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Toxicokinetics of selenium in the slider turtle, *Trachemys scripta*

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Abstract Selenium (Se) is an essential element that can be harmful for wildlife. However, its toxicity in poikilothermic amniotes, including turtles, remains poorly investigated. The present study aims at identifying selenium toxicokinetics and toxicity in juvenile slider turtles (age: 7 months), *Trachemys scripta*, dietary exposed to selenium, as selenomethionine *SeMet*, for eight weeks. Non-destructive tissues (i.e. carapace, scutes, skin and blood) were further tested for their suitability to predict selenium levels in target tissues (i.e. kidney, liver and muscle) for conservation perspective. 130 juvenile yellow-bellied slider turtles were assigned in three groups of 42 individuals each (i.e. control, $SeMet_1$ and $SeMet_2$). These groups were subjected to a feeding trial including an eight-week supplementation period SP_8 and a following 4-week elimination period EP_4. During the SP_8, turtles fed on diet containing 1.1 ± 0.04, 22.1 ± 1.0 and 45.0 ± 2.0 μg g^{-1} of selenium (control, $SeMet_1$ and $SeMet_2$, respectively). During the EP_4, turtles fed on non-supplemented diet. At different time during the trial, six individuals per group were sacrificed and tissues collected (i.e. carapace, scutes, skin, blood, liver, kidney, muscle) for analyses. During the SP_8 (Fig. 1), both $SeMet_1$ and $SeMet_2$ turtles efficiently accumulated selenium from a SeMet dietary source. The more selenium was concentrated in the food, the more it was in the turtle body but the less it was removed from their tissues. Moreover, SeMet was found to be the more abundant selenium species in turtles' tissues. Body condition (i.e. growth in mass and size, feeding behaviour and activity) and survival of the $SeMet_1$ and $SeMet_2$ turtles seemed to be unaffected by the selenium exposure. There were clear evidences that reptilian species are differently affected by and sensitive to selenium exposure but the lack of any adverse effects was quite unexpected.

Keywords *Trachemys scripta* · Turtles · Selenium · Selenium species · In vivo exposure

Christelle Dyc and Johann Far have contributed equally to this work.

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Introduction

Selenium is a naturally occurring element that was first discovered as a toxic in 1817 and then as an essential compound in the late 1950s (Wisniak 2000). Living animals mainly accumulate selenium through their diet as organic (i.e. selenomethionine *SeMet* and selenocysteine *SeCys*) or inorganic (i.e. selenite and selenate) selenium, and either store it within tissues or excrete it as methylated species (Dumont et al. 2006; Reilly 2006). Most of the selenium requirement is provided by SeMet and SeCys in a lesser extend. SeCys is specifically incorporated into

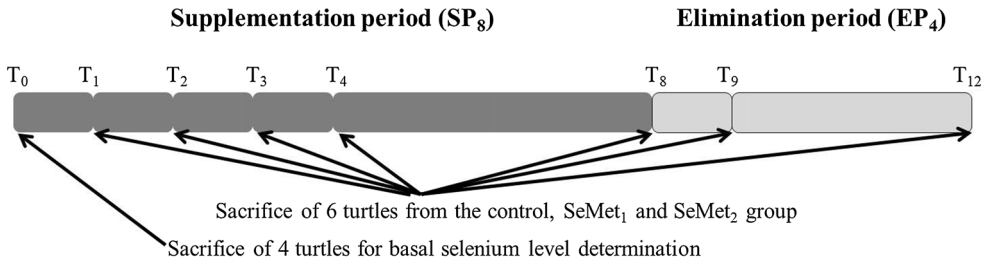

Fig. 1 Design of the feeding trial. T, Time of tissues collection in weeks. The feeding trial included a supplementation period of 8 weeks (i.e. SP_8) followed by an elimination period of 4 weeks (i.e. EP_4). Six turtles from each turtle group (i.e. control, $SeMet_1$ and $SeMet_2$) were sacrifice at each collection time, from T_1 to T_{12}. At T_0, four turtles were sacrificed

proteins while SeMet is unspecifically put in place of its amino acid analogue, i.e. methionine, without clear distinction and in a concentration-dependent way (Moroder 2005). SeMet was so considered as the primary organic selenium form relevant for bioaccumulation and toxicity in wildlife (Fan et al. 1998; Schrauzer 2000).

The toxicity of selenium mainly inducts an oxidative stress that disturbs the metabolism of the antioxidant glutathione (e.g. the oxidized to reduced glutathione ratio *GSSG:GSH*), activity of antioxidant enzymes (e.g. glutathione peroxidase *GPx* and superoxide dismutase *SOD*) and/or promotes lipid peroxidation (Hoffman 2002). Embryotoxicity was the most reported adverse effect associated with selenium exposure and occurred as reduced hatching rate and hatchlings' survival, and/or teratogenicity in aquatic birds (Hoffman 2002). Selenium toxicity was further associated with higher hepatic GSSG:GSH ratio, increased GPx activity in plasma and liver, and reduced SOD activity in liver, kidney and muscle of birds (Hoffman et al. 1989, 1996; Wang et al. 2011).

Globally speaking, much remains to be discovered in the field of selenium toxicity and especially in poikilothermic amniotes commonly known as reptiles (Janz et al. 2010; Sparling et al. 2010; Young et al. 2010; Perrault et al. 2013). Nevertheless, laboratory-controlled studies have provided evidences that reptiles may be likewise affected by selenium exposure. Physiological impairments, including embryotoxicity have been reported (Hopkins et al. 1999; Ganser et al. 2003; Hopkins et al. 2005a; Rich and Talent 2009). The leopard gecko *Eublepharis macularius* dietary exposed to selenium as selenite, at level of 4.6 μg g^{-1} of sand mixture (i.e. ~0.21 μg of selenium per gram of body mass per day), showed depressed growth in mass (Rich and Talent 2009). Additional biological impairments (i.e. reductions of food ingestion, food conversion efficiency and growth in size) were described for lizards that daily consumed 0.43 μg of selenium per gram of body mass. Liver histological abnormalities were also suspected for water snakes *Nerodia fasciata* and western fence lizards *Sceloporus occidentalis* dietary exposed to selenium as SeMet, at levels ranging from 11 to 23 μg g^{-1} of diet (Hopkins et al. 2001, 2005b).

To the best of authors' knowledge, such controlled studies were not investigated in other reptilian species including turtles. However, field studies have indicated the potential toxicity of selenium in these vertebrates as documented for American alligator *Alligator mississipiensis* (Roe et al. 2004) and marine turtles (Lam et al. 2006; van de Merwe et al. 2009; Perrault et al. 2013; Dyc et al. 2015). For the first time, the present study investigated the toxicity and kinetics of selenium towards freshwater turtle specie, the slider turtle *Trachemys scripta*. Juvenile slider turtles were used as model candidates and dietary exposed to selenium as SeMet under laboratory-controlled conditions and for eight weeks. For assessing selenium toxicity to turtles, biological endpoints (namely survival, straight carapace length SCL, straight carapace width SCW, body mass) were recorded during acclimation and feeding trial. Non-destructive tissues (i.e. carapace, scutes, skin and blood) were tested for their suitability to be used as biomonitoring tools for evaluating the selenium exposure in turtles under field and laboratory conditions.

Materials and methods

Ethic statement

In the present study, all turtles were treated humanely and their welfare was optimised. The methodology for housing and euthanasia was approved by the Animal Care and Use Committee of the University of Liège in Belgium (file number 1091).

Turtle housing and husbandry

On September 2010, 130 one-month old yellow-bellied slider turtles, *Trachemys scripta*, were purchased from a pet store and arbitrarily placed per pair in rectangular plastic tanks (30 × 20 × 14 cm^3). The same day,

individuals were weighted and measured (i.e. Straight Carapace Length *SCL* and Straight Carapace Width *SCW*), and identified with a unique ID number. Their mass and SCL (minimum–maximum) ranged from 6.2 to 13.3 g and from 3.1 to 4.1 cm, respectively (Supplementary Data: Table S1).

Turtles were housed in agreement with the national authorities of Animal care (CCPA, 2006). Slider turtles are semi-aquatic turtles relying on their behavioural thermoregulation to maintain optimal body temperature and to ensure vital basic processes. Therefore, they need a temperature-controlled basking area and fluorescent tubes (i.e. JBL Solar Reptil Sun T8) emitting a full spectrum light (i.e. UVA and UVB) to prevent calcium and vitamin deficiencies. An oak driftwood was used as basking area, the room temperature was kept around 29–32 °C and a 12 h-photoperiod cycle was achieved. To minimise the energy expenditure of turtles for air breathing, the water height was adjusted to their mean carapace width (i.e. ~3 cm-height). Turtles were fed each morning around 10.00 a.m. with specific food (ZoodMed Hatchling formula, Biotop S.P.R.L. with 43 % of protein), aquariums were cleaned thereafter and water renewed.

Study design

Turtles were first acclimatized to laboratory conditions and feeding processes (i.e. quantity, frozen nature and timing) for six months. To better control the ingested food quantity, each turtle was fed alone by placing a plastic separation into the tank (Suppl. Fig. 1).

The day before the beginning of the feeding trial (March 2011), turtles were weighted and measured. Mean body mass and SCL (±standard error *SE*) was 53.8 ± 0.7 g and 3.1 ± 0.1 cm, respectively (Supplementary Data Table S1). Four individuals were sacrificed for determining the baseline level of selenium (T_0). The remaining turtles (n = 126) were then arbitrarily distributed into three groups (i.e. control, $SeMet_1$ and $SeMet_2$) counting 42 individuals each. The feeding trial lasted 12 weeks and included two periods, an eight-week supplementation period (SP_8) and a four-week elimination period (EP_4). During the SP_8, each turtle fed on the food stock according to its group belonging (i.e. basal diet, $SeMet_1$ or $SeMet_2$ diet). During the EP_4, the basal diet used for the acclimation period was given to each turtle. SCL, SCW and body mass during the feeding trial are provided in supplementary data (Tables S2 and S3).

Choice of the selenium species

The naturally occurring organic L-form of selenium, i.e. seleno-L-methionine (SeMet), was chosen for exposure due to its readily bioaccumulation through the food web (Fan et al. 2002). The SeMet concentrations used for the food supplementation were within the range of those reported as inducing no lethal effects in birds and reptiles (Hoffman 2002; Rich and Talent 2009). Exposure concentrations were based on mathematical estimation available for the lizard *Eublepharis macularius* (Rich and Talent 2009) and were expected to affect the turtles' body condition (i.e. growth in mass and SCL, feeding activity). The estimated low-observed-adverse-effect level (LOAEL) affecting growth in mass in lizard (Rich and Talent 2009) was used for the first diet treatment, i.e. $SeMet_1$ food stock. The $SeMet_1$ stock was supplemented with 64.2 μg of SeMet per gram of diet on a dry weight basis (d.w.) corresponding to 25.7 μg g^{-1} d.w. of selenium. The concentration affecting the whole lizard body condition (i.e. food ingestion, growth in mass and SCL, food conversion efficiency; Rich and Talent 2009) was used for the second diet treatment, i.e. $SeMet_2$ food stock. The $SeMet_2$ stock was supplemented with 134 μg g^{-1} d.w. of SeMet corresponding to 58.8 μg g^{-1} d.w. of selenium.

The supplementation of the $SeMet_1$ and $SeMet_2$ food stocks with SeMet suggested a supplementation with the methionine amino acid (Met) as well. Therefore, for reducing the number of variables, the food given to the control group during the SP_8 was supplemented with that amino acid.

Since two exposure groups were assigned (i.e. $SeMet_1$ and $SeMet_2$), two sub-control groups were assigned (i.e. Met_1 and Met_2) and two additional food stocks were prepared containing only Met supplement, 47.8 and 100.3 μg g^{-1} d.w. of Met, respectively. The Met concentration was estimated from the methionine fraction in the $SeMet_1$ and $SeMet_2$ diet stocks. Met concentrations in the Met_1 and $SeMet_1$ food stocks were therefore similar (i.e. 47.8 μg g^{-1} d.w. of Met), as were in the Met_2 and $SeMet_2$ ones (i.e. 100.3 μg g^{-1} d.w. of Met).

To resume, four food stocks were used for feeding the turtles during the SP_8 (i.e. $SeMet_1$, $SeMet_2$, Met_1 and Met_2 food stocks) while a sole food stock (i.e. basal diet) containing neither additional SeMet nor Met was used for feeding turtles from every groups during the acclimation period and the EP_4.

Preparation of the food

A commercial diet was used (i.e. ZooMed Hatchling Formula) for preparing the five stocks ($SeMet_1$, $SeMet_2$, Met_1, Met_2 and basal diet). The turtle pellets were reduced into powder and deionized water containing thickener agent (i.e. carboxymethylcellulose; 4 % of the total pellet mass) was added. For the Met and SeMet food stocks, the required quantity of Met or SeMet powder (Sigma-Aldrich

Co., Belgium) was added into the deionized water. No additional SeMet or Met was added for the basal diet. The resulting dough was then pressed to form spaghetti-like strands and the reconstituted food was dried at room-temperature for 72 h. All food stocks were stored in a −20 °C freezer until use.

Feeding of the turtles

Each turtle was fed with a food quantity (in gram) in agreement with their diet requirement (i.e. ~4 % of the individual body weight; CCPA, 2006). During the feeding trial, turtles were weighed every two weeks and the daily feed allowance was adjusted accordingly. Once a week, the spaghetti-like strands were broken into small pieces and individual daily diet rations were packed (i.e. one pack per day and per turtle). Food rations were let into freezer until use (i.e. −20 °C) and varied between (mean ± SE) 0.28 ± 0.01 and 0.32 ± 0.01 g at the beginning and the end of the study, respectively.

Biological endpoints and tissue collection

Turtles were daily monitored for illness and mortality, and weighted every two weeks. A growth index (Eq. 1) was calculated for each individual by considering its weight at the beginning and the end of the feeding trial:

$$\text{Growth Index } (\%) = \left(\frac{\text{Final Body Weight} - \text{Initial Body Weight}}{\text{Initial Body Weight}}\right) \times 100 \quad (1)$$

Six turtles from both SeMet groups and three from both Met groups were sacrificed one, two, three, four, eight, nine and 12 weeks after the beginning of the feeding trial (i.e. at T_1, T_2, T_3, T_4, T_8, T_9 and T_{12}). Sacrifices were therefore done during the SP_8 (i.e. T_1, T_2, T_3, T_4 and T_8) and EP_4 (i.e. T_9 and T_{12}). The day prior sacrifice, each turtle was weighted and measured (i.e. SCL and SCW). The day of sacrifice, turtles (n = 18) were euthanized by cerebral commotion and beheading. Blood, liver, kidney, pectoral muscle, skin, carapace and scutes were removed and kept frozen (i.e. −20 °C) for analyses.

Due to the possibility of (a) selenium diffusion from the food into the water and (b) incomplete consumption of the food by turtles, the selenium dose truly assimilated by turtle cannot be accurately determined. To overcome this issue, the uneaten food was daily collected from each turtle tank and let drying for ~72 h. The resulting dry weight was subtracted to the dry weight of the food given to each individual. The effective consumption was determined by dividing the calculated ingested food by the individuals' mass.

Selenium analyses

After being frozen in a liquid nitrogen bath for 10 min, the tissues (i.e. blood, liver, kidney, pectoral muscle, skin, carapace and scutes) collected at each collection time (i.e. T_1, T_2, T_3, T_4, T_8, T_9 and T_{12}) were lyophilised (Benchtop 3L Sentry Virtis, New York, USA) and the dry weight calculated. Approximately 100 mg d.w. of liver and carapace, and 50 mg d.w. of other tissues were digested in Teflon tubes with a solution containing 1 ml of 30 % hydrogen peroxide, 2 ml of 65 % concentrated nitric acid and 5 ml of deionized water. (Due to inadequate tissue quantity, analyses were not performed in scutes collected at T_1.) Tubes were then place in a microwave oven (Microwave Labstation) for 35 min. After cooling, the mineral deposits were diluted with deionized water in volumetric flask to a final volume of 50 ml (i.e. liver and carapace) or 15 ml (i.e. other tissues) and kept at room temperature. Food samples from each treatment (i.e. Met_1, Met_2, $SeMet_1$ and $SeMet_2$) were also subjected to analysis at three times all along the feeding trial (i.e. T_0, T_4 and T_9). Samples for total selenium were analysed by Inductively Coupled Plasma-Mass Spectrometry (ICPMS, Elan DRC II, PerkinElmer Inc.) equipped with a Dynamic Reaction Cell (methane at 0.5 ml ml^{-1} was used as reaction gas) and certified reference materials (i.e. DOLT3-dogfish liver, NIST 1566b-oyster tissue, NIST 2976-mussel tissue, NIST 1577c-bovin liver, BCR414-Plancton and Whole Blood L3) were used as quality and precision controls. Mean percentage recoveries in certified reference materials ranged from 95 to 124 % for Se.

Selenium species identification (speciation)

Due to a limited availability of the turtle tissues, selenium speciation were only investigated in blood, liver, muscle and skin at some of the collection times (i.e. T_2, T_4, T_8 and T_{12}). Besides, analyses were only performed in the control and $SeMet_2$ turtle group.

The determination of selenium species (i.e. SeMet, selenocysteine *SeCys* and inorganic selenium *In.Se*) in the turtle tissues was described elsewhere (Far et al. 2016). Briefly, 50–100 mg of whole tissue and selenium-containing proteins (i.e. SeMet and SeCys residues) were denaturized by concentrated 2 ml urea solution (7 M) and SeCys proteins were stabilized by iodoacetamide (20 µmol) alkylation after reduction by dithiothreitol (8 µmol), 15-times diluted with TRIS buffer (50 mM, pH 7.5) and then digested by *Streptomyces griseus* protease XIV (roughly 3UI) and *Candida rugosa* lipase VII (roughly 630 UI) overnight (37 °C). Samples were filtered through ultrafiltration membrane (nanoSEP 3 kDa cut-off). The resulting extracts were injected into a strong anion-

exchange high-performance liquid chromatography (SAX-HPLC PRP-X100) coupled to the ICPMS (methane was used as reaction gas) by using volatile buffer (ammonium acetate, 20–200 mM) and pH (9–5) gradient at 0.95 ml min^{-1}. Quantification were performed using external calibration of the appropriate standards (commercially available or synthetized by reduction-alkylation) and by peaks area integration (trapezoidal rule) using a home-made macro for Lotus Note (IBM). Authentic standards were used for SeMet quantification and certified material (Se-enriched yeast reference material, SELM-1) was used as quality control.

Statistical analysis

Statistical analyses were conducted by using the Statistica 9.0 software (StatSoft Inc.). Data were expressed as mean ± standard error (SE) for $n \geq 4$, and as minimum–maximum for $n \leq 3$.

The measured concentrations in selenium species (i.e. selenium, SeMet, SeCys, In.Se) were grouped by tissue (i.e. liver, kidney, pectoral muscle as target tissues or destructive sampling, and, blood, skin, scutes and carapace as non-invasive sampling or non-destructive tissues), turtle group (i.e. control, $SeMet_1$ or $SeMet_2$) and collection time (i.e. T_1, T_2, T_3, T_4, T_8, T_9 and T_{12}). Considering the low sample size, statistical differences between concentrations in selenium species (i.e. SeMet, SeCys and In.Se) were only tested in the blood.

For a given tissue and turtle group, concentrations were compared between each collection time (e.g. hepatic selenium levels in SM_2 turtles: T_1 vs T_2, T_1 vs T_8). For a given tissue and collection time, comparisons were tested between groups (e.g. hepatic selenium concentration at T_1: control vs $SeMet_1$ vs $SeMet_2$). Statistical analyses were done by means of one factor ANOVA and a two-tailed *T* test was used for comparing groups in pairs. Shapiro–Wilk normality test was employed in all cases and data were log-transformed if necessary prior to application of statistical tests.

The three turtle groups (i.e. control, $SeMet_1$ and $SeMet_2$) were considered as a single group and both periods (i.e. SP_8 and EP_4) were included in the correlation analyses. Correlations between concentrations measured in non-destructive tissues were tested by using two-tailed Pearson test. The determination coefficient (r^2) is the explicable value and the hardiness of the correlation was estimated as followed: strong correlation for $r \geq 0.68$ and moderate correlation for $0.35 < r < 0.68$ (Taylor 1990). As proposed for low sample size (Singh and Nocerino 2002), a *p* value of 0.2 was used for significance in statistical analyses.

Results

Dietary selenium and body condition

For a given food stock, selenium concentrations did not vary throughout the feeding trial (*t* Test, $p > 0.05$; Table 1). The effective assimilation of selenium (i.e. total and SeMet) and the turtle growth index increased along the feeding trial for every individual (F-test, $p < 0.05$). However, no statistical differences between groups were reported for these biological endpoints (Normal Z, $z < 1.96$). The tested selenium concentrations did not cause mortality or affect the turtles' body condition (i.e. mass, weight, SCL, SCL and mass ratio).

Selenium in the control subgroups (i.e. Met_1 and Met_2)

In each Met group, the accumulation pattern of selenium was constant throughout the feeding trial. Kidney accumulated the higher selenium concentration (t Test, $p < 0.05$) followed by muscle (t Test, $p < 0.05$) > blood ≥ liver > skin > carapace ≥ scutes. Concentrations were further higher in liver than in skin, and higher in skin than in carapace and scutes (t Test, $p < 0.05$). For a given tissue, similar selenium concentrations were observed in the Met1, Met2 and T0 (i.e. turtles sacrificed before the feeding trial) groups (T test, $p > 0.05$). Therefore, these groups were combined under a unique "control group" name in the following sections of this paper and in tables (Tables 2, 3, 4).

Total selenium: kinetics and comparison

As a reminder, no values were available for the Se concentration at T_1 for scutes due to inadequate quantity of tissue.

In $SeMet_1$ (Fig. 2a) and $SeMet_2$ (Fig. 2b) groups, tissue Se concentrations increased over the course of the SP_8 and decreased in most tissues during the EP_4 (i.e. from T_8 to T_{12}), and as soon as T_9 in most of them (Fig. 2a, b; *T* test, $p < 0.2$).

During the SP_8, a similar accumulation pattern of Se of invasive sampling and non-destructive tissues was reported in the turtle body of both SeMet groups (Figs. 3, 4, respectively). The highest selenium concentration was measured in their kidney, followed by muscle and blood. Carapace and scutes accumulated the lowest selenium concentrations (Fig. 4).

Throughout the feeding trial, selenium concentrations were higher in both SeMet turtle groups than in controls (*T* test, see Tables 1, 2). Nonetheless, the $SeMet_2$ turtles

Table 1 Selenium concentration (μg g^{-1} d.w.) in target tissues (i.e. liver, kidney and muscle) collected from the control, $SeMet_1$ and $SeMet_2$ turtle groups

n = 6[$]	Liver Mean ± SE Median Min–Max			Kidney Mean ± SE Median Min–Max			Muscle Mean ± SE Median Min–Max		
	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$
T_1	1.53 ± 0.09	4.01 ± 0.53	4.47 ± 0.85	3.56 ± 0.24	10.58 ± 1.74	9.38 ± 2.54	1.88 ± 0.31	6.46 ± 1.30	6.13 ± 0.98
	1.50	3.62	4.80	3.30	9.70	11.65	1.75	5.10	6.90
	1.12–2.03	1.63–7.54	1.22–7.34	2.80–4.90	2.90–23.40	0.04–17.90	1.00–3.40	3.10–13.70 (5)[$]	2.70–9.10
		a	a		a	a		a	a
T_2	1.33 ± 0.08	6.42 ± 0.31	10.04 ± 0.42	3.15 ± 0.13	17.28 ± 0.93	24.28 ± 1.74	1.23 ± 0.12	8.04 ± 0.52	15.40 ± 1.99
	1.42	6.42	10.25	3.15	17.45	25.30	1.10	8.20	15.50
	0.87–1.57	5.29–7.46	8.22–11.70	2.60–3.90	14.60–10.10	17.90–29.70	0.90–1.90 (5)[$]	5.70–10.4 (5)[$]	7.20–29.6 (7)[$]
		a, b, c	a, c		a, b, c	a, c	a	a, b	a, c
T_3	1.40 ± 0.09	7.54 ± 0.27	11.70 ± 1.27	3.07 ± 0.08	21.60 ± 0.86	40.04 ± 1.57	1.40 ± 0.12	12.82 ± 0.63	22.50 ± 1.89
	1.42	7.39	13.25	3.00	22.75	39.70	1.40	12.85	21.30
	1.04–1.88	6.68–8.98	7.60–14.7	2.80–3.40	17.80–24.30	35.30 – 44.00 (5)[$]	0.90–1.90	9.80–14.90	16.70–27.20 (5)[$]
		a, b, c	a		a, b, c	a, c		a, b, c	a, c
T_4	1.32 ± 0.08	10.52 ± 0.99	16.16 ± 1.30	2.80 ± 0.11	23.10 ± 0.76	56.74 ± 3.41	1.32 ± 0.05	17.84 ± 1.34	27.80 ± 2.11
	1.45	10.14	15.70	2.70	22.40	44.70	1.30	16.80	30.30
	0.95–1.49	6.75 – 17.00	12.20–19.60 (5)[$]	2.50–3.30	20.00–26.60	36.30–55.60 (5)[$]	1.10–1.60	15.00–22.70 (5)[$]	21.60–31.70 (5)[$]
		a, b, c	a, c	c	a, b	a, c		a, b, c	a, c
T_8	1.43 ± 0.06	10.76 ± 0.71	18.27 ± 1.12	3.00 ± 0.15	30.17 ± 1.02	52.87 ± 1.92	1.48 ± 0.05	24.00 ± 0.98	38.75 ± 4.37
	1.43	10.07	17.60	2.80	29.55	51.20	1.50	23.20	39.40
	1.09–1.77	8.63–14.20	14.10–23.40	2.60–3.90	25.60–35.30	47.10–63.90	1.20–1.70	20.90–29.70	21.40–54.20
		a, b	a		a, b, c	a	c	a, b, c	a, c
T_9	1.53 ± 0.08	8.23 ± 0.97	17.55 ± 2.20	3.02 ± 0.06	20.67 ± 2.16	45.63 ± 1.56	1.37 ± 0.12	15.52 ± 3.14	34.43 ± 3.67
	1.49	8.27	15.35	3.00	21.80	45.35	1.20	13.65	32.95
	1.16–1.87	4.31–13.20	9.90–33.70	2.60–3.40	7.70–27.90	38.50–52.60	1.10–1.90	3.10–29.00	21.50–46.60
		a, b, c	a		a, b, c	a, c		a, b, c	a

Table 1 continued

n = 6[$]	Liver Mean ± SE Median Min–Max			Kidney Mean ± SE Median Min–Max			Muscle Mean ± SE Median Min–Max		
	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$
T_{12}	1.50 ± 0.12	6.35 ± 0.65	14.02 ± 1.41	2.67 ± 0.08	14.92 ± 1.62	28.08 ± 3.33	1.20 ± 0.05	16.65 ± 2.72	35.45 ± 5.73
	1.36	6.80	15.70	2.60	17.20	32.10	1.20	19.40	43.30
	1.16–1.94	1.77–8.94	4.61–18.00	2.40–3.20	3.00–19.00	7.10–38.60	1.00–1.50	2.70–24.60	4.40–50.30
		a, b, d	a, d	c, d	a, b, c, d	a, c, d	cd	a, b, c, d	a, c

(a) Statistical difference between the SeMet turtles group (i.e. $SeMet_1$ or $SeMet_2$) and controls. (b) Statistical difference between the $SeMet_1$ and $SeMet_2$ turtle groups. (c) Statistical difference between two consecutive collection times, for a given group (i.e. control or $SeMet_1$ group or $SeMet_2$). (d) Statistical difference between the concentrations measured at the beginning (i.e. T_8) and end (i.e. T_{12}) of the EP_4

[$] Six turtles were usually sacrificed except when mentioned into brackets

[*] Samples were only available from the second week

[#] Since three samples were only available, statistical analysis were not run between $SeMet_2$, controls, and $SeMet_1$ turtles

accumulated higher selenium levels in their tissues than $SeMet_1$, and as soon as the first week of Se supplementation in destructive and non-invasive sampling (Figs. 3, 4 respectively). Selenium concentrations in both SeMet groups remained higher than those in controls at T_{12}, as well as than those measured at T_1. (*T* test, $p < 0.2$; Tables 1, 2).

Selenium speciation: kinetics and comparison

As a reminder, tissues were not available before T_2 for Se speciation. Besides, due to the low sample size, comparisons between groups were based on the highest concentrations measured in the turtle tissues (i.e. $n \leq 3$). Therefore, results were discussed as a global trend.

Whatever the collection time, the main Se species identified in the $SeMet_1$ and $SeMet_2$ turtle's tissues was the SeMet species. The highest SeMet concentration was measured in muscle (Table 3). Interestingly, liver was the tissue that accumulated the lowest SeMet concentration over the course of the feeding trial.

Muscle was further observed as the preferential tissue accumulating SeCys (Table 3). Nonetheless the SeCys concentration increased over the feeding trial in all tested tissues (Table 3).

At the contrary, the In.Se did not follow the same trends than the SeMet and SeCys accumulation for the tested tissues. The increase of In.Se found on the tissues was observed after T_8. Whatever the collection time, skin accumulated the lowest In.Se concentration.

Considering the low sample size, comparisons were based on the highest values. The $SeMet_2$ turtles concentrated more SeMet, SeCys and In.Se than the controls (Table 3). In blood, mean SeMet concentration increased throughout the feeding trial (i.e. from T_2 to T_{12}) while concentrations increased up to T_8 before slightly decreasing in the other tissues during the elimination period (i.e. T_{12}) (i.e. liver, muscle and skin). Similar trend was also observed for SeCys and In.Se.

During the EP_4, SeMet tended to decrease faster in skin than in other tissues. The contribution percentage of each selenium species (i.e. %SeMet, %SeCys and %In.Se) was estimated to the sum of all of them. Globally speaking, SeMet was the main species in the control and SeMet2 turtle tissues. The second species was SeCys and inorganic Se in the control and SeMet2 turtle tissues, respectively. However, pattern was In.Se > SeMet ≥ SeCys in the control liver and SeMet > SeCys > In.Se in the SeMet2 muscle.

Correlation analysis for non-destructive tissues

The scute tissues excluded, strong ($r^2 > 0.68$) and positive relationships (Fig. 5) were observed between selenium

Table 2 Selenium concentration (μg g^{-1} d.w.) in nondestructive tissues (i.e. skin, carapace, scutes and blood) collected from the control, $SeMet_1$ and $SeMet_2$ turtle groups

n = 6[$]	Skin Mean ± SE Median Min–Max			Carapace Mean ± SE Median Min−Max			Scutes* Mean ± SE Median Min−Max			Blood Mean ± SE Median Min−Max		
	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$
T_1	1.22 ± 0.11	3.52 ± 0.66	4.08 ± 0.60	0.43 ± 0.05	1.58 ± 0.20	1.37 ± 0.21				1.50 ± 0.14	3.45 ± 0.44	5.89 ± 0.93
	1.07	2.79	4.08	0.46	1.75	1.25				1.33	3.64	6.66
	0.96–1.70	1.43–8.33	1.64–6.11	0.26–0.62	0.68–2.18	0.64–2.09				1.03–2.09	1.54–4.59	2.15–10.10
		a	a		a	a					a, b	a
T_2	0.68 ± 0.03	6.05 ± 0.45	10.04 ± 0.75	0.55 ± 0.05	2.62 ± 0.14	4.02 ± 0.27		1.42 ± 0.11		1.22 ± 0.10	6.64 ± 0.11	10.13 ± 0.86
	0.65	6.05	9.52	0.69	2.89	4.06		1.46		1.07	6.48	9.38
	0.57–0.79	4.28–7.58	7.04–13.40	0.44–0.74	2.10–3.24	3.22–4.93 (5)[$]	0.04–0.04 (3)[$,#]	1.16–1.60 (4)[$]	2.17–2.98 (3)[$,#]	1.00–1.74	6.42–7.46	7.25–15.20
	c	a, b, c	a, c	c	a, b, c	a, c					a, b, c	a, c
T_3	1.11 ± 0.05	7.24 ± 0.63	12.00 ± 1.53	0.40 ± 0.03	2.44 ± 0.21	3.34 ± 0.43	0.56 ± 0.03	2.13 ± 0.22	2.82 ± 0.18	1.31 ± 0.02	8.37 ± 0.32	15.08 ± 1.02
	1.14	7.50	13.40	0.37	2.47	3.76	0.57	2.01	2.91	1.29	8.43	14.60
	0.82–1.29	5.09–8.97	0.79–16.4	0.31–0.62	1.61–3.10	1.07–4.43 (5)[$]	0.38–0.68	1.40–2.93	2.23–3.40 (5)[$]	1.25–1.46	6.81–10.20	12.10–18.10 (5)[$]
	c	a, b	a, c	c	a, b	a		a, b, c	a		a, b, c	a
T_4	1.04 ± 0.02	9.27 ± 0.55	15.27 ± 1.8	0.53 ± 0.02	4.42 ± 0.39	7.23 ± 0.88	0.42 ± 0.06	1.97 ± 0.17	3.22 ± 0.49	1.15 ± 0.03	9.6 ± 0.76	18.30 ± 1.59
	1.03	9.75	16.40	0.50	4.62	7.78	0.46	1.89	2.72	1.14	10.15	16.10
	0.95–1.15	6.01–10.8	2.10–23.60	0.41–0.68	2.10–6.11	1.05–11.20 (5)[$]	0.04–0.64 (5)[$]	1.40–2.72	1.76–5.11	0.98–1.29	6.02–12.30	15.40–23.70 (5)[$]
		a, b, c	a, c		a, b, c	a, c		a, b	a		a, b	a
T_8	0.99 ± 0.08	12.55 ± 0.46	17.61 ± 2.04	0.55 ± 0.02	5.97 ± 0.35	9.73 ± 0.59	0.48 ± 0.06	2.64 ± 0.07	7.06 ± 1.03	1.38 ± 0.03	13.13 ± 0.32	25.72 ± 1.21
	1.07	12.60	19.15	0.56	6.85	9.02	0.53	2.63	6.66	1.39	13.00	24.85
	0.61–1.25	10.40–14.90	2.63–25.90	0.40–0.63	4.19–7.18	7.12–12.4	0.001–0.68 (5)[$]	2.34–2.93	4.08–11.8	1.25–1.52	11.80–15.50	21.00–34.60
		a, b, c	a		a, b, c	a		a, b, c	a, c	c	a, b, c	a, c
T_9	1.17 ± 0.06	8.66 ± 1.11	20.15 ± 1.41	0.53 ± 0.05	4.25 ± 0.63	8.69 ± 0.37	0.71 ± 0.03	2.36 ± 0.19	3.93 ± 0.45	1.41 ± 0.07	9.14 ± 1.14	17.84 ± 1.26
	1.19	10.04	21.10	0.64	3.68	8.06	0.71	2.32	3.42	1.49	9.48	18.20
	0.94–1.50	1.09–11.40	14.60–25.20	0.32–0.71	1.40–6.76	7.33–10.10	0.53–0.94	1.52–3.37	2.78–6.79	1.16–1.60	3.48–12.70	9.83–22.20
		a, b, c	a		a, b, c	a	c	a, b	a, c		a, b, c	a, c
T_{12}	0.92 ± 0.05	6.87 ± 0.83	13.38 ± 1.70	0.49 ± 0.01	3.68 ± 0.40	7.38 ± 1.18	0.37 ± 0.09	1.98 ± 0.15	3.19 ± 0.36	1.30 ± 0.07	10.46 ± 1.12	19.41 ± 2.34
	0.94	7.75	15.80	0.51	4.00	4.92	0.51	1.86	3.32	1.36	11.75	22.85
	0.75–1.07	1.29–9.81	3.59–17.60	0.42–0.57	0.88–4.73	1.14–10.90 (5)[$]	0.04–0.57 (4)[$]	1.41–3.08	1.28–4.68	1.06–1.53	2.24–13.60	4.24–25.60

Table 2 continued

n = 6[$]	Skin			Carapace			Scutes*			Blood		
	Mean ± SE			Mean ± SE			Mean ± SE			Mean ± SE		
	Median			Median			Median			Median		
	Min–Max			Min–Max			Min–Max			Min–Max		
	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$	Control	$SeMet_1$	$SeMet_2$
	c	a, b, c, d	a, c, d	d	a, b, d	a	c	a, b, d	a, d		a, b, d	a, d

(a) Statistical difference between the SeMet turtles group (i.e. $SeMet_1$ or $SeMet_2$) and controls. (b) Statistical difference between the $SeMet_1$ and $SeMet_2$ turtle groups. (c) Statistical difference between two consecutive collection times, for a given group (i.e. control or $SeMet_1$ group or $SeMet_2$). (d) Statistical difference between the concentrations measured at the beginning (i.e. T_8) and end (i.e. T_{12}) of the EP_4

[$] Six turtles were usually sacrificed except when mentioned into brackets

* Samples were only available from the second week

[#] Since three samples were only available, statistical analysis were not run between $SeMet_2$, controls, and $SeMet_1$ turtles

concentrations measured in the non-destructive tissues (i.e. blood, skin and carapace) and those in the target ones (i.e. liver, kidney and muscle). The strongest correlations were observed between concentration in target tissues and those in carapace (Fig. 5, from section A to C), skin (Fig. 5, from section G to I) and blood (Fig. 5, from section J to L). Only medium ($0.35 < r < 0.68$) and positive correlations were observed between concentrations in target tissues and those in scutes (Fig. 5, from section D to F).

Discussion

Selenium exposure and related adverse effects in turtles

Se concentrations may pose a considerable risk to turtles through reduced egg viability (Lam et al. 2006). However, no experimental data were available about selenium toxicity towards young developing turtles. The present study reported the ability of turtles to efficiently accumulate selenium as SeMet from a SeMet dietary source and in a dose-dependent way. Indeed, the more SeMet was concentrated in the food, the more it was in the turtles' tissues; and tissue levels increased throughout the feeding trial (Figs. 3, 4). The slight increase of SeCys and In.Se levels during the SP_8 (Table 3) further suggested the turtles' ability to convert the ingested SeMet as SeCys into proteins or as selenite, eventually complexed to proteins (Dumont et al. 2006).

No adverse effect was associated with selenium exposure in the SeMet turtle group. Rather, individuals have grown normally in size and mass (CCPA, 2006). Their feeding behaviour seemed not affected. The turtles looked healthier as the feeding trial progressed. Snakes and lizards were likewise unaffected by dietary level as high as $\sim$23 μg g^{-1} of selenium as SeMet (Hopkins et al. 2004, 2005b). Nevertheless, this was quite surprising since we used dietary selenium concentrations reported as affecting biological endpoints in the leopard gecko *E. macularius* (Rich and Talent 2009). This could be explained by difference in selenium chemical forms and/or exposure duration used for the SeMet turtle and lizard studies. The leopard gecko fed on food supplemented with inorganic selenium as sodium selenite (Se(IV)) for less than 1 month. The SeMet turtle groups were dietary exposed to organic SeMet for 2 months. Although adverse associated with Se(IV) seems to occur faster than with SeMet (i.e. delay of one week), these factors cannot exclusively account for the lack of adverse effects in the present study (Heinz et al. 1988). Conversely, hepatic glutathione metabolism and lipid peroxidation appeared more affected by SeMet exposure than by Se(IV) in birds (Hoffman et al. 1989). As

Table 3 Concentration in selenium species (i.e. SeMet, SeCys and inorganic Se) in tissues collected from the $SeMet_2$ and control turtles

Tissue	Turtle group	Mean ± SE Median Min–Max (Number of samples)											
		T_2			T_4			T_8			T_{12}		
		SeMet	SeCys	In.Se	SeMet	SeCys	In.Se	SeMet	SeCys	In.Se	SeMet	SeCys	In.Se
Blood	$SeMet_2$	2.1* ± 0.5	0.3 ± 0.1	0.6 ± 0.04	8.7* ± 0.8	0.7* ± 0.1	0.6 ± 0.1	13.6* ± 1.3	0.9* ± 0.1	2.4* ± 0.6	18.2* ± 3.0	0.8 ± 0.2	0.9 ± 0.3
		2.5	0.5	0.1	8.7	0.8	0.7	12.0	0.8	1.9	19.7	0.8	0.7
		0.6–3.0	0.01–0.5	0.06–0.2	6.9–11.6	0.4–0.8	0.2–0.8	11.4–18.2	0.6–1.1	1.2–4.7	10.3–23.0	0.2–1.2	0.5–1.6
		(5)			(5)			(6)			(4)		
	p-value*	0.031 746			0.043 329	0.125 541		0.002 165	0.002 165	0.064 935	0.028 571		
	Control	0.5 ± 0.1	0.4 ± 0.05	0.08 ± 0.01	0.3 ± 0.04	0.05 ± 0.02	0.1 ± 0.02	0.6 ± 0.05	0.5 ± 0.06	0.1 ± 0.06	0.3 ± 0.1	0.4 ± 0.1	0.05 ± 0.03
		0.4	0.5	0.08	0.3	0.04	0.1	0.5	0.5	0.4	0.3	0.3	0.05
		0.3–1.0	0.4–0.7	0.05–0.1	0.1–0.3	0.02–0.1	0.06–0.2	0.4–0.7	0.2–0.6	0.07–0.4	0.1–0.6	0.1–0.7	N.D.–0.1
		(5)			(5)			(6)			(4)		
Liver	$SeMet_2$	2.0–5.4	0.2–0.7	N.D.–0.2	0.02–4.8	0.1–0.9	ND–0.003	5.3 ± 1.0	1.3 ± 0.3	3.1 ± 0.6	3.7–6.5	1.2–1.6	1.2–1.5
								4.7	1.4	2.6			
								3.4–8.1	0.4–1.8	2.4–5.0			
		(3)			(3)			(4)			(3)		
	Control	0.27	0.10	0.16	0.19–0.24	N.D.	0.21–0.26	0.02–0.12	0.07–0.17	0.23–0.31	0.04–0.09	0.07–0.17	0.28–0.30
		(1)			(3)			(2)			(3)		
Muscle	$SeMet_2$	8.3 ± 0.9	1.6 ± 0.3	0.4 ± 0.1	14.1–17.0	N.D.–5.1	0.03–4.0	55.0–77.8	3.4–7.0	0.5–2.1	60.2–74.3	2.4–4.6	0.6–0.7
		8.1	1.3	0.4									
		5.2–11.4	0.9–2.7	0.2–0.7									
		(6)			(3)			(2)			(3)		
	Control	1.3–1.3	0.3–0.5	0.13–0.14	1.0	0.2	0.07	2.5	0.4	0.1	2.0 ± 0.32	0.3 ± 0.08	0.2 ± 0.08
											2.0	0.3	0.2
											2.0–2.7	0.3–0.4	0.2–0.3
		(2)			(1)			(1)			(4)		
Skin	$SeMet_2$	5.7	0.3	0.1	9.7–10.7	0.50–0.8	0.9–1.5	14.9–20.7	0.8–2.5	0.4–0.7	11.5–13.1	0.6–0.6	0.4–0.4
		(1)			(2)			(2)			(2)		
	Control	0.3	0.1	N.D.	0.4 ± 0.05	0.2 ± 0.05	0.1 ± 0.02	N.A.			0.1	0.04	0.03
					0.4	0.2	0.1						
					0.3–0.5	0.05–0.3	0.01–0.1						
		(1)			(4)						(1)		

For n > 3, mean ± SE and median values were reported in the table. For n ≤ 3, minimum–maximum values were only reported

* Statistical differences with the control and associated p-value

Tables 4 Concentration factor (tissue/dietary selenium) of tissues collected from the $SeMet_1$ and $SeMet_2$ turtles during the elimination period (i.e. EP_4)

	Liver		Muscle		Kidney		Blood		Skin		Carapace		Scutes	
	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$	$SeMet_1$	$SeMet_2$
T_8	0.49	0.41	1.09	0.86	1.37	1.18	0.59	0.57	0.57	0.39	0.27	0.22	0.12	0.16
T_9	0.37	0.39	0.70	0.77	0.94	1.01	0.41	0.40	0.39	0.45	0.19	0.19	0.11	0.09
% to T_8^a	76	95	64	90	69	86	70	70	68	115	70	86	92	56
T_{12}	0.29	0.31	0.75	0.79	0.68	0.62	0.47	0.43	0.31	0.30	0.17	0.16	0.09	0.07
% to T_8^a	59	76	69	92	50	53	80	75	54	77	63	73	75	44

The selenium levels were 22.1 ± 1.0 and 45.0 ± 2.0 in the $SeMet_1$ and $SeMet_2$ food, respectively

[a] The percentage of selenium elimination between T_8 and T_9, and T_8 and T_{12}

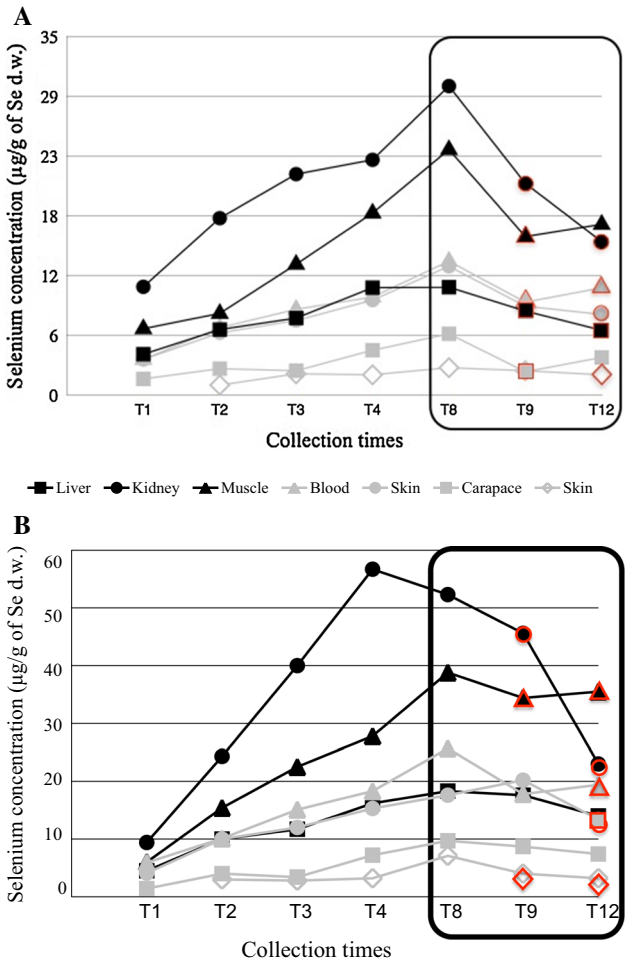

Fig. 2 Pattern of the selenium concentration (µg g^{-1} of Se d.w.) over the course of the feeding trial in the target and non-destructive tissues collected from the $SeMet_1$ (**a**) and $SeMet_2$ (**b**) turtle groups. *Black outlines* target tissues, i.e. liver, kidney and muscle; *grey outlines* non-destructive tissues, i.e. blood, skin, carapace and scutes; *non boxed outlines* from T_1 to T_8, supplementation period (SP_8); *black boxed outlines* from T_8 to T_{12}, elimination period (EP_4); *red thickened data symbol at T_9* statistical differences with selenium concentration at T_8; *red thickened data symbol at T_{12}* statistical differences with selenium concentration at T_8 (Color figure online)

previously suggested in reptiles, the hepatic selenium level measured in the SeMet turtle groups could be associated with sublethal effects such as an increase of the GPx indicating cellular damage (Ganser et al. 2003). GPx uses SeCys in its active site and the slight increase observed for the hepatic levels of SeCys (based on maximal concentration) throughout the feeding trial could make possible such assumption. Likewise, histopathological alterations in kidney could occur for the reported concentrations (Tashjian et al. 2006).

Selenium kinetics

The dietary selenium dose had no effect on the selenium kinetics since both dietary exposed groups, i.e. $SeMet_1$ (Fig. 2a) and $SeMet_2$ (Fig. 2b), shown similar accumulation pattern in tissues. Selenium was preferentially accumulated in the kidney of the turtles from both SeMet groups and was consistent with other studies in reptiles (Hopkins et al. 2002, 2004) but differed from those in birds which reported a preferential hepatic storage of selenium (Albers et al. 1996; Franson et al. 2007). Differences in selenium kinetics between birds and turtles were further indicated in blood and muscle. For similar dietary selenium level, the SeMet turtle groups accumulated higher and lower selenium levels in their muscle and blood than birds, respectively. In addition, one and 12 weeks were needed to birds for reaching 95 % of the selenium dietary level in liver and muscle, respectively (Heinz et al. 1990). More than 48 and 100 % of the dietary level were reached in the $SeMet_1$ liver and muscle at T_8 (Fig. 2a), respectively. Likewise, 41 and 81 % were reached in the $SeMet_2$ liver and liver (Fig. 2b), respectively.

The renal partitioning of selenium could suggest that turtles coped with a metabolic excess of selenium enhancing its rate of glomerular filtration (Oster and Prellwitz 1990; Windisch 2002). Such selenium excess

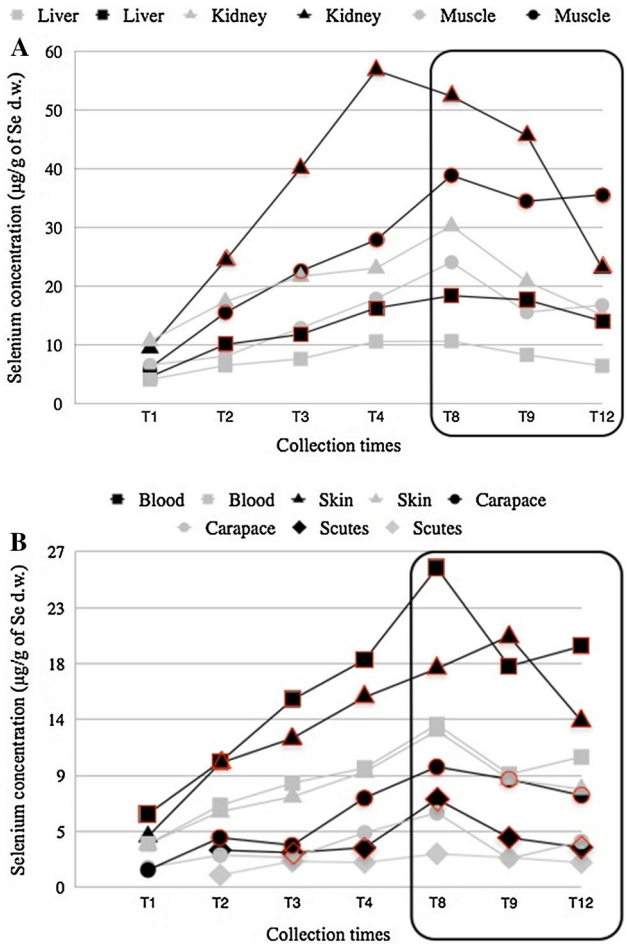

Fig. 3 Pattern of the selenium concentration (µg g^{-1} of Se d.w.) over the course of the feeding trial in the target tissues collected from the $SeMet_1$ (**a**) and $SeMet_2$ (**b**) turtle groups. *Target tissues* liver, kidney and muscle; *non-destructive tissues* blood, skin, carapace and scutes; *Grey outlines* $SeMet_1$ group; *black outlines* $SeMet_2$ group; *non boxed outlines* from T_1 to T_8, supplementation period (SP_8); *black boxed outlines* from T_8 to T_{12}, elimination period (EP_4); *red thickened data symbol* statistical differences between each group (Color figure online)

could further indicate an internal steady state, which was only observed in liver from T_4 to T_8 (Fig. 2a, b) (Schrauzer 2000). Nevertheless, equilibrium may have been missed in other tissues due to the lack of available samples between T_4 and T_8 (i.e. at T_5, T_6 and T_7). The reported renal partitioning in the turtles could also indicate that the dietary selenium levels were not toxic for the turtles and even good for their metabolism (Oster and Prellwitz 1990). Indeed, adequate supplementation of selenium was indicated to enhance renal filtration and selenium accumulation (Oster and Prellwitz 1990). That would be quite surprising considering that adverse effects were previously associated with similar dietary selenium levels in birds and lizards (Hoffman 2002; Rich and Talent 2009).

The dietary selenium dose differently affected the selenium elimination in turtles. Indeed, the more selenium was concentrated in diet, the more it was in the turtles' tissues but the less efficiently it was removed from them. By calculating a concentration factor for each tissue (i.e. selenium concentration in tissue/selenium concentration in diet, Table 4), the $SeMet_1$ turtles group eliminated selenium more readily than the $SeMet_2$ turtle group. This observation contrasts with results from birds for which the more selenium was concentrated in liver, the faster it was removed (Heinz et al. 1990). A lower metabolism and/or activity of detoxifying enzymes in the SeMet turtles groups than in birds was therefore suggested. Evidences arguing towards such hypothesis were that turtles needed more than 28 days (i.e. EP_4) for coming back to 50 % of their basal selenium level (Table 4) while birds needed around 10 and 24 days for reaching such levels in their blood and muscle, respectively (Heinz 1993).

In previous reptilian studies, snakes and lizards were dietary exposed to lower or similar selenium levels (i.e. between 11.36 and 22.70 µg g^{-1} d.w., Table 5) than the $SeMet_1$ turtles (i.e. 22.1 ± 1.0 µg g^{-1} d.w., Table 5), and

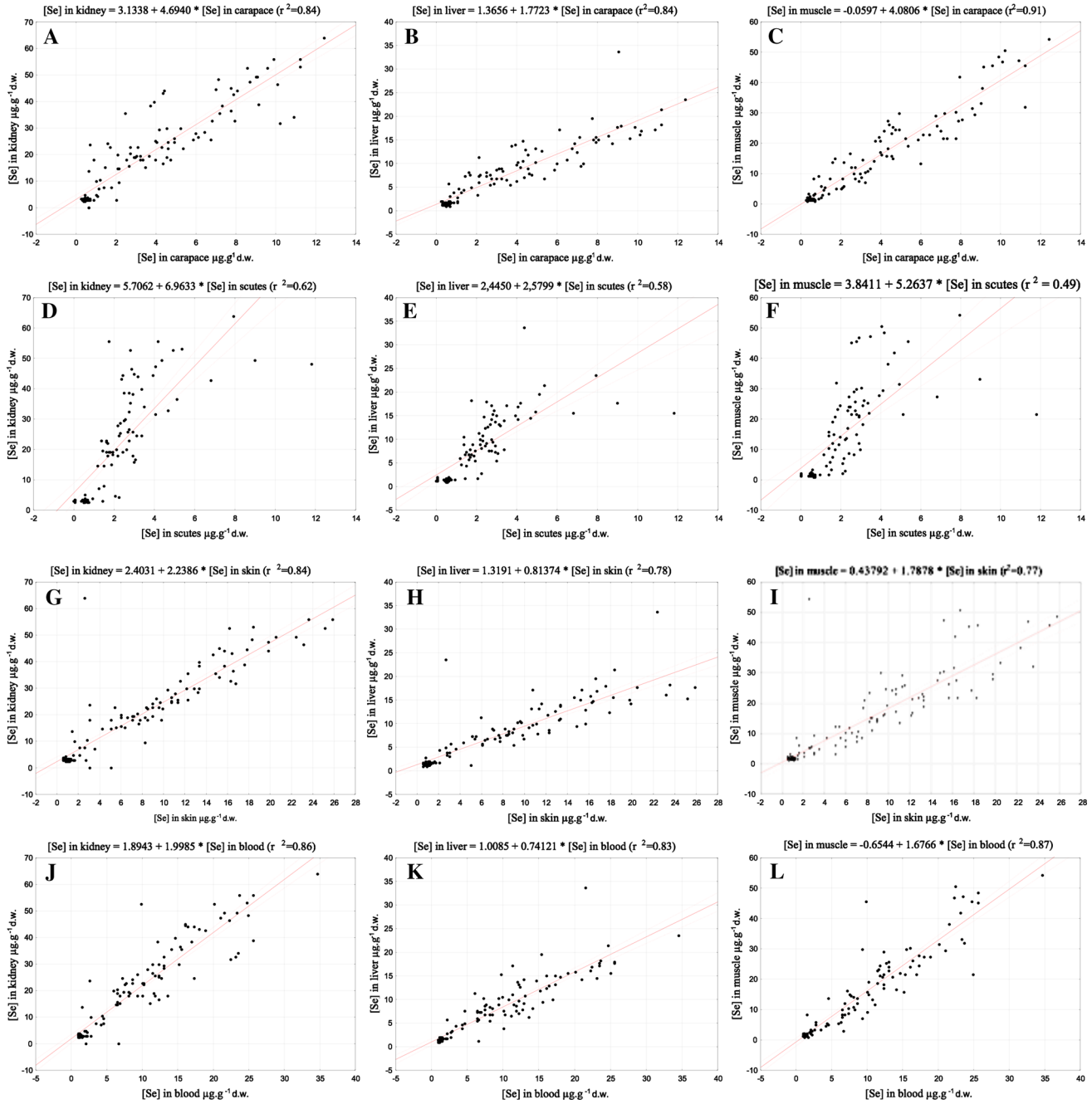

Fig. 4 Pattern of the selenium concentration (µg g^{-1} of Se d.w.) over the course of the feeding trial in the non-destructive tissues collected from the $SeMet_1$ and $SeMet_2$ turtle group. *Target tissues* liver, kidney and muscle; *non-destructive tissues* blood, skin, carapace and scutes; *Grey outlines* $SeMet_1$ group; *black outlines* $SeMet_2$ group; *non boxed outlines* from T_1 to T_8, supplementation period (SP_8); *black boxed outlines* from T_8 to T_{12}, elimination period (EP_4); *red thickened data symbol* statistical differences between each group (Color figure online)

to lower levels than the $SeMet_2$ turtles of the present study (i.e. 45.0 ± 2.0 µg g^{-1} d.w, Table 5). For the lowest selenium concentration in the snakes' and lizards' diet (i.e. <15 µg g^{-1} d.w., Table 5), similar selenium levels were reported in their liver and in the $SeMet_1$ turtles' one (i.e. ~11.0 µg g^{-1} d.w., Table 5). As expected, the $SeMet_2$ turtles concentrated more selenium in their liver than the $SeMet_1$ turtles. Surprisingly, turtles from both SeMet groups concentrated less selenium in their liver than the two species snakes feeding on diet supplemented with around 23.0 µg g^{-1} d.w. of selenium (i.e. similar to $SeMet_1$ diet level and lower to $SeMet_2$ diet level; Table 5).

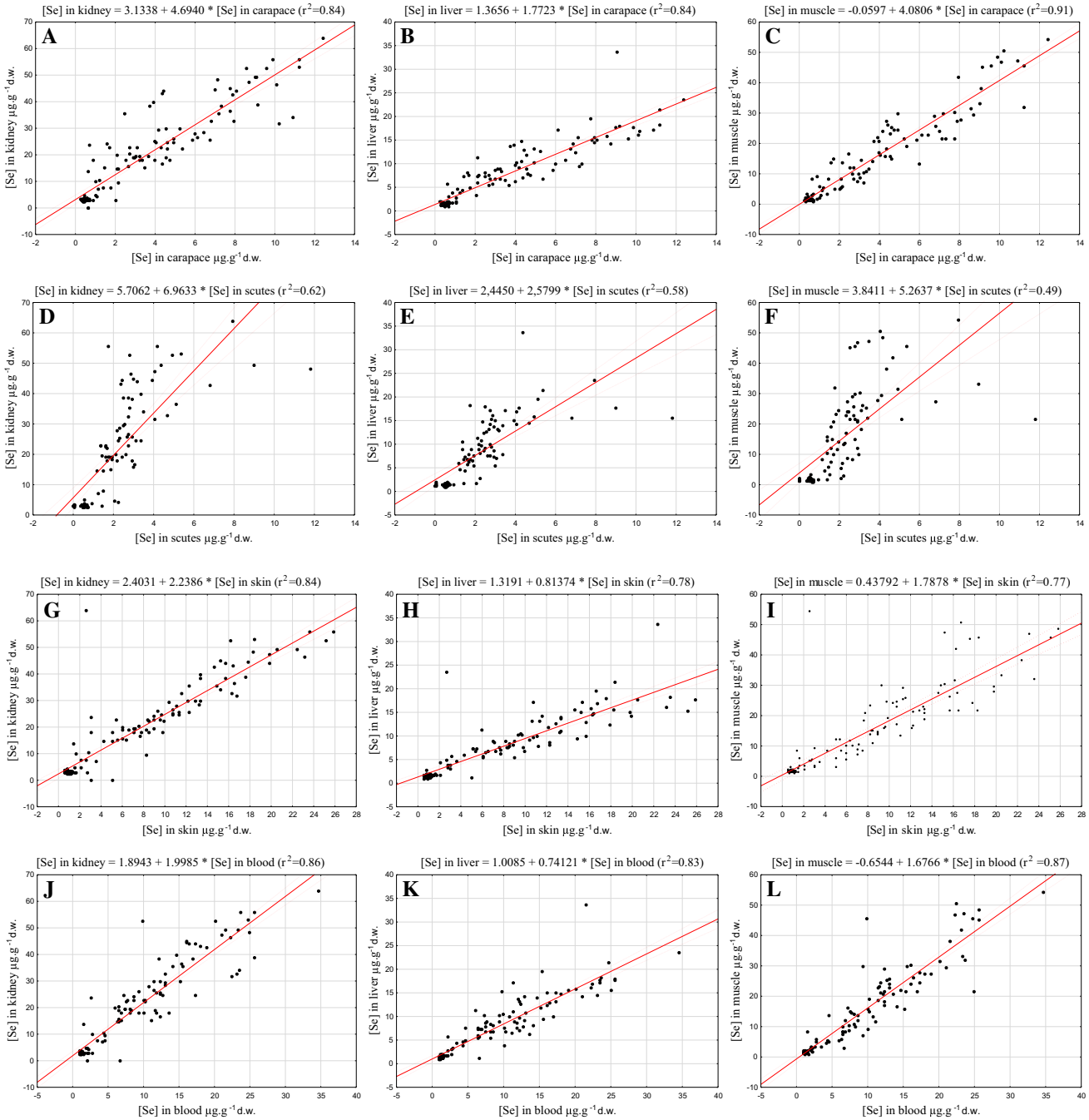

Fig. 5 Predictions (*solid line*) of selenium concentrations (in µg of Se g^{-1}) in target tissues (i.e. kidney, liver and muscle) from concentrations measured in non-destructive tissues (i.e. blood, skin, carapace and scutes) collected from turtles. The three turtle groups (i.e. control, $SeMet_1$ and $SeMet_2$) were considered as a single group and both periods (i.e. SP_8 and EP_4) were included in the correlation analyses. Points are the original data used in the regression analyses expressed on a dry mass basis

Indeed, expectations would be that the hepatic selenium levels were similar in the snakes and $SeMet_1$ turtles, while the liver's $SeMet_2$ turtles was higher concentrated in selenium than the snakes' one. In addition, selenium levels were globally higher in kidney from both SeMet turtle groups than in the snakes' one (Table 5). Altogether, these differences can be explained by (a) species-related factors, (b) the exposure duration suggesting that long exposure (i.e. $\geq$10 months in snakes) enhances the selenium sequestration in liver and related toxic effects, as well as (c) a likely higher ability of juvenile slider turtles to deal with selenium exposure by enhancing renal filtration (Heinz et al. 1990; Oster and Prellwitz 1990).

Table 5 Selenium concentrations (μg g^{-1} d.w.) in tissues collected from various reptiles species including turtles from the present study

Species	Selenium form Selenium source	Concentration in the food (μg g^{-1} dry mass)	Feeding trial duration (months)	Tissue concentrations (μg g^{-1} dry mass)
Yellow-bellied slider turtle	Seleno-L-methionine	$SeMet_1$: 22.1 ± 1.0	2	*At T_8*
Trachemys scripta scripta	Commercial pellets			Kidney: 30.17 ± 1.02
The present study				Liver: 10.76 ± 0.71
				Muscle: 24 ± 0.98
				Blood: 13.13 ± 0.32
		$SeMet_2$: 45.0 ± 2.0		Kidney: 52.87 ± 1.92
				Liver: 18.27 ± 1.12
				Muscle: 38.75 ± 4.37
				Blood: 25.72 ± 1.21
Brown house snake	Seleno-L,D-methionine	Treatment 1: 12.52 ± 0.32	10	Kidney: ~20.0
Lamprophis fuliginosus	Injected into thawed mice			Liver: ~11.0
(Hopkins et al. 2004)				Gonads: ~12.0
		Treatment 2: 22.95 ± 0.37		Kidney: ~32.0
				Liver: ~20.0
				Gonads: ~21.0
				Females/males
Banded water snake	Total Se	Treatment 1: ~11.36	24	Kidney: 16.0 ± 1.5/ 21.1 ± 6.6
Nerodia fasciata	Fish from coal ash-contaminated site			Liver: 11.6 ± 0.5/ 10.8 ± 0.6
(Hopkins et al. 2002)				Gonads: 10.0 ± 0.3/ 3.1 ± 0.2
		Treatment 2: ~22.70		Kidney: 25.9 ± 1.2/ 32.0 ± 1.0
				Liver: 24.1 ± 0.5/ 24.2 ± 2.4
				Gonads: 17.6 ± 1.3/ 19.1 ± 1.1
Western fence lizard *Sceloporus occidentalis*	Seleno-L,D-methionine	Overall mean: 14.7	~3	*All individuals*
(Hopkins et al. 2005b)	Supplemented crickets *A. domestica*			Tail: 7.6 ± 0.2
				Females/males
				Liver: 11.0 ± 1.0/ 13.0 ± 1.0
				Gonads: 14.0 ± 0.5/ 11.0 ± 0.5
Mallard ducks	Seleno-L,D-methionine	10 in wet weight	1.5	Liver: 66.0
Anas platyrhynchos	Commercial diet			Muscle: 31.5
				Blood: 84.0

Non-destructive tissues as indicators

Developing low invasive biomonitoring tools for estimating pollutant exposure in wildlife is of current concern worldwide, especially for highly protected species such as marine turtles (e.g. Guirlet et al. 2008; Hopkins et al. 2013). In this context, non-destructive collection techniques were proposed in the present study. The suitability of collecting blood, skin, scutes and carapace to assess the selenium level in target tissues (i.e. liver, kidney and muscle) was tested. Each of the analysed tissues accumulated selenium, in a dose- and time-dependant way making the method viable for the present purpose (Fig. 2a, b).

Similar model was reported for snakes and allowed the estimation of selenium levels in tissues (i.e. gonads, kidney, liver and eggs) from those in tail and blood (Hopkins et al. 2005a). By reporting the selenium levels measured in the $SeMet_1$ and $SeMet_2$ skins at T_8 (i.e. 12.55 and

17.61 μg g^{-1} d.w. in the $SeMet_1$ and $SeMet_2$ turtles, respectively; Table 2), this snake model predicted selenium concentrations similar than those effectively measured in the SeMet turtles' livers (i.e. ~14.0 and ~18.0 μg g^{-1} d.w. in the $SeMet_1$ and $SeMet_2$ turtles, respectively) but lower than those measured in their kidneys. Indeed, this snake model predicted renal selenium levels of ~20.0 and ~34.0 μg g^{-1} d.w. for the $SeMet_1$ and $SeMet_2$ turtles, respectively while the measured concentrations were 30.17 and 52.87 μg g^{-1}, respectively (Table 1). By reporting the blood selenium levels measured in the $SeMet_1$ and $SeMet_2$ at T_8 (i.e. 13.13 and 25.72 μg g^{-1} d.w. in the $SeMet_1$ and $SeMet_2$ turtles, respectively; Table 2), the snake model predicted quite similar concentrations measured in the SeMet turtles' kidney (i.e. ~30.0 and >50.0 μg g^{-1} d.w. in the $SeMet_1$ and $SeMet_2$ turtles, respectively) but higher levels than those effectively measured in the turtles' livers. Indeed, the model predicted hepatic levels of ~16.0 and >30.0 μg g^{-1} d.w. for the $SeMet_1$ and $SeMet_2$ turtles, respectively while the measured concentrations were 10.76 and 18.27 μg g^{-1}, respectively (Table 1). As expected, the comparison of the turtle and snake models suggested that confounding factors (e.g. species belonging and physiology) most likely influenced the selenium kinetics in these organisms.

The turtle model was proposed to assess the selenium exposure in turtles rather than precisely determine levels in a given tissue. Therefore, statistical relationships (Fig. 5) were provided for estimating the selenium levels in the turtles' target tissues (i.e. liver, kidney and muscle) from those measured in non-destructive tissues (i.e. blood, skin, carapace and scutes). Then, selenium levels in target tissues can be compared to available toxic thresholds for assessing the turtle health risk to selenium exposure. As an example, Perrault and co-authors (2011) reported selenium levels ranging from 12.1 to 69.7 μg g^{-1} d.w. in blood collected from Florida leatherback turtle (*Dermochelys coriacea*) hatchlings (conversions were made from wet to dry weight by assuming a blood moisture of 90 %). Our model describing the relationship between selenium level in blood and liver (Fig. 5, section K) predicted that these hatchlings would have hepatic selenium levels ranging from 9.9 to 52.6 μg g^{-1} d.w. Likewise, selenium levels ranging from 0.7 to 91 μg g^{-1} d.w. were reported in blood of juveniles green marine turtles (*Chelonia mydas*) from Australia (van de Merwe et al. 2010). These individuals would have selenium levels ranging from 1.5 to 68 μg g^{-1} d.w. in their liver (Fig. 5, section K). Then, freshwater turtles accumulating 2.6–3.4 μg g^{-1} d.w. of selenium in their blood (Bergeron et al. 2007) would accumulate hepatic levels ranging from 2.9 to 3.5 μg g^{-1} d.w. (Fig. 5, section K).

Unfortunately, selenium toxic thresholds were not available for turtles but were for close related species, i.e. birds (Hoffman 2002; Janz et al. 2010). These bird studies reported reduced growth and survival, alteration of the glutathione metabolism and lipid peroxidation for hepatic selenium levels exceeding 20 μg g^{-1} dw. Our present model predicted selenium levels up to 53 and 68 μg g^{-1} d.w. in marine turtles' liver raising question about potential selenium toxicity. Nonetheless, we previously reported that turtles looked healthier along with the feeding trial. Therefore, they could better manage with selenium toxicity than other vertebrates (e.g. snakes, lizards), birds included. The bird model could thus overestimate the turtle response to selenium exposure.

Conclusion

The present study was the first to investigate dietary selenium exposure in freshwater turtles. During the SP_8 (Fig. 1), both $SeMet_1$ and $SeMet_2$ turtles efficiently accumulated selenium from a SeMet dietary source. The more selenium was concentrated in the food, the more it was in the turtle body but the less it was removed from their tissues. Moreover, SeMet was found to be the more abundant selenium species in turtles' tissues (Table 3). Body condition (i.e. growth in mass and size, feeding behaviour and activity) and survival of the $SeMet_1$ and $SeMet_2$ turtles seemed to be unaffected by the selenium exposure. There were clear evidences that reptilian species are differently affected by and sensitive to selenium exposure but the lack of any adverse effects was quite unexpected. Ecotoxicological investigations and toxic thresholds are still being lacking in reptiles and preclude any definitive conclusions. Selenium toxicity most likely occurred through other pathways that were not investigated in the present study (e.g. hepatic histopathological lesions).

Many confounding factors may influence the selenium toxicity in turtles such as the development stage, sex, route of exposure, selenium chemical form and/or the occurrence of other pollutants. Besides, questions remain concerning the use of laboratory models in field situations, even for close related species. Nevertheless, the present study aimed at proposing reliable tools for evaluating the selenium exposure rather than at precisely predicting levels in tissues. In a conservational context, the use of carapace and skin for assessing selenium exposure should warrant further attention. These can be easily collected from living and dead individuals and pollutant levels were expected to less fluctuate along with the animal life history than in other tissues such as blood.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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