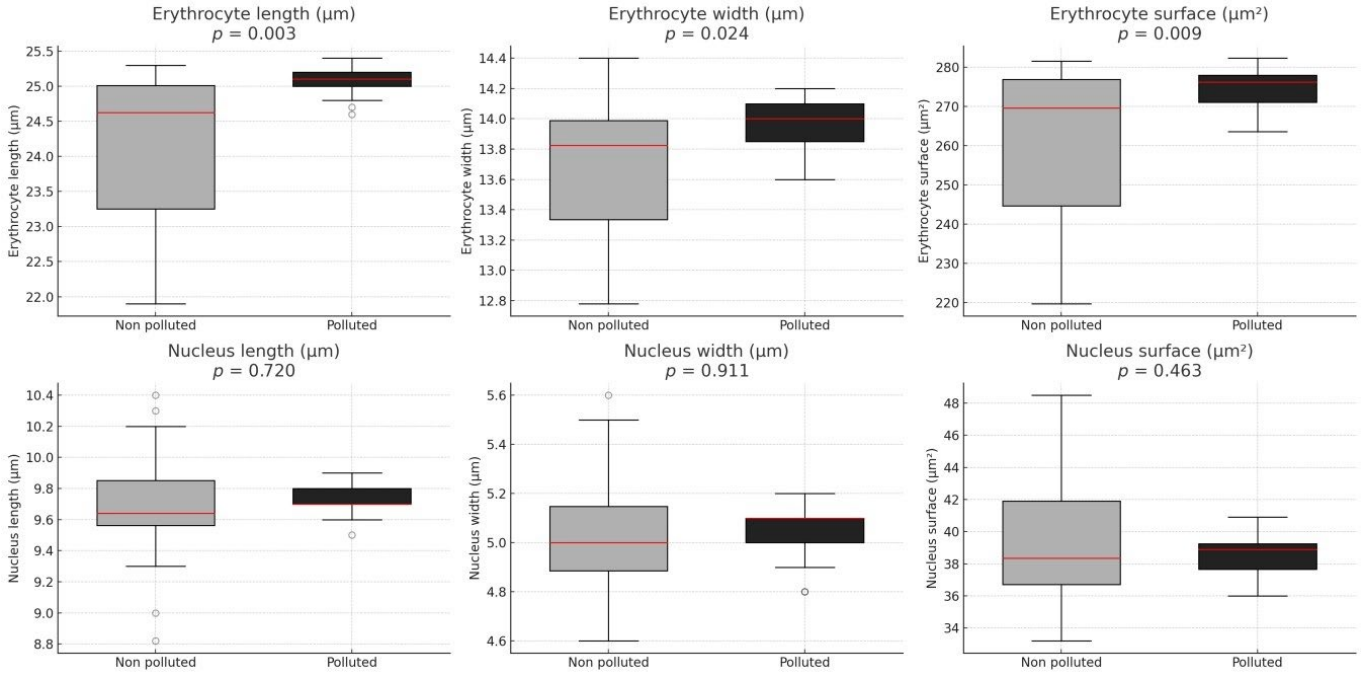

Fig.2. Comparative morphometric analysis of erythrocytes and nuclei between a non-polluted and a polluted site in *Mauremys leprosa*.

same result for *M. leprosa* in a Portuguese population, where heterophils dominated the leukocyte population at 61.53%, followed by lymphocytes at 28.89%. The average lymphocyte value for the Brabtia population is 28.11%, which is closer to the value found by Marques et al. (2025) in turtles from Porto d'Abrigo in Portugal. At the degraded site of Bouhamra, values are similar to those reported by Hidalgo-Vila et al. (2007) for *Mauremys leprosa* in Spain, with a high percentage of heterophils (61.53%) and a low percentage of lymphocytes (12.25). However, Hidalgo-Vila et al. (2007) did not provide information on the specific pollutants or their concentrations. Instead, they focused on haematological and biochemical parameters as indicators of environmental influence. By contrast, the presence of elevated levels of heavy metals such as manganese, nickel, iron, arsenic, and lead, together with detectable amounts of cadmium and mercury in Bouhamra, suggests that toxicological stress is the main factor underlying the observed haematological alterations (Christin et al., 1999; Sparling et al., 2010). Changes induced by stress lead to an increase in the number of heterophils while decreasing the number of lymphocytes. Given that the numbers of heterophils and lymphocytes are affected by stress in opposing directions, researchers frequently regard this ratio as an indicator of the stress response (Campbell, 1998; Santoro et al., 2020; Davis et al., 2008; Maxwell, 1993). The average H/L ratio value at the Brabtia site is 1.60, whereas at Bouhamra it is strongly higher at 4.39. This significant difference ($p = 0.00$) suggests that stress levels are higher among the population at the polluted site. At both sites, sex-related differences in the H/L ratio were negligible, suggesting that environmental conditions exert a stronger influence on immune modulation than sexual dimorphism. Notably, at the polluted site, both males (4.26 ± 1.10) and females (4.55 ± 0.80) displayed comparably high H/L values, underscoring the systemic impact of pollutants across individuals. This leukocyte profile shift is a well-documented physiological reaction to chronic stressors such as pollution, infection, or habitat disturbance (Campbell, 1998; Santoro et al., 2020). The percentage of eosinophils is higher at the pristine site (12.68%) than at the degraded site (2.89%), and there is a highly significant difference between the two environments ($p < 0.001$). As observed in some pollution studies (Mitchell, 1982; Claver and Quaglia, 2009; Kaiser et al., 2015), a decrease in eosinophils in a polluted environment may suggest chronic immunosuppressive stress, which can inhibit the normal production or mobilization of these cells. Such responses have often been associated with exposure to heavy metals, pesticides, and industrial effluents, all of which are known to interfere with hematopoiesis and immune regulation (Christin et al., 1999; Sparling et al., 2010). The reduction in eosinophil counts therefore appears to represent a sensitive biomarker of long-term pollutant exposure, reflecting sublethal effects that may compromise the overall health and disease resistance of exposed organisms.

This difference could also be due to by the presence of the most widespread reptilian blood parasite, *Haemogregarina stepanowi* Danilewsky, 1885, in the population of the pristine site (personal communication). This factor may explain the higher percentage of eosinophils at this site, given that these cells are linked with defenses against parasites (Rupley, 1997; Kiesecker, 2002). A significant difference ($p < 0.001$) was also observed between populations at polluted and pristine sites in monocyte percentages. The percentage of these cells is twice as high at the Bouhamra pollution site (19.47%). The high proportion of heterophils and monocytes, combined with a reduction in lymphocytes (12.8%), suggests an increased mobilization of nonspecific cellular defenses, which is typical of a degraded environment.

Figure 3 shows the different blood cell types observed in the blood smears of turtles. In individuals from the Brabtia site (A, B), oval-shaped erythrocytes with a central basophilic nucleus are visible, along with several types of leukocytes: lymphocytes, monocytes, heterophils, eosinophils, and basophils with granule-rich cytoplasm. In individuals from the Bouhamra site (C), the same cell categories are present, particularly heterophils, together with nucleated thrombocytes, often occurring in clusters.

Individuals from the polluted site had larger erythrocytes (average length: 25.07 ± 0.21 μm; surface area: 274.95 ± 5.72 μm^2) than those from the unpolluted site (average length: 24.10 ± 1.20 μm; surface

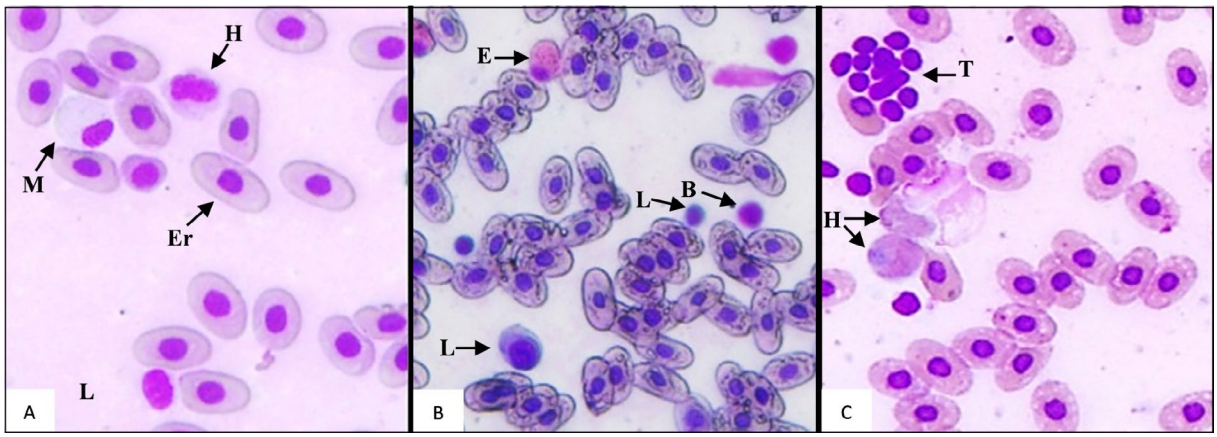

Fig.3. Photomicrographs of peripheral blood cells of the Mediterranean pond turtle (*Mauremys leprosa*) stained with May-Grünwald-Giemsa from Brabtia (A, B) and Bouhamra (C). Erythrocyte (Er); heterophil (H); thrombocyte (T); monocyte (M); eosinophil (E); basophil (B) and lymphocyte (L) (© Frih A.).

area: 260.97 ± 19.74 μm²). However, nuclear dimensions (NL and NW) and the NL/NW ratio were similar at both sites, suggesting that nuclei are less sensitive to environmental disturbances than the cytoplasm. These values are significantly higher than those measured in other European populations. Perpiñán and Sánchez (2009) found *Mauremys leprosa* turtles at the Catalonian Reptile and Amphibian Rehabilitation Centre in Barcelona, Spain, with erythrocytes measuring 20.53 ± 1.35 μm in length and 11.01 ± 0.94 μm in width. Nucleus values were also lower, at 6.81 ± 0.75 μm in length and 5.40 ± 0.45 μm in width. In Turkey, *Mauremys rivulata* also exhibited lower values: an average erythrocyte length of 19.3 μm, a width of 12.3 μm, and a nucleus length of 6.7 μm and a width of 5.9 μm (Çiçek et al., 2015). The same observation applies to *Emys orbicularis* in Turkey, with erythrocytes measuring 20.1 μm in length and 12.7 μm in width. The nucleus is 7.2 μm in length and 6.1 μm in width.

The nucleus-to-cytoplasm ratio (N/C) is a valuable morphometric indicator that reflects the balance between the nuclear and cytoplasmic volumes of erythrocytes. Recent advances in cell biology have emphasised the importance of the nuclear-to-cytoplasmic (N/C) ratio as a key factor in determining cell function. This ratio links DNA content with cell size, biosynthetic capacity and cell-cycle progression (Balachandra et al., 2022). Alterations to this ratio have been observed in situations of cellular stress, such as oxidative damage and toxic exposure, and are linked to reduced proliferative capacity or the onset of senescence (Neumann and Nurse, 2007; Levy and Heald, 2010). In aquatic vertebrates, changes in nuclear morphology and the N/C ratio have been documented as sensitive indicators of contaminant exposure, particularly in environments enriched with heavy metals and organic pollutants (Da Silva Souza and Fontanetti, 2006; Cavas and Ergene-Gözükara, 2005). In this study, the N/C ratio was slightly higher in individuals of the species *Mauremys leprosa* from the unpolluted site (Brabtia: 0.15 ± 0.01) than in those from the polluted site (Bouhamra: 0.14 ± 0.00). This difference was statistically significant ($p = 0.028$). A lower N/C ratio may indicate cell enlargement resulting from cytoplasmic expansion, which can be caused by environmental stressors such as exposure to pollutants. These results are consistent with previous studies on reptiles and other vertebrates, in which environmental contaminants have been associated with morphological changes in erythrocytes, including a decrease in the N/C ratio (Metin et al., 2006; Petrov and Stepanyan, 2016). Therefore, the observed reduction in the N/C ratio in turtles from the polluted site could indicate cytoplasmic hypertrophy, which is a sign of environmental stress. Thus, integrating differential leukocyte counts, the heterophil-to-lymphocyte ratio, and nuclear-to-cytoplasmic measurements may provide a more comprehensive assessment of the physiological stress induced by chronic pollution in freshwater turtles.

5. Conclusion

Our results confirm that alterations in haematological parameters can serve as ecological sentinels for diagnosing the impact of pollution on freshwater turtle populations. Leukocyte profiles and erythrocyte morphometric characteristics offer a dual, integrated immunological and physiological approach to assessing health status. It would be relevant to extend this analysis by considering other factors, such as age, season, and specific pollutant concentrations. Future studies could also explore gene expression associated with the immune response to refine the interpretation of cytological data.

Conflict of Interest

The authors declare no conflict of interest.

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