Chemosphere 220 (2019) 432–441

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Morphological and physiological assessments reveal that freshwater turtle (*Mauremys leprosa*) can flourish under extremely degraded-polluted conditions

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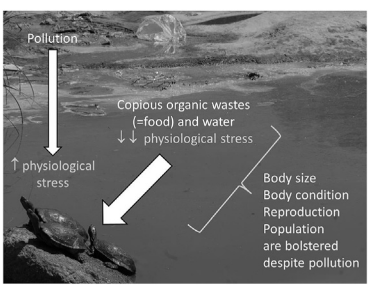
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 October 2018
Received in revised form
11 December 2018
Accepted 19 December 2018
Available online 20 December 2018

Handling Editor: Jim Lazorchak

Keywords:
Bio-indicator
Body condition
CORT
Stress
Testosterone
T4

ABSTRACT

Freshwater turtles are long-lived sedentary organisms used as biological sentinels to assess anthropogenic perturbations in freshwater-ecosystems; notably because pollutants tend to accumulate in their tissues. Pollution has detrimental effects in sea turtles, but studies in freshwater turtles have provided contrasted results: several species have been impacted by habitat perturbation and pollution while others not. It is important to explore this issue since freshwater turtles are threatened worldwide. We compared two populations of the stripe necked terrapin (*Mauremys leprosa*) in a relatively pristine area (piedmont of the Atlas mountain) *versus* an extremely degraded-polluted area (sewers of a large city) in Morocco. All morphological and physiological proxies showed that turtles were able to cope remarkably well with highly degraded-polluted habitat. Population density, body size, and body condition were higher in the sewers, likely due to permanent water and food availability associated with human wastes. Stress markers (e.g. glucocorticoids) provided complex results likely reflecting the capacity of turtles to respond to various stressors. Reproductive parameters (testosterone level, indices of vitellogenesis) were lower in the relatively pristine area. The deceptive overall image provided by these analyses may hide the disastrous human impact on rivers. Indeed, *Mauremys leprosa* is the only aquatic vertebrate able to survive in the sewers, and thus, might nonetheless be a pertinent indicator of water quality, providing that the complexity of eco-physiological responses is considered.

1. Introduction

Pollution and urbanization are major threats to freshwater

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https://doi.org/10.1016/j.chemosphere.2018.12.142
0045-6535/© 2018 Published by Elsevier Ltd.

ecosystems (Dudgeon et al., 2006). A recent review based on >7000 vertebrates and invertebrates species showed that extinction risk was significantly higher for freshwater compared to terrestrial species (Collen et al., 2014). This worrying outcome could have been expected because freshwater ecosystems are heavily exploited, polluted and fragmented; at the same time, they are particularly vulnerable to global changes. They suffer from the combination of multiple anthropogenic stressors with a limited resilience (Woodward et al., 2010). Beside global tendencies, strong differences among species in response to environmental stressors reveal complex effects. For instance, eco-physiological differences (e.g. in thermal tolerance) explain why some closely-related species decline, or not, under similar environmental constraints (Helmuth et al., 2005; Dhillon et al., 2013; Campos et al., 2018). It is therefore important to accurately examine how peculiar species cope (e.g. adapt or decline) with environmental perturbations to better understand population trends.

Freshwater turtles provide a patent illustration of the difficulties in predicting how species and populations respond to anthropogenic perturbations. Like other chelonians, they are long-lived organisms that exhibit slow pace life history traits; population viability depends on elevated adult survivals (Heppell, 1998; Blamires and Spencer, 2013). Many species are threatened by pollution, habitat destruction, invasive species and harvesting (Minton, 1968; Polo-Cavia et al., 2008; Doupe et al., 2009; Spencer et al., 2018). Whereas most freshwater turtles decline worldwide (e.g. due to overharvesting; Turtle Conservation Fund, 2002), others possess features that may be advantageous in highly perturbed environments. They display great behavioral plasticity, high tolerance to physiological stressors, and they can even survive repeated extreme environmental fluctuations (Lutz et al., 2003; Refsnider and Janzen, 2012; Jergenson et al., 2014). Not all organisms show equivalent adaptability to rapidly changing conditions however (Burgin and Ryan, 2008), many freshwater turtle species are impacted by brutal climatic changes for example (Ihlow et al., 2012). Yet, literature reports cases of species that actually benefited from human perturbations (Rees et al., 2009; Germano, 2010; Roe et al., 2011). This taxonomic inconsistency poses difficulties to guide conservation plans. Global models ignore peculiarities and are of little use at a local scale, notably to set up practical actions.

Indeed, no theoretical framework can embrace the diversity of turtle life history traits in order to predict which species will sustain, or not, the variability of human perturbations. Therefore, it is essential to assess the status of populations across contrasted situations and to examine the effect of proximal factors (e.g. habitat quality, pollution) to derive information about the resilience and adaptability of a wide spectrum of species. This may help to refine conservation priorities and propose effective field actions. Few studies compared populations exposed to different levels of anthropogenic disturbance; objectives and methodologies varied, and not univocal outcomes further limit interpretation (Shelby and Mendonca, 2001; Luiselli and Akani, 2003; Baker and Kjellerup, 2016; Gibbs et al., 2017; Cochran et al., 2018).

A better understanding of anthropogenic impacts is important to assist freshwater turtle conservation, but also because chelonians are excellent biological probes to track contamination thanks to their sedentary habits, long life expectancy, and propensity to accumulate pollutants (Golden and Rattner, 2003; Piña et al., 2009; Hopkins et al., 2013; Adel et al., 2015; Slimani et al., 2018).

We compared two populations of the stripe-necked terrapin (*Mauremys leprosa*) in an arid region of Morocco where climatic changes and human activities severely threaten freshwater ecosystems. We selected two distant albeit connected sites, a major affluent of an important river: 1) upstream in relatively pristine area at the piedmont of the Atlas *versus* 2) downstream in area where the sewers of a large city are discharged in the main river. We expected individuals from the extremely degraded and polluted site (sewers) to display smaller body size, lower body condition and a higher expression of stress markers (e.g. high glucocorticoid levels, low sex steroid levels as observed in birds; Tartu et al., 2014) compared to those from the more pristine site. Lessons from previous studies prompt caution however; turtles may benefit from resources provided by sewers (Germano, 2010). Overall, using a large data set, we aimed to clarify the possible impact of extreme habitat perturbation in a reptile species that tightly depends on river ecosystems. This issue is important because rivers are heavily threatened by anthropogenic activities and climate changes in North Africa, notably in Morocco (Schilling et al., 2012; Tramblay et al., 2013).

2. Materials and methods

2.1. Study sites

The study was carried out in a river ecosystem of Morocco, respectively upstream and downstream with strong anthropogenic perturbations. Fig. 1 and S1 illustrate the contrast between the two sites. Below, we provide additional information:

A) Upstream: the first site, named ZAT, is situated in the Oued Zat (31°32‘N; 7°38‘W), 37 km southeast of Marrakech, 2 km upstream the city of Ait Ourir, at the piedmont of the Atlas Mountain. This site is poorly urbanized, few small villages are scattered upstream. The river runs freely from the Atlas to the study site. Strong seasonal flooding creates a large riverbed (~200 m wide) with multiple meanders and ponds. Various types of vegetation along with various substrates (e.g. pebbles, silt, sand …) offer abundant shelters and create a mosaic of microhabitats that spread from the riverbed to the riverbanks. Turtles can move freely between temporary or permanent riverbeds, ponds, patches of riparian forest and small meadows. This station was considered as relatively pristine: the landscape is shaped by natural elements while macroscopic indices of human perturbation are limited to few plastic wastes. Turtles were searched for along 1,000 m of this complex habitat (in a surface covering broadly 76 ha), targeting ponds and small connecting streams where they spend most of their time. The Oued Zat is a major affluent of the Oued Tensift.

B) Downstream: the second station, named TENSIFT, is situated in the river Oued Tensift that crosses the city of Marrakech (31°42‘N; 8°04‘W). The riverbanks have been deeply remodeled and natural habitats almost totally destroyed. This site admits an immense open dump. Several small patches of riverbank are cultivated and intensively patrolled by herds of sheep and numerous feral dogs. The riverbed of the Oued Tensift is dry during most of the year, except in the study site where it receives a continuous flow of water from the wastewater treatment plant (WWTP) of Marrakech. It also collects abundant waste and continuous leachates from the open dump. Landfill leachates have been estimated at 2800 m^3/ha/yr (the dump covers more than 30 ha), a considerable quantity flows directly into the river, especially during rainfall, as a result the water is dark brown and highly polluted (Hakkou et al., 2001). The high degree of degradation of this section of the Tensift river has been clearly documented (Imziln and Barakate, 1997; El Gharmali et al., 2004; Ghallabi et al., 2011; Oufline et al., 2012; Hakkou,

Fig. 1. Contrast between two study sites upstream (top picture) and downstream (bottom picture) with a strong anthropogenic disturbance in the river ecosystem. Top picture: ZAT is a major affluent of TENSFIT. The habitat is relatively natural and the water flows from the Atlas Mountain without crossing urbanized (except small villages) or industrialized areas. Many aquatic vertebrates and invertebrates occur in this river. Bottom picture: TENSIFT runs through a large city, Marrakech. The study site is at the outskirts of the city with its main sewers. The water comes from untreated and treated water (a waste water plant is situated behind the vegetation on the right top corner), and from the leachates of a huge open dump that covers most of the horizon of the picture. Abundant macroscopic wastes are continuously discharged in the dark river, including organic material consumed by turtles. Turtles are virtually the only aquatic vertebrates found in this area.

2001). Turtles were searched for in the main riverbed in a surface covering approximately 22ha.

Mean annual temperature and precipitation are 350 mm and 21.2 °C in ZAT, 242 mm and 21.4 °C in TENSIFT; both stations belong to the arid bioclimatic zone (Mokhtari et al., 2013).

2.2. Study species

The stripe-necked terrapin, or Mediterranean pond turtle (*Mauremys leprosa*), is widely distributed in river ecosystems of Northern Africa and Southwestern Europe (Fritz et al., 2006). Previous studies provide information on morphology, ecology and physiology (Keller, 1998; Segurado and Araújo, 2004; Muñoz and Nicolau, 2006; Bertolero and Busack, 2017). Like other freshwater turtles (Meyers-Schöne et al., 1993; Blanvillain et al., 2007; Hopkins et al., 2013; Schneider et al., 2015), this species has been used as a biological probe to assess water quality (Héritier et al., 2017), notably to track mercury contamination across a wide geographic area (Slimani et al., 2018).

2.3. Field procedures

The sampling period extended from April 2012 to May 2016 (most samples were collected in spring). Individuals were caught by hand or with baited fish-traps, measured and subjected to different procedures. Most individuals ($N = 1153$) were marked (using a code of notches on the marginal scutes) and released. A small number of individuals were not marked ($N = 44$ [due to technical problem]; N-total = 1197). A subsample of 98 individuals was used for physiological investigations. Precisions are provided below, yet because we employed a wide range of techniques we do not expose all details here; instead we refer to published material where the procedures are presented.

Morphology: Individuals were weighed using an electronic balance (±0.1 g). Straight shell length was measured with a caliper (±1 mm). Body condition (mass scaled by size) was calculated as the residuals from the regression between mass and size (log-transformed data). Sex was determined with secondary sexual traits, notably the shape of the plastron and of the tail (Muñoz and Nicolau, 2006; Bonnet et al., 2010). Most individuals, including juveniles, exhibited marked secondary sexual traits and could be classified according to sex. Very small individuals (<45 mm) could not be classified. The proportion of classified individuals increased with size and was easy above 100 mm. Size at maturity along with sexual dimorphism are variable across populations (review in Lovich et al., 2010). Our data confirm this variability among 14 populations (unpublished). For conciseness we classified males smaller than 80 mm and females smaller than 120 mm as juveniles. Although arbitrary (not all individuals mature at the same size) this categorization facilitated comparisons.

Population size: From January 2013 to August 2014; 2 mark-recapture sessions (1 day) were setup every month in ZAT and TENSIFT (every 15 days, 40 sessions in total). Each captured individual was checked for its identity, marked if new (see above), and immediately released. Overall, during this period, 899 turtles were marked and 115 recaptured. The survey period encompassed the breeding season and provided sufficient time for individuals to move across neighboring areas. Thus we used an open model (acceptance of population flows) to estimate population size as recommended for freshwater turtles (Lindeman, 1990; Langtimm et al., 1996; Koper and Brooks, 1998; Hamer et al., 2016). The open population model (POPAN) was appropriate because the study sites were open, the duration of the study was relatively long, and the close-test program (Stanley and Richards, 2004) suggested that both populations were open.

Physiological markers: Blood samples were taken in the field to assess different physiological parameters during the reproductive season (March–May). On average, 58% of the blood samples originated from TENSIFT (range 47%–68% depending upon each hormone or metabolite). The exact timings at capture and when the blood was retrieved were recorded to take into account handling stress, measured as the time elapsed since capture. The blood was immediately centrifuged and the plasma stored in liquid nitrogen (details in Slimani et al., 2018). Corticosterone plasma level (CORT, $N = 89$) was the main proxy of stress assayed (Selman et al., 2012). We also assayed testosterone plasma level (T, $N = 79$) because polluted and chronic stressful conditions can damper sex steroid levels (Moore et al., 1991). We also examined thyroid hormone levels (thyroxine-T4 and triiodothyronine-T3, $N = 50$) because these hormones provide useful information regarding the general metabolism and reproductive status of turtles, although limited information is available under natural conditions (Licht et al., 1985; Kohel et al., 2001). Hormone levels were measured in the CEBC-laboratory using radio immunology assays (Naulleau et al., 1987; Bonnet et al., 2016a, b).

We assayed, on 60 individuals, the plasma levels of several metabolites, ions and of one enzyme (i.e. glucose, albumin, total proteins, cholesterol, triglycerides, uric acid, urea, phosphorus, calcium, iron, Alkaline phosphatase) in the CEBC-laboratory using a Pentra-C200 (HORIBA). Technical details are provided in Bonnet et al. (2016a). Variations in metabolite, ion and enzyme plasma levels reflect complex underlying physiological processes. Part of these variations is influenced by health status, reproduction and environmental conditions (Ehsanpour et al., 2015; Bonnet et al., 2016a). Thus plasma parameters are useful to assess how chelonians respond to environmental stressors (Sibeaux et al., 2016).

2.4. Analyses

Comparisons of morphological traits and physiological parameters between sites were performed with generalized linear models (GLM). Dependent variables were often skewed toward low values for physiological parameters. Thus, prior analyses, they were subjected to transformation (log, Box-Cox) when appropriate; the resulting normal probability plots of the residuals were generally linear (error terms were thus normally distributed in this analysis). However, for several highly skewed variables (notably hormones) we used a Poisson distribution of untransformed data and a log-link function. Most T3 samples were below the detection range, this hormone was not considered. Several individuals were blood sampled more than once for CORT, T, and T4 ($N = 29$, $N = 23$, and $N = 22$ pseudo-replicates respectively), and blood samples were taken from different puncture-sites (e.g. jugular, sub-carapacial cervical plexus). Further, handling-stress duration was variable. All these factors can influence results (e.g. due to lymph heamodilution or stress, Bonnet et al., 2016a). Therefore we performed preliminary analyses to examine to what extent these confounding factors could affect the outcomes. Most exerted significant effects on plasma concentrations, but none modified significantly our main results. Nonetheless, for conciseness, we have presented results obtained on a restricted sample size (discarding pseudo-replicates and highly heamodiluted blood samples) and we used handling duration when appropriate (e.g. CORT or T4). Including all samples (pseudo-replicates notably) in the analyses modified several *P*-values (lower P due to larger sample size) but did not change any conclusion. Not all parameters were assayed in each individual (e.g. we limited the blood volume taken in small terrapins); in addition some information (e.g. body mass) was sometimes missing; generating minor fluctuations of sample sizes.

Model selection of population size estimates was based on Akaike information criterion (AIC). By default Mark software ranks models from the lowest AIC and the least number of parameters (Cooch and White, 2006). Nevertheless, life history traits and field conditions should be considered during selection. Thus, we chose the model where the probability of capture and recapture varies with time due to strong seasonal effects and a constant probability of survival due to the very high annual survival rate of chelonians.

We used MARK 8.1 to estimate population sizes, other statistical analyses were performed using SPSS-18.0.0 and Statistica-12 (StatSoft France, 2013; www.statsoft.fr.).

3. Results

3.1. Morphology, sex and age

Turtles were larger (hence heavier) and exhibited higher body condition in TENSIFT compared to ZAT (Table 1; Figs. 2–4). Females exhibited higher body condition compared to males, and adults exhibited higher body condition than juveniles. Interactions between sex, age and sites revealed constantly higher body condition in TENSIFT than in ZAT (Table 1; Figs. 3 and 4). Overall, the local conditions that prevail in the most degraded and polluted site were systematically associated with a tendency of turtles to store larger body reserves.

Population structure strongly differed between the sites; juveniles were more abundant in ZAT where they represented 34.1% of the individuals captured *versus* 8.4% in TENSIFT ($Chi^2 = 112.2$, $df = 1$, $P < 0.001$; Fig. 2). Operational sex ratio (OSR, based on adults only)

Table 1
Parameter estimates using GLM analysis to explore the effect of sex, age and site (ZAT vs TENSIFT) on body size and body condition (mass scaled by size) in *Mauremys leprosa* freshwater turtle. Data were log transformed prior analyses. Significant effects are indicated in bold. Note that 87 small individuals could not be sexed and were not included in this analysis.

Source	Effect	Df	MSq	F	P
Body size	Intercept	1, 1084	13.510	337,435.2	**<0.001**
	Sex	–	2.243	419.5	**<0.001**
	Age	–	5.332	997.0	**<0.001**
	Site	–	0.258	48.2	**<0.001**
	Sex*Age	–	0.001	0.3	0.613
	Sex*Site	–	0.001	0.1	0.709
	Age*Site	–	0.016	3.0	0.085
Body condition	Intercept	1, 1039	2192.161	49,659.8	**<0.001**
	Sex	–	20.091	455.1	**<0.001**
	Age	–	39.024	884.0	**<0.001**
	Site	–	3.134	71.0	**<0.001**
	Sex*Age	–	0.217	4.91	**0.027**
	Sex*Site	–	0.001	0.02	0.982
	Age*Site	–	0.077	1.75	0.187

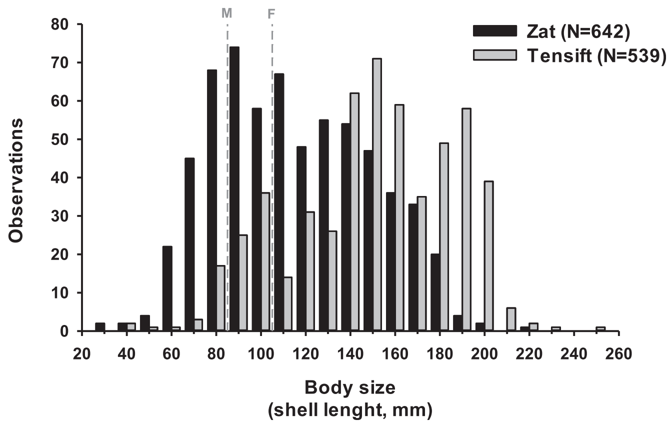

Fig. 2. Body size distribution of *Mauremys leprosa* freshwater turtle in ZAT (black bars) and TENSIFT (grey bars). The vertical dashed line indicates body size at maturity for males (M) and females (F). Statistics were performed on log-transformed data (see Table 1).

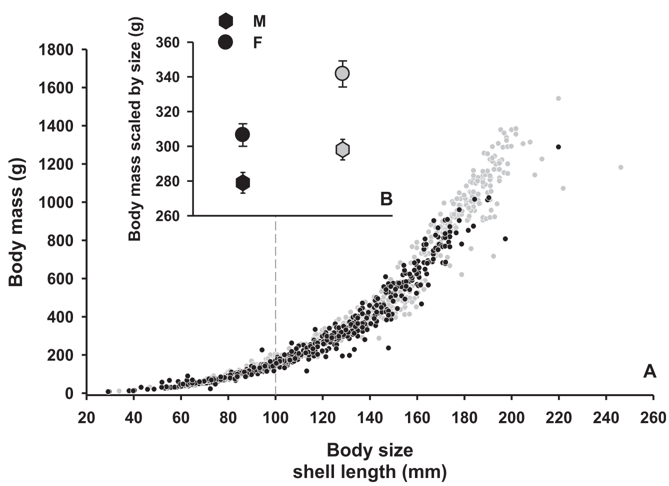

Fig. 3. Relationship between body mass and body size in ZAT (black circles) and TENSIFT (grey circles) turtles. The inserted panel provides mean values (±SD) of the residuals values from the regression to facilitate the visual comparison between sexes and sites (body mass scaled by size is an equivalent of body condition). TENSIFT individuals attain larger body size and are relatively heavier. Statistics were performed on log-transformed data (see Table 1).

was biased toward males, but it diverged between sites: adult females represented 35.8% of the individuals captured in ZAT *versus* 44.7% in TENSIFT ($Chi^2 = 7.62$, df = 1, P = 0.006).

3.2. Population size

Population size estimates were respectively 1289 ± 392 turtles in TENSIFT and 577 ± 54 turtles in ZAT (Supplementary Table S1). Thus, population density (number of individuals/ha) was more than eight times higher in TENSIFT than in ZAT (58.5 ind/ha and 7.6 ind/ha respectively).

3.3. Physiological markers

Handling stress (time elapsed since capture) exerted a strong positive influence on CORT (Table 2). GLM analysis revealed site and sex effects for CORT levels (Table 3; Fig. 5). Mean-CORT was low in the degraded-polluted (TENSIFT) site and high in the relatively pristine site (ZAT), but this effect was particularly marked in

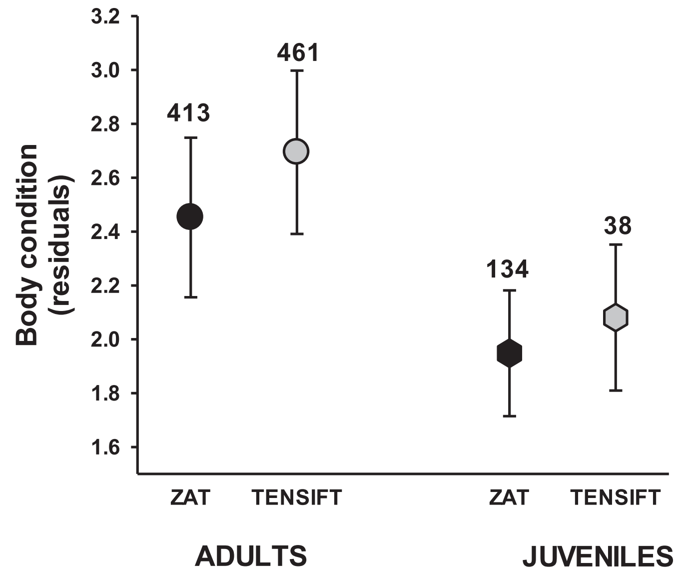

Fig. 4. Mean body condition (±SD, mass scale by size: residuals from the regression between body mass against body size) in adult (circles) and juveniles (hexagons) freshwater turtles (*Mauremys leprosa*) studied in two contrasted sites: a relatively pristine site (ZAT, black symbols) and a highly polluted site (TENSFIT, grey symbols). See Table 1 for statistics.

Table 2
Parameter estimates using GLM analysis (using Poisson distribution and a log link function) to explore the effect of sex and site (ZAT vs TENSIFT) on corticosterone concentrations (CORT) measured in the plasma of freshwater turtles. Time represents the time elapsed between capture and blood sampling (handling stress duration). Significant effects are indicated in bold.

Effect	Estimation	SE	Wald Stat	P
Intercept	0.697	0.190	13.510	**<0.001**
Time	5.516	2.210	6.228	**0.013**
Sex	0.060	0.135	0.196	0.658
Site	0.354	0.129	7.596	**0.006**
Sex*Site	0.258	0.129	4.016	**0.045**

Table 3
Parameter estimates using GLM analysis (using Poisson distribution and a log link function) to explore the effect of sex and site (ZAT vs TENSIFT) on testosterone concentrations (T) measured in the plasma of freshwater turtles. Significant effects are indicated in bold.

Effect	Estimation	SE	Wald Stat	P
Intercept	1.114	0.151	54.714	**<0.001**
Time	−11.849	2.300	26.537	**<0.001**
Sex	−1.171	0.136	74.048	**<0.001**
Site	−0.388	0.138	7.864	**<0.01**
Sex*Site	0.156	0.137	1.200	0.255

females.

Handling stress exerted a negative influence on T. Mean-T was higher in males (7.2 ± 1.7 [SE] $ng.ml^{-1}$, N = 26) compared to females (Table 3), but it was very low in females (0.7 ± 0.1 [SE] $ng.ml^{-1}$, N = 30). In males, mean-T was higher in TENSIFT (8.6 ± 2.0 [SE] $ng.ml^{-1}$, N = 17) than in ZAT (4.6 ± 3.1 [SE] $ng.ml^{-1}$, N = 9).

We found significant sex and site effects for T4 without effect of handling stress (Table 4). Mean T4 was higher in males (ZAT: 3.7 ± 1.7 ng ml^{-1}, N = 3; TENSIFT: 3.0 ± 0.7 ng ml^{-1}, N = 8) than in females (ZAT: 2.9 ± 0.5 ng ml^{-1}, N = 9; TENSIFT: 1.1 ± 0.2 ng ml^{-1}, N = 8), and higher in the relatively more pristine site (Table 4).

Concentrations of the plasma metabolites involved in

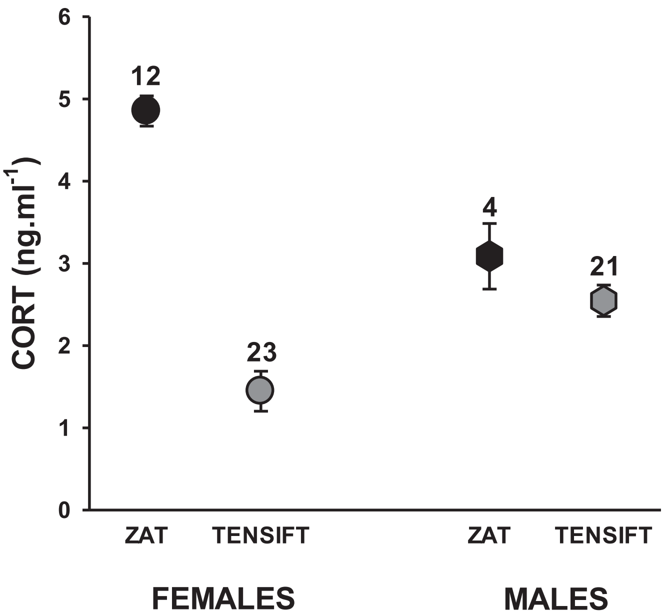

Fig. 5. Mean plasma concentrations (±SE) of corticosterone (CORT) measured in female (circles) and male (hexagons) freshwater turtles (*Mauremys leprosa*) studied in two contrasted sites: a relatively pristine site (ZAT, black symbols) and a highly polluted site (TENSFIT, grey symbols). See Table 2 for statistics.

Table 4
Parameter estimates using GLM analysis (using Poisson distribution and a log link function) to explore the effect of sex and site (ZAT vs TENSIFT) on thyroxin concentrations (T4) measured in the plasma of freshwater turtles. Significant effects are indicated in bold.

Effect	Estimation	SE	Wald Stat	P
Intercept	0.879	0.205	18.362	**<0.001**
Time	0.332	2.472	0.018	0.893
Sex	−0.296	0.139	4.472	**0.034**
Site	0.296	0.133	4.974	**0.026**
Sex*Site	0.179	0.132	1.838	0.175

vitellogenesis (e.g. plasma lipids, proteins, Calcium and Phosphorus ions that are linked to the production of vitellogenin) were systematically higher in females compared to males; although the difference was not always significant (three cases among 6, Table 5). All these markers of vitellogenesis were systematically higher in TENSIFT compared to ZAT, but often not significantly (2 cases among 6, Table 5). This site difference was mostly due to the high values found in TENSIFT females, although TENSIFT males also tended to exhibit (often slightly) higher values compared to ZAT males (Table 5).

As expected for reptiles, plasma concentrations were low for urea and high for uric acid (Table 5). Mean values for these catabolism markers were significantly higher in ZAT than in TENSFIT, and in males compared to females for the uric acid (Table 5).

Mean phosphatase concentration was higher in males and in TENSIFT; mean plasma iron concentration was higher in ZAT (Table 5).

4. Discussion

The risk of assessing a wide range of parameters that do not necessarily respond in the same way to environmental constraints (morphology, population size, plasma concentrations of different hormones, metabolites, enzyme, and ions) is to obtain disparate information and blurred results. This difficulty was overcome through the comparison of large samples collected in contrasted sites; analyses revealed complex albeit consistent patterns. As expected freshwater turtles were abundant in the relatively pristine site, but surprisingly they were flourishing in a highly degraded habitat (the sewers of a large city amidst a huge open dump) without showing peculiar sign of physiological disorder or stress. Below, we briefly review the main parameters assessed, and then question the validity of using *Mauremys leprosa* as a “good biological model of water quality” (Héritier et al., 2017).

4.1. Morphological traits

Individuals living in the most degraded habitat (TENSIFT) were larger and exhibited higher body condition index (mass scaled by size) compared to those from the pristine area (ZAT). Considering that relatively pristine natural habitats provide baseline values for the species, our results suggest that *Mauremys leprosa* actually withdraws benefits from the mixture of wastewater and organic material discharged in the river. We witnessed individuals feeding on diverse wastes (e.g. remains of meals embedded in paper or plastic bags, disposable nappies). We don't know how turtles process this resource, they inevitably ingest indigestible matter (e.g. plastic debris, pollutants), but they successfully learned to exploit it, attaining large body size and storing abundant body reserves. There is no other food source than wastes in TENSIFT; there is a

Table 5
Summary of the effects of sex and site (ZAT vs TENSFIT) on the concentrations of metabolites, ions, and one enzyme measured on the plasma of free-ranging turtles sampled in two sites (ZAT and TENSIFT). A broad role, or context, of each parameter assayed is provided: “vitell” stands for vitellogenesis, “met” for metabolism, “cat” for catabolism, “He St” for health status, “cont” for contamination (note that albumin is also involved in the oncotic pressure regulation). Mean concentrations (±SE) are provided for females from ZAT (FZ), females from TENSFIT (FT), males from ZAT (MZ), and males from TENSFIT (MT). Sample sizes are provided in brackets. Mean values are expressed as mmol/L except total proteins (Tot proteins) that are expressed in g/L. Phosphatase means alkaline phosphatase. Results from GLM analyses are not presented for conciseness. Instead, the direction of the comparisons is provided: F stands for female, M for male, Z for ZAT, T for TENSFIT. Significant effects are indicated in bold and underscored. The last column indicates if the interaction between sex and site (Int) is significant (NS for non-significant).

Parameter	Role/Context	FZ (N = 24)	FT (N = 14)	MZ (N = 8)	MT (N = 14)	Sex	Site	Int
Triglycerides	Vitell	4.4 ± 0.7	8.1 ± 1.6	1.2 ± 0.4	3.6 ± 0.9	**F > M**	**T > Z**	NS
Cholesterol	Vitell	2.9 ± 0.4	3.9 ± 0.6	2.5 ± 0.5	2.7 ± 0.4	F > M	T > Z	NS
Calcium	Vitell	3.0 ± 0.3	3.0 ± 0.4	2.1 ± 0.4	2.1 ± 0.2	F > M	T > Z	NS
Phosphorus	Vitell	0.6 ± 0.1	1.1 ± 0.3	0.3 ± 0.1	0.5 ± 0.1	F > M	T > Z	NS
Tot proteins	Vitell	27.0 ± 2.5	29.6 ± 6.0	24.9 ± 5.2	25.7 ± 1.7	**F > M**	T > Z	NS
Albumin	Vitell	12.9 ± 1.5	14.2 ± 2.5	11.0 ± 2.6	14.1 ± 1.0	**F > M**	**T > Z**	**<0.01**
Glucose	Met	2.8 ± 0.2	3.8 ± 0.5	7.8 ± 1.0	5.9 ± 0.4	**M > F**	T > Z	NS
Uric acid	Cat	52.0 ± 9.1	22.0 ± 5.2	57.1 ± 12.1	46.5 ± 4.2	**M > F**	**Z > T**	**<0.01**
Urea	Cat	2.9 ± 0.5	3.3 ± 0.7	4.8 ± 1.4	2.4 ± 0.4	M > F	**Z > T**	**<0.01**
Phosphatase	He St	24.6 ± 3.8	35.8 ± 6.3	32.5 ± 10.3	44.7 ± 4.8	**M > F**	**T > Z**	NS
Iron	Met/Cont	9.9 ± 6.8	4.6 ± 0.9	6.0 ± 2.3	5.2 ± 0.7	F > M	**Z > T**	**<0.01**

severe scarcity of prey in TENSIFT (e.g. aquatic insects, fish, amphibians or water snakes regularly encountered in ZAT were almost totally absent; unpublished) while macroscopic water vegetation was scarce. The sewers of TENSIFT are not an exception in providing abundant not-natural resources to freshwater turtles. The largest *Mauremys leprosa* individuals were captured in the sewers of another large city, FEZ, while sewers were often the best place to observe turtles across Morocco (supplementary material A; Slimani et al., 2018; unpublished data).

4.2. Population density and structure

Results on population density mirror morphological analyses. Despite difference in population structure, all age/sex classes were well represented in both sites, suggesting that turtles succeed to reproduce at a rate compatible with population viability in each case (i.e. no sign of population decline, like a strong decrease of sighting probabilities, in both sites visited over 10 years). The lack of dense recapture data precludes assessing age/sex specific recruitment, growth or survival rates for instance, and thus to perform accurate demographic comparison between sites (Arsovski et al., 2018a, b). Especially during the early development stages that are particularly sensitive to environmental perturbations (Thompson et al., 2018). Yet, broad effects were clearly visible. Dense groups of turtles were observed basking in TENSIFT (and in other sewers, supplementary figure S1) but not in ZAT. In TENSIFT, the capacity to process individuals represented our main limitation in the field while in ZAT substantial searching effort was necessary to collect them (easily spotted from distance, turtles rapidly escape when approached). Expectedly, estimated population size and density were markedly higher in TENSFIT. Body size correlates with fecundity while body condition positively influences long-term reproductive output in freshwater turtles (Zuffi et al., 1999; Litzgus et al., 2008). These proximal factors may explain the high population density in TENSIFT. Further, the proportion of adult females was higher in TENSIFT. Interestingly, similar trends have been reported in the painted turtles in the USA, with significantly higher proportions of mature females in the most urbanized areas (Bowne et al., 2018).

4.3. Physiological proxies

Physiological assessments are in line with morphological and population investigations. CORT (scaled by time) was more elevated in ZAT compared to TENSIFT, especially in females. CORT increases during egg production in turtles (Hamann et al., 2002). This may superficially indicate a higher reproductive rate in ZAT than in TENSIFT. But corticosterone is not the primary stimulator of vitellogenesis in chelonians; this role is played by estradiol (Mahmoud and Licht, 1997). An elevation of CORT is not specific to reproduction but is rather involved in the mobilization of body resources during demanding phases like reproduction, starvation, migration, or more generally under stressful conditions (Charmandari et al., 2005). Further, during egg production female sea turtles tend to decrease their CORT responsiveness (Jessop, 2001). High CORT in ZAT may reflect the necessity for females to mobilize their body reserves in a constraining environment whilst high food income diminishes this requirement in TENSIFT. Further investigations are needed to tease apart the influence of reproductive status, body condition and environment on CORT during vitellogenesis in *Mauremys*; but low CORT recorded in TENSIFT associated with very high population density and large body size suggests that individuals living in the sewers were physiologically less stressed. In both sexes, T4 and iron concentrations were more elevated in ZAT, suggesting a higher metabolic rate in individuals living in the relatively pristine site. Perhaps turtles in ZAT need to forage more actively? Unpublished radio-tracking data show that mean home range is broadly 2.5 larger in individuals monitored in ZAT compared to TENSIFT; indicating a differential access to resources. Whatever the case, low CORT and low T4 recorded in TENSIFT, show that turtles sampled in the sewers do not experience stressful conditions in terms of energy budget.

The negative impact of handling-stress on T we observed has been previously documented in turtles (Jessop et al., 2002). High T (scaled by time) in TENSIFT males further supports the notion of a relatively less stressful environment in the sewers. Although the small sample size of ZAT-males for T4 ($N = 3$) calls for caution, female and male hormonal results were convergent: highly degraded and polluted habitat is physiologically less challenging for *Mauremys leprosa* compared to a more natural habitat, likely because it provides abundant food and water supply. In contrast, temporary ponds between ZAT and TENSIFT (connecting river sections) sites provide little resources and host only few small individuals (Slimani et al., 2018).

Vitellogenesis is a very demanding process in ectothermic reptiles (Van Dyke and Beaupre, 2011). Vitellogenin is a phospholipoprotein linked to calcium synthetized by the liver under estradiol stimulation (Ho et al., 1982). The production and release of this yolk precursor correlates with an increase of circulating lipids, calcium, phosphorous, and proteins (Lagarde et al., 2003); higher concentrations were found precisely in females *Mauremys leprosa* in spring. High concentrations observed in TENSIFT fit in well with the notion that food resources were more accessible in the sewers, lowering the need for individuals to rely on high CORT and T4 to mobilize body reserves in order to sustain elevated metabolism and anabolism during reproduction. Besides, high uric acid and urea concentrations in ZAT support the notion of more intense mobilization of body reserves in the relatively pristine site.

Overall, physiological parameters were in agreement with the fact that body size and population density were bolstered in the sewers. The wide geographic distribution of *Mauremys leprosa* confirms that this species is able to cope with contrasted conditions, ranging from temperate-cool to extremely arid-hot climates, or from fresh to brackish waters (Bertolero and Busack, 2017). Populations of different freshwater turtle species also adapt to degraded or artificial habitats, including sewers (Sidis and Gasith, 1985; Souza and Abe, 2000; Spinks et al., 2003; Rees et al., 2009; Germano, 2010; Polo-Cavia et al., 2010; Roe et al., 2011; Stokeld et al., 2014), but others do not (Luiselli and Akani, 2003; Nasri et al., 2008; Chen and Lue, 2009; Yadollahvand and Kami, 2014). Studying underpinning mechanisms of population vitality divergences is crucial. But measuring all possible factors involved (e.g. ecological, demographic, physiological) is an insurmountable task, especially on the long term. Body condition is a relevant, integrative, and easily accessible parameter to monitor populations (Lecq et al., 2014), especially as it provides information congruent with physiological parameters (Sibeaux et al., 2016; this study). Recent studies have challenged this view however.

Indeed, body condition was considered as a “misleading indicator of health status” because in altered habitat, western pond turtles exhibit both high body condition and a depressed immune response (Polo-Cavia et al., 2010). Similarly, water pollution stimulated gene expression of major enzymes involved in detoxification processes in *Mauremys leprosa* (Héritier et al., 2017). These studies stated that animals are more stressed in polluted habitats. We reached differing conclusions, superficially at least. We notably observed high alkaline phosphatase levels in the most polluted site (TENSIFT). In vertebrates, this ubiquitous enzyme exerts multiple physiological roles *via* key phosphorylation cascades. Increasing plasma concentrations are usually the markers of an augmentation of hepatic protective functions against toxins or bacterial infections (Poelstra et al., 1997). Thus, the ZAT - TENSIFT comparison may actually indicate that pollution is stressful for turtles, and that assaying key enzyme levels like alkaline phosphatase is useful to assess habitat quality (Meyer et al., 2013). Although not investigated, negative impacts of habitat degradation and pollution on the defensive physiology of *Mauremys leprosa* against biological and chemical threats in TENSIFT are likely. Thus, we do not dispute that pollution is stressful. Instead, we argue that in this species, well-nourished individuals are capable of deploying efficient responses as indicated by hormonal and metabolic assessment, of attaining large size, and to reproduce successfully. Otherwise it would be impossible to explain convergent prosperity indices observed in the field.

The discrepancy between previous studies and the current one merely stands in interpretations. To caricaturize, we may pose a naive question: are contaminated, large and well-nourished individuals in a poorer overall condition than non-contaminated, small and starving individuals? Evolutionary ecologists may consider the trade-off between detoxification costs and reproductive success to appraise adaptiveness and population status. Veterinarians may rather consider alterations of defensive systems as an indication of illness. Both perspectives are correct. In other words, perhaps TENSIFT turtles have to deploy a physiological arsenal to combat pollution, but thanks to abundant energy supply they successfully adapt to deteriorated environmental conditions and can maintain most physiological parameters within the expected range for the species. Indeed, among the blood biochemical parameters used in this study (Table 5), seven (including glucose) have been previously assessed in healthy *Mauremys leprosa* in a natural reserve in Spain (Table 1 in Hidalgo-Vila et al., 2007); current mean values fall within, or remain very close to, the published range.

Across their wide distribution range *Mauremys* turtles have to physiologically respond to pollution, but also to various abiotic and biotic stressors, including non-anthropogenic ones (e.g. natural predation, food shortage). Their formidable adaptability may explain why they are successful, providing that they can find abundant food and water supplies. Reporting a physiological response to an environmental stressor independently from other traits (toxicity, disease, lack of reproduction, low survival), is insufficient to conclude that the health status of the turtles is dramatically challenged. Beside physiological defensive responses, broad physiological traits and population status should be assessed; but this is rarely the case.

5. Conclusion

Mauremys leprosa is a particularly resistant freshwater turtle (a 'modest' equivalent to *Rattus norvegicus* among rodents). It is often the only aquatic vertebrate remaining in the most degraded habitats. This resistance is underpinned by measurable phenotypic and physiological strategies. Consequently, we concur with Hériter et al. (2017) that it is a suitable biological model to monitor freshwater ecosystems across their immense distribution range. This species should be rather considered as a biological probe than a bio-indicator however (Slimani et al., 2018). Before we can use physiological assays to gauge the degree of anthropogenic disturbance, additional investigations are needed. Indeed, most of the physiological responses we can observe evolved before the Anthropocene and can result from various natural biotic and abiotic stressors that may trigger complex effects.

Author contributions

Conceptualization: all authors. Data curation: all authors. Formal analysis: MSEH and XB; Assays: XB; Funding acquisition: TS & EHEM; Field work: all authors; Project administration: TS and EHEM; Writing original draft: MSEH & XB; Writing review & editing: all authors.

Funding

This work was supported by the Hassan II Academy of Sciences and Technology - Morocco (ICGVSA project).

Acknowledgements

We thank C.L. Michel, F. Brischoux, T. Lechevalier, M. Bonnet, S. Lecq, and D. Desmonts for their help during field work, and to B. Michaud, C. Trouvé and C. Parenteau for assays.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.12.142.

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