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Stable isotope analysis successfully identifies wild-caught individuals of threatened Asian freshwater turtles in illegal trade

Yik-Hei Sung [a,b,*], Jia Huan Liew [c], Wing Sing Chan [d], Amy Wing Lam Fok [b], Julia Leung [b,e], Ho Fung Wong [f], David M. Baker [d,g], Timothy C. Bonebrake [d], Caroline Dingle [h], David Dudgeon [d], Nancy E. Karraker [i], Anthony Lau [b], Violaine A. Colon [j], Ioannis Magouras [k], Gary Ades [l], Paul Crow [l], Liz Rose-Jeffreys [l], Ricky Spencer [m], Jonathan J. Fong [b,**]

[a] *School of Health, Sciences and Society, University of Suffolk, Ipswich, UK*
[b] *Science Unit, School of Interdisciplinary Studies, Lingnan University, Hong Kong SAR*
[c] *School of Natural Sciences, University of Tasmania, Sandy Bay, Tasmania, 7005. Australia*
[d] *School of Biological Sciences, The University of Hong Kong, Pok Fu Lam, Hong Kong SAR*
[e] *Department of Veterinary Clinical Sciences, City University of Hong Kong, Hong Kong SAR*
[f] *Ocean Futures Research Cluster, School of Science, Technology & Engineering, University of the Sunshine Coast, 90 Sippy Downs Dr, Sippy Downs, QLD 4556, Queensland, Australia*
[g] *The Swire Institute of Marine Science, The University of Hong Kong, Cape D'Aguilar Road, Shek O, Hong Kong SAR*
[h] *Biology Department, Capilano University, North Vancouver, British Columbia, Canada*
[i] *Department of Natural Resources Science, University of Rhode Island, Kingston, RI, USA*
[j] *Durrell Wildlife Conservation Trust, Trinity, Jersey JE3 5BP, UK*
[k] *Veterinary Public Health Institute, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[l] *Kadoorie Farm and Botanic Garden, Tai Po, Hong Kong SAR*
[m] *School of Science, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia*

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ABSTRACT

Laundering of wild-caught animals as captive-bred is a frequent practice in the illegal wildlife trade. Stable isotope analysis is a promising tool for distinguishing wild and captive-bred animals. We use Hong Kong freshwater turtles to test the effectiveness of using stable isotopes to differentiate wild and captive-bred individuals. In this study, we compared five stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$) in claw samples across four highly threatened species: *Cuora trifasciata*, *Mauremys reevesii*, *Platysternon megacephalum*, and *Sacalia bealei*. We found non-overlapping $\delta^{13}C$ and $\delta^{15}N$ values for all species; combined $\delta^{13}C$ and $\delta^{15}N$ isotopic profiles resulted in a 100 % accuracy in identifying the sources of turtles. Through repeated sampling of seized *P. megacephalum*, we estimate 95 % turnover rates of 46.3 months for $\delta^{13}C$ and 32.8 months for $\delta^{15}N$, suggesting that wild-caught individuals can be identified up to two years after capture. Lastly, we apply the stable isotope method in true wildlife seizures. These seizures are unique because some individuals possessed microchips from our long-term population study, so were unambiguously from the wild. The isotopic profiles of seized turtles clustered with those of wild populations, providing forensic evidence that supported the prosecution of suspects for illegal

* Correspondence to: University of Suffolk, JH304, James Heir Building, Ipswich IP3 0BW, UK.
** Correspondence to: Lingnan University, Room 116/6, Lau Chung Him Building, Tuen Mun, Hong Kong.
E-mail addresses: h.sung@uos.ac.uk (Y.-H. Sung), jonfong@ln.edu.hk (J.J. Fong).

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trade and/or possession. Overall, our study demonstrates the effectiveness of $\delta^{13}C$ and $\delta^{15}N$ in differentiating wild and captive freshwater turtles. We advocate for using isotopic profiling in future seizures and expanding its application to more taxa and geographic locations to support wildlife trade management and prevent illegal exploitation of wild organisms globally.

1. Introduction

Unsustainable wildlife trade is a major threat to biodiversity worldwide (Bennett et al., 2002; Hoffmann et al., 2010), leading to population declines and disruption of ecosystem function (Coleman and Williams, 2002). To prevent unsustainable trade, most countries regulate cross-border wildlife trade using the framework provided by the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) (Reeve, 2002). CITES regulates trade by listing species in three appendices (I, II, III), with most threatened species listed in Appendix I. The regulation of wild-caught and captive-bred status differs for species listed in the Appendix II: captive-bred individuals can be legally traded, while the sale of wild-caught individuals is prohibited. Illegal traders exploit this policy by fraudulently declaring wild-caught specimens as captive-bred (Lyons and Natusch, 2011). The burden of proof falls to enforcement authorities, and it is difficult to impossible to differentiate captive-bred from wild-caught individuals. If officials cannot identify the source (wild-caught vs. captive-bred) of an individual, it is impossible to enforce laws against illegal trade and possession of wild-caught individuals (Sung et al., 2024). Fraudulent declaration and the lack of wildlife forensic tools undermines CITES's effectiveness and leads to over-exploitation of wild populations (Challender et al., 2015; Nijman and Shepherd, 2015).

A reliable forensic tool is needed for effective enforcement against misdeclaration and laundering of wild-caught specimens in trade (Brasileiro et al., 2023). Many studies have tested the effectiveness of stable isotope analysis (SIA) as a wildlife forensic tool to distinguish wild and captive animals. Brasileiro et al. (2023) reviewed 47 studies (55 species) and found that wild and captive animals could be differentiated in 84 % of studies across a wide range of taxa, including birds (Andersson et al., 2021), fish (Anderson et al., 2010; Chaguri et al., 2017; Vizzini et al., 2010), frogs (Dittrich et al., 2017), and lizards (van Schingen et al., 2016). Captive animals generally have higher $\delta^{13}C$ and $\delta^{15}N$ values because of their more consistent, nutrient-rich diet that is often rich in C_4 plants (van Schingen et al., 2016). Three other isotopes ($\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$) have rarely been used in wildlife forensics (Brasileiro et al., 2023), but have potential applications as tracers.

Freshwater turtles and tortoises are overexploited and frequently illegally traded; consequently, they are among the world's most endangered animals (Sigouin et al., 2016; Turtle Taxonomy Working Group, 2021). Turtle farming in China is widespread and intensive (Shi et al., 2008), which provides the opportunity to fraudulently claim wild-caught turtles as captive-bred to be traded "legally", a practice that is prevalent for some endangered species (Shi et al., 2008; Sung et al., 2024). There is a critical need to develop reliable tools to distinguish wild from captive-bred turtles to effectively regulate the turtle trade. However, applying SIA to turtle trade regulation has two major barriers. First, most species lack a SIA database for wild-caught and captive-bred turtles. To our knowledge, SIA has only been tested for differentiating wild and captive-bred Wood Turtles (*Glyptemys insculpta*) (Hopkins et al., 2022, 2023), Red-eared Sliders (*Trachemys scripta*) (Hill et al., 2020), and Philippine Forest Turtles (*Siebenrockiella leytensis*) (Brandis et al., 2023). The first three studies found SIA to be effective, while the last did not. Without species-specific datasets and studies, it is challenging to confidently differentiate wild from captive-bred individuals. Second, it is important to determine the turnover rates of isotopes in body tissues (Vander Zanden et al., 2015), *i.e.* the speed at which the isotope signal changes due to dietary shifts, which will determine how long the isotopic signature retains information about wild source after the individual has been removed from the wild. A slower turnover rate extends the period for which wild source can be detected. However, empirical data are rare because diet-switching experiments are challenging to implement due to the long time required (Haywood et al., 2019). Data on stable isotope turnover rates are only available for a single turtle species (*Trachemys scripta*), and these studies found that it takes at least six months for $\delta^{15}N$ and $\delta^{13}C$ to reach equilibrium (stabilizing to reflect the isotopic signatures of the new diet) in claw tissue following a dietary change (Aresco et al., 2015; Seminoff et al., 2007). However, the turnover time for $\delta^{13}C$ remains uncertain, and turnover rates may vary between species and geographical regions (Rosenblatt and Heithaus, 2013; Vander Zanden et al., 2015).

In this study, with a valuable opportunity to access samples from wild, captive, and seized individuals of threatened freshwater turtles, we address three objectives. First, we advanced the use of SIA in wildlife forensics by increasing the number of turtle species and tracers analyzed. Specifically, we compared isotopic signatures of five elements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$) in four native species from Hong Kong: two that are critically endangered (*Cuora trifasciata* and *Platysternon megacephalum*) and two endangered (*Mauremys reevesii* and *Sacalia bealei*). Second, we analyzed the turnover rate of stable isotope signatures in wild, confiscated *P. megacephalum* kept in captivity. Lastly, we applied this technique to provide proof-of-concept data by analyzing the isotope profiles of seized turtles to identify the source of turtles from turtle seizures in Hong Kong, including individuals of known source that were microchipped during our population studies.

2. Methods

2.1. Study species and sampling

We obtained tissue samples from both wild and captive freshwater turtles in Hong Kong Special Administrative Region, China, focusing on four native species: the Golden Coin Turtle (*C. trifasciata*), Chinese Pond Turtle (*M. reevesii*), Big-headed Turtle

(*P. megacephalum*), and Beale's Eyed Turtle (*S. bealei*). *Cuora trifasciata* is listed as critically endangered by IUCN (Fong et al., 2020). The species occurs only in South China and has likely been extirpated from large portions of its former range. No comprehensive diet study has been conducted, but it is probably omnivorous like its congeners, feeding on a variety of animals and plants. *Mauremys reevesii* is listed as endangered and is distributed in North Korea, South Korea, and China (van Dijk, 2011). It inhabits lowland shallow ponds and marshes, including agricultural areas. This species is omnivorous, with snails potentially making up a large part of its diet. *Platysternon megacephalum*, also assessed as critically endangered, ranges from China to Cambodia, Lao, Myanmar, Vietnam, and Thailand (Fong et al., 2021). This species inhabits higher gradient hillstreams and feeds primarily on fruits, insects, crabs, and mollusks (Sung et al., 2016). *Sacalia bealei* is listed as endangered and is endemic to southeastern China (Asian Turtle Trade Working Group, 2000). It occupies rocky hillstreams and feeds mainly on fruits, other plant matter, and insects (Sung et al., 2021).

We have conducted long-term turtle surveys in Hong Kong to study turtle distributions and monitor their populations since 2009. During the surveys we capture and uniquely mark each individual (shell notching, microchip), measure (carapace length and weight), and record location of capture. In 2015, we started collecting claw samples from these wild turtles for SIA. To protect these species from poaching, we do not disclose the locations of wild populations. For each species, claw samples from wild individuals were collected from at least three study sites and sampling covers different sex and age classes (adult male, adult female, and juvenile). Claw samples from captive individuals were collected from animals maintained in captivity for over two years (Aresco et al., 2015; Hopkins et al., 2022) by conservation organizations and universities: Hong Kong Society of Herpetology Foundation for *S. bealei*, Kadoorie Farm and Botanic Garden for *C. trifasciata* and *P. megacephalum*, and Lingnan University for *C. trifasciata*, *M. reevesii*, and *S. bealei*. We also included samples from three wildlife seizure cases in Hong Kong involving 47 *P. megacephalum* and *S. bealei* in October 2018, May 2022, and July 2023. Each of these cases involved an individual of known wild provenance from Hong Kong (based on the presence of microchips identifying them as individuals from our population studies).

For each turtle, we obtained two to four claw tips (< 5 mm) from the hind limbs using a pet nail clipper (Marchand et al., 2018). We chose the use of claws because they can be non-invasively sampled and are metabolically inert, which can provide more stable dietary information indicative of an animal's source (Bearhop et al., 2003). Turtle claws, similar to those of birds (Bearhop et al., 2003), grow from the base, so claw tips represent older tissue and reflect less recent dietary input compared to the claw base. All claw samples of wild turtles were collected between March and November during their active period. For seized turtles, claw samples were collected within two weeks of seizure. To process the samples for SIA, the claw samples were first rinsed with distilled water to remove any potential contaminants. Then, the nail samples were oven-dried at 60 °C for 48 h. The samples were then stored in airtight vials and kept in a dehumidifier before further processing. Next, we homogenized the dried samples using a mortar and pestle or homogenizer (Bioprep-24, Hangzhou Allsheng Instruments Co. Ltd, Hangzhou City, China). We did not remove lipids from the samples due to the low lipid content in claws (Sung et al., 2021). Subsequently, the samples were weighed (1–3 mg) and transferred to tin capsules for measurement of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, and silver capsules for measurement of $\delta^{2}H$ and $\delta^{18}O$. For $\delta^{2}H$ and $\delta^{18}O$ analyses, samples were allowed to equilibrate to atmospheric moisture for one to two weeks before analysis.

2.2. Stable isotope analysis

Isotopic measurements were taken using a Thermo EA-Isoprime Precision Isotope-ratio Mass Spectrometer (for measurement of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and an Elemental Pryo cube EA/Isoprime Precision Isotope-ratio Mass Spectrometer (for measurement of $\delta^{2}H$ and $\delta^{18}O$) at the Stable Isotope Laboratory of The University of Hong Kong. Standards used in the analysis included USGS-40, USGS-41a, and acetanilide for $\delta^{13}C$ and $\delta^{15}N$, USGS-42, IAEA-S1, and IAEA-S2 for $\delta^{34}S$, and Kudu Horn Standard (KHS), and Caribou Hoof Standard (CB), and benzoic acid for $\delta^{2}H$ and $\delta^{18}O$. The isotope samples were analyzed in two periods: in 2019–2020 only $\delta^{13}C$ and $\delta^{15}N$ were measured, and in 2021–2024 all isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$) were measured. Therefore, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$ data are unavailable for some samples because some samples were exhausted after the first analysis period.

2.3. Data analysis

We graphically compared the stable isotope values between wild and captive individuals using biplots of two isotopic pairs ($\delta^{13}C$ and $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$). We generated the biplots using ggplot2 package (Wickham, 2016) in software R (R Core Team, 2023). We compared stable isotope values between wild and captive turtles separately for each species and isotope using *t*-tests for normally distributed data or Mann-Whitney *U* tests for non-normally distributed data (Zar, 1999).

Next, we fitted penalized logistic regression models and applied the k-fold cross-validation (10-fold) technique to assess the probability of correctly assigning individuals to their source (wild vs. captive) based on four different isotope combinations: (1) $\delta^{13}C$ and $\delta^{15}N$; (2) $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$; (3) $\delta^{2}H$ and $\delta^{18}O$; and (4) $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$ (Hopkins et al., 2022). The analyses were done using the package glmnet (Friedman et al., 2021) in software R.

Lastly, to estimate the turnover rate of $\delta^{15}N$ and $\delta^{13}C$, we obtained repeated samples from five confiscated individuals of *P. megacephalum* cared for by the rescue team at the Kadoorie Farm and Botanic Garden from May 2022 to May 2024. We sampled the turtles at three time points: 0th, 4th and 15th month. The turtles were subsequently relocated, and no further sampling was done. To estimate the potential saturation value resembling isotopic ratios of captive-bred *P. megacephalum*, we averaged isotopic ratios of three additional individuals that were kept in the same facility and maintained on a same, consistent diet (comprised of fish, shrimp, clams, insects, meat, fruits, and commercial turtle pellets) for at least three years. To calculate turnover rates, we used a first-order exponential equation (Downs et al., 2014):

$$\delta_t = ae^{-\lambda t} + c,$$

where δ_t is the isotopic value at time t, a is the difference in isotopic value between the initial and equilibrium stage, λ is the first-order rate constant, and c is the isotopic value at saturation (*i.e.*, the equilibrium stage). We used a nonlinear least squares regression to estimate λ, using all available data points and the average saturation point. Additionally, we used 1000 bootstrap iterations to estimate 95 % confidence intervals of the mean isotopic values over time. From these values, we determined the 50 % (half-life) and 95 % turnover of isotopic values using the following equation (Downs et al., 2014):

$$t_{\alpha/100} = \ln(1 - \alpha/100)/\lambda,$$

where $t_{\alpha/100}$ is time required to reach α% turnover. We ran the analysis using the nlstools package (Baty et al., 2015) in R.

3. Results

3.1. Isotopic signatures between wild and captive individuals

In total, we sampled 126 wild, 57 captive, and 47 seized individuals across the four study species (Fig. 1). We found that $\delta^{13}C$ and $\delta^{15}N$ values were significantly higher in captive than in wild turtles for all four species (Table 1, Fig. 2). We had sufficient samples to conduct statistical tests of $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$ for *C. trifasciata* and *S. bealei* only. For both species, $\delta^{34}S$ signatures were significantly higher in wild compared to captive individuals, while $\delta^{2}H$ values were significantly lower in wild individuals. Wild and captive individuals differed significantly in $\delta^{18}O$ values for *S. bealei* only, with captive individuals having higher values.

Isotopic biplots revealed distinct, non-overlapping clusters for wild and captive individuals across all species for $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3A–3D), and $\delta^{2}H$ and $\delta^{18}O$ (Figs. 3E and 3F). All seized turtles, with and without microchips, clustered with wild individuals (Figs. 3A and 3B).

3.2. Classification accuracy

Using penalized logistic regression models and the k-fold cross-validation technique, we found that the combination of $\delta^{13}C$ and $\delta^{15}N$ achieved 100 % accuracy in assigning wild-caught and captive animals across all species (Table 2). For *C. trifasciata* and *S. bealei*, where sufficient sample sizes were available to test other isotope combinations, the accuracies were 100 % for all isotope combinations except for $\delta^{2}H$ and $\delta^{18}O$ in *S. bealei* (97 % accuracy).

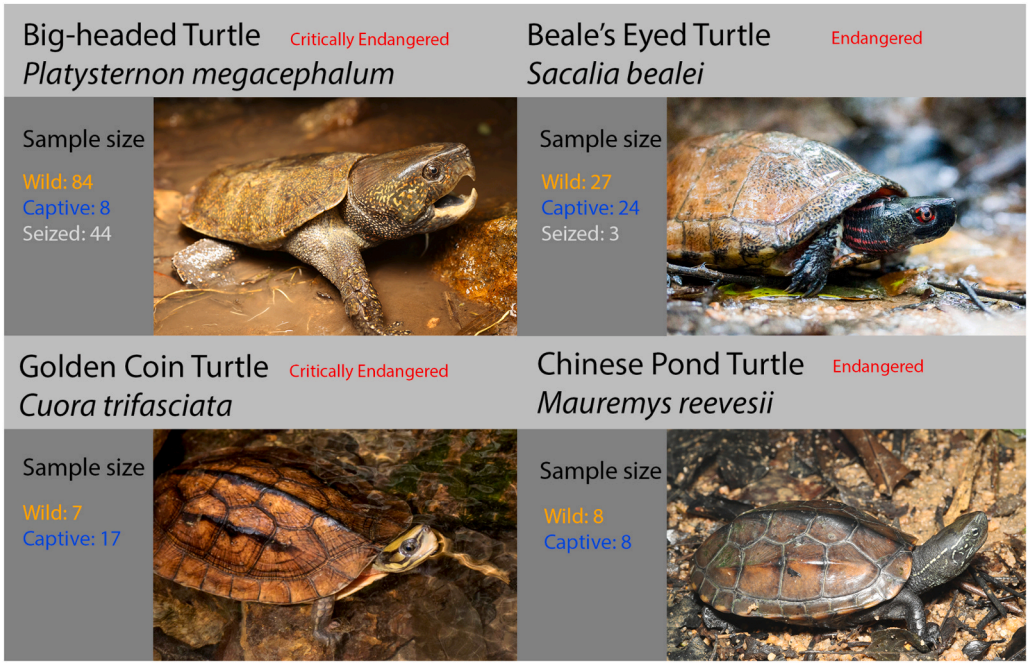

Fig. 1. Number of wild, captive, seized individuals sampled, and their IUCN Red List conservation status, for the four species of freshwater turtles studied, including *Cuora trifasciata*, *Mauremys reevesii*, *Platysternon megacephalum*, and *Sacalia bealei*.

Table 1
Stable isotope values (‰; mean ± SD, sample size in parentheses) of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$), hydrogen ($\delta^{2}H$), and oxygen ($\delta^{18}O$) in claw samples collected from wild and captive individuals for four species of freshwater turtles (*Cuora trifasciata*, *Mauremys reevesii*, *Platysternon megacephalum*, and *Sacalia bealei*). Comparisons between wild and captive turtles were performed using *t*-tests for normally distributed data or Mann-Whitney *U* tests for non-normally distributed data. Asterisks indicate results using Mann-Whitney *U* tests. Tests were not conducted (denoted with dashes) for combinations where the sample size of either wild or captive individuals was less than three. Bold in the last column indicates significant test results.

Species	Wild	Captive	Test result (*t* or *U*, *p*)
$\delta^{13}C$			
C. trifasciata	−25.3 ± 0.4 (7)	−18.1 ± 0.8 (17)	***119, < 0.001**
M. reevesii	−24.0 ± 0.5 (8)	−19.7 ± 1.0 (8)	***64, < 0.001**
P. megacephalum	−23.8 ± 1.3 (84)	−18.1 ± 1.0 (8)	***672, < 0.001**
S. bealei	−26.0 ± 0.7 (27)	−20.1 ± 0.5 (24)	***720, < 0.001**
$\delta^{15}N$			
C. trifasciata	5.8 ± 1.4 (7)	10.1 ± 0.5 (17)	***119, < 0.001**
M. reevesii	7.6 ± 1.1 (8)	9.7 ± 0.8 (8)	**4.27, < 0.001**
P. megacephalum	5.3 ± 1.0 (84)	10.4 ± 0.7 (8)	***672, < 0.001**
S. bealei	5.0 ± 1.2 (27)	9.3 ± 0.6 (24)	***648, < 0.001**
$\delta^{34}S$			
C. trifasciata	7.8 ± 1.3 (7)	10.3 ± 1.1 (17)	***110, 0.002**
M. reevesii	-	10.5 ± 1.5 (8)	-
P. megacephalum	7.9 ± 0.6 (22)	9.1 ± 1.6 (2)	-
S. bealei	7.6 ± 1.0 (18)	9.0 ± 0.6 (24)	**5.42, < 0.001**
$\delta^{2}H$			
C. trifasciata	−54.1 ± 4.7 (4)	−68.3 ± 7.7 (17)	**−4.73, 0.002**
M. reevesii	-	−51.1 ± 7.6 (8)	-
P. megacephalum	−47.9 ± 7.0 (14)	−62.9 ± 8.7 (2)	-
S. bealei	−43.1 ± 8.6 (19)	−66.3 ± 5.1 (16)	**−9.84, < 0.001**
$\delta^{18}O$			
C. trifasciata	8.8 ± 0.2 (4)	9.5 ± 1.7 (17)	*38, 0.753
M. reevesii	-	11.6 ± 1.4 (8)	-
P. megacephalum	9.1 ± 0.7 (14)	9.8 ± 1.3 (2)	-
S. bealei	9.7 ± 0.7 (19)	11.4 ± 0.9 (16)	**6.16, < 0.001**

3.3. Turnover rate in *P. megacephalum*

For the turnover rate estimate in *P. megacephalum*, the 50 % turnover rate for $\delta^{13}C$ is 10.2 months (95 % confidence interval = 7.4–16.5), and the 95 % turnover rate is 46.3 (32.0–71.2) months (Fig. 4). The 50 % turnover rate of $\delta^{15}N$ is 7.5 (5.4–12.2) months, while the 95 % turnover rate is 32.4 (23.4–52.6) months (Fig. 4).

4. Discussion

We provide data to support the application of SIA as a wildlife forensic tool to distinguish the source (wild vs. captive-bred) of individuals across four highly threatened freshwater turtle species from Hong Kong (*C. trifasciata*, *M. reevesii*, *P. megacephalum*, and *S. bealei*). We demonstrate that the combined use of $\delta^{13}C$ and $\delta^{15}N$ offers a promising tool for differentiating wild and captive individuals across all four species. Further, our estimates on isotopic signal turnover rates in claw tissue indicate that SIA can reliably determine the source of the animal up to two years after capture. Lastly, we demonstrate the effectiveness of SIA in prosecuting wildlife crimes, by applying it to three wildlife seizure cases in Hong Kong, each involving at least one individual of known source. We elaborate on these points below, highlighting their implications for law enforcement efforts against the illegal wildlife trade.

4.1. Applying stable isotope analysis in wildlife forensics

Across the four native, endangered species examined, biplots of $\delta^{13}C$ and $\delta^{15}N$ signals revealed distinct, non-overlapping isotopic profiles between wild and captive individuals, achieving 100 % classification accuracy. Our findings accord with previous studies that highlight the potential of $\delta^{13}C$ and $\delta^{15}N$ to differentiate between wild and captive individuals (Brasileiro et al., 2023; Hopkins et al., 2022). In addition to $\delta^{13}C$ and $\delta^{15}N$, we explored the potential of $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$ as supplementary tracers. These analyses were limited by smaller sample sizes, but in general these tracers were more similar between wild and captive-bred individuals than those of $\delta^{13}C$ and $\delta^{15}N$. Nonetheless, there was no overlap in $\delta^{34}S$ and $\delta^{2}H$ between wild and captive-bred individuals of *C. trifasciata* and *S. bealei.* Although further data are needed, our findings highlight the potential of $\delta^{34}S$ and $\delta^{2}H$ as useful tracers for wildlife forensics.

Our study is timely given a 2021 amendment to wildlife regulations in mainland China; captive-bred individuals can be legally traded for over 300 animal species (Sung et al., 2024), including the four turtle species included in this study. This regulatory change has created opportunities for laundering of wild-caught animals in the trade. We demonstrate that SIA can effectively differentiate wild-caught and captive-bred individuals of these four turtle species, and we recommend that SIA be immediately adopted in mainland China to detect wild-caught turtles laundered through trade and turtle farms.

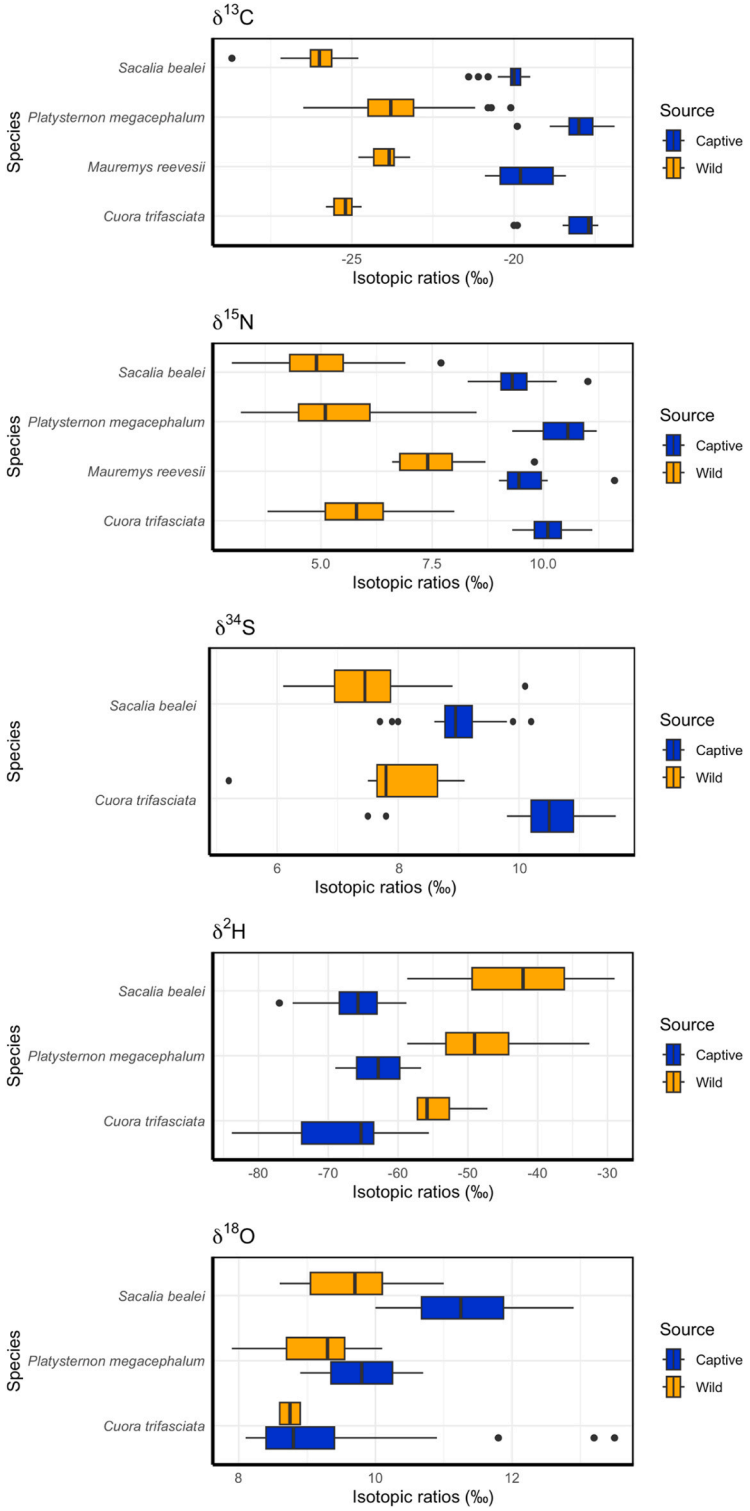

Fig. 2. Median stable isotope values (‰) of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$), hydrogen ($\delta^{2}H$), and oxygen ($\delta^{18}O$), in claw samples of wild (orange) and captive (blue) individuals in four species of freshwater turtles (*Cuora trifasciata*, *Mauremys reevesii*, *Platysternon megacephalum*, and *Sacalia bealei*).

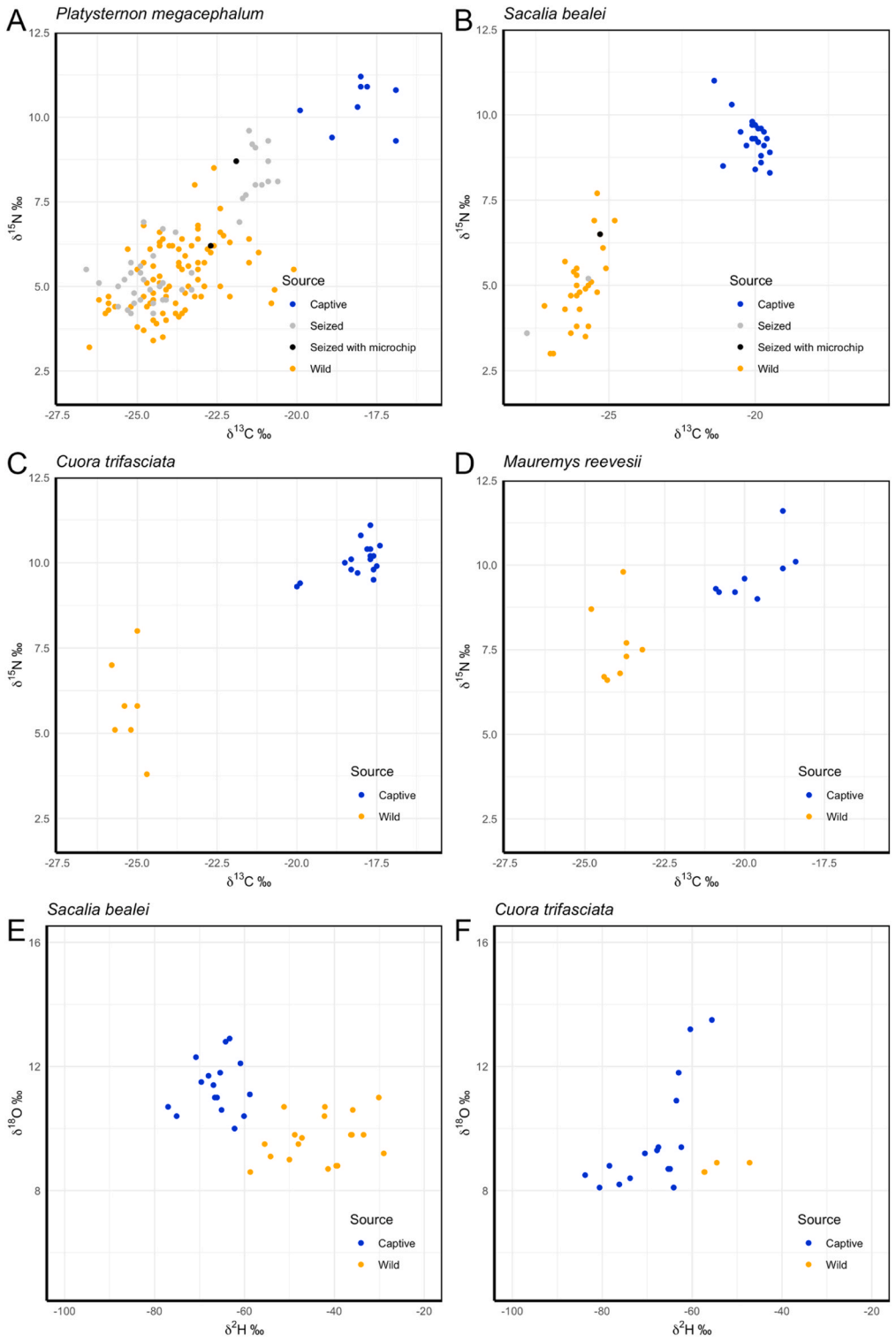

Fig. 3. Biplots of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values (‰) (A–D), and hydrogen ($\delta^{2}H$) and oxygen ($\delta^{18}O$) values (E and F) in claw samples of wild (orange), captive (blue), and seized (grey) individuals in four species of freshwater turtles (*Cuora trifasciata*, *Mauremys reevesii*, *Platysternon megacephalum*, and *Sacalia bealei*). In panels A and B, black dots indicate the three individuals (two *Platysternon megacephalum* and one *Sacalia bealei*) that were microchipped when seized by the enforcement authority, indicating their unambiguity of being wild caught from Hong Kong.

Table 2
Accuracy (%) of distinguishing sources (wild vs. captive) for four species of freshwater turtles based on different isotope combinations in claw samples. The tests were done using penalized logistic regression and a k-fold cross-validation technique. Tests were not conducted (denoted with dashes) for combinations where the sample size of either wild or captive individuals was less than three.

Species	$\delta^{13}C$, $\delta^{15}N$	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	$\delta^{2}H$, $\delta^{18}O$	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, $\delta^{18}O$
Cuora trifasciata	100	100	100	100
Mauremys reevesii	100	-	-	-
Platysternon megacephalum	100	-	-	-
Sacalia bealei	100	100	97	100

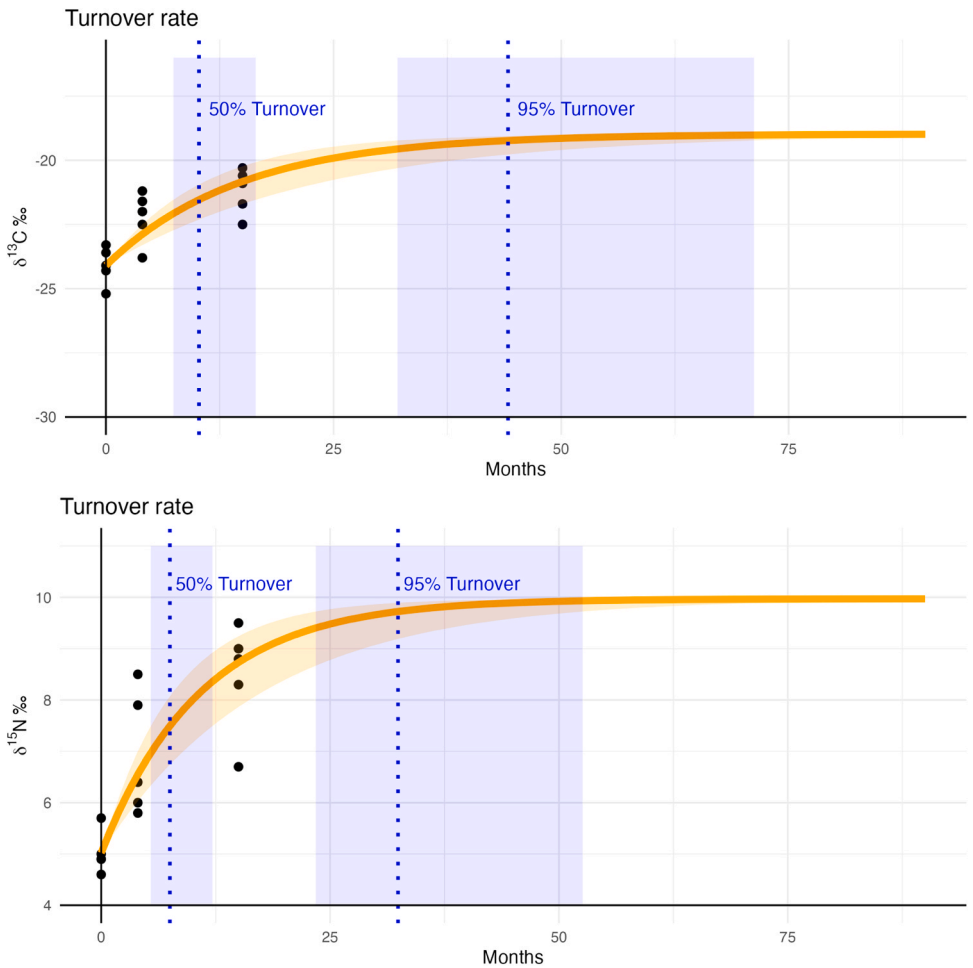

Fig. 4. 50 % and 95 % turnover rates of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in five seized *Platysternon megacephalum*. Samples were collected at three time points (0th, 4th, and 15th months) after confiscation. The shaded areas (blue for turnover rate, orange for mean isotopic signal) represent the 95 % confidence intervals estimated using bootstrapping.

4.2. The effects of turnover rates on the use of isotopic tracers

To estimate isotopic turnover rate in claw tissue of turtles, we performed repeated measurements of isotope profiles of five seized *P. megacephalum* individuals in captivity. We found that the 50 % turnover rates for $\delta^{15}N$ and $\delta^{13}C$ were 7.5 and 10.2 months, respectively. These turnover rates are among the longest reported in animals; for comparison, the estimated turnover rate is five months in the much larger American alligator (*Alligator mississippiensis*) (Rosenblatt and Heithaus, 2013), and six months in the Red-eared Slider (*T. s. scripta*; the only other freshwater turtle studied to date) (Aresco et al., 2015; Seminoff et al., 2007). There are two

potential explanations for the slow turnover rates in *P. megacephalum*. First, *P. megacephalum* may have a lower metabolic rate as it lives in cool, rocky mountain streams, which may slow its isotopic incorporation (Thomas and Crowther, 2015). Second, *P. megacephalum* may require more time to acclimatize to captive environments, resulting in reduced food intake and a slower turnover in the isotopic composition of tissues.

For the 95 % turnover rates, the lower values of the confidence interval ($\delta^{15}N = 23.4$ months, $\delta^{13}C = 32.0$ months) indicate that *P. megacephalum* retains the wild-caught stable isotope signal for at least 23 months after being kept in captivity. These findings suggest that SIA of claw tissue is effective for identifying wild-sourced turtles captured up to nearly two years prior, enhancing its application in wildlife trade regulation. The duration is potentially longer when using both $\delta^{13}C$ and $\delta^{15}N$ together. This extended detection window not only supports enforcement of cross-border trade but also aids domestic regulation. For example, in Hong Kong, possession permits are required for CITES I and wild-caught CITES II specimens. SIA can be used to screen captive colonies to identify CITES II turtles illegally sourced from the wild, helping stop laundering of wild-caught turtles as captive-bred in trade.

4.3. Application of SIA in Hong Kong seizures

We worked with the Hong Kong Government on three real wildlife seizure cases in Hong Kong. The goal was to use SIA as a wildlife forensics tool to collect data suitable for the court of law for prosecution of suspected wildlife crime. The number of seized individuals was 3–24. In all three cases (luckily for the legal process and unluckily for the wild populations), the seized turtles included one individual in each case (total two *P. megacephalum*, one *S. bealei*) with a microchip from our long-term population study, providing unambiguous evidence of their wild source from Hong Kong. This case study would not be possible without the presence of microchipped individuals, highlighting the conservation value of long-term monitoring of wild populations. We measured the isotopic profiles of all seized individuals (microchipped and not microchipped) and found them to be similar to their wild counterparts (Fig. 3A, B). These results provided evidence that helped with the prosecution of suspects for illegal hunting and/or possession of these wild-sourced specimens.

Despite the high accuracy of SIA in differentiating wild-caught and captive-bred turtles, it is important to recognize two potential limitations: (1) the observed isotopic signatures of wild turtles may reflect local dietary and environmental conditions specific to a geographic area, and (2) the isotopic profiles of captive-bred turtles could vary with their diet (Hopkins et al., 2022). We believe these limitations can be effectively addressed in two ways. First, studies should be extended to build a larger database for comparison of more species, across a broader geographic range, under a wider range of captive conditions, and include testing of additional tracers (*e.g.*, other isotopes and elemental markers) (Hopkins et al., 2023). Second, SIA should be integrated with complementary methods to increase the robustness of investigations into the source of seized turtles, such as DNA analysis identifying geographic origin and fecal analysis identifying wild dietary items. Coupled with the relatively low cost of bulk $\delta^{13}C$ and $\delta^{15}N$ analysis (Haywood et al., 2019), SIA is an efficient, cost-effective approach to identify wild-caught turtles in future seizure cases in Hong Kong.

5. Conclusions

Turtles are among the most threatened and illegally traded taxonomic groups, with the laundering of wild-caught turtles as captive-bred being prevalent and impossible to detect by simple observation. Stable isotope analysis offers a robust, yet currently underused tool to determine the source of turtles, helping to close a significant loophole in combating illegal wildlife trade. This study provides proof-of-concept for using SIA of claw samples to differentiate wild and captive turtles in Hong Kong. We advocate for collaboration between researchers and enforcement authorities to apply SIA to identify turtles that are being illegally traded or possessed. With proper permits, researchers, wildlife center staff, and law enforcement officials can quickly and easily collect claw samples and store them until SIA can be undertaken, as demonstrated in this study. We encourage partnerships between laboratories capable of performing SIA and officials involved in turtle confiscations to advance SIA in a timely manner, ensuring that fewer wild turtles are moved through the illegal trade. With additional testing to build a more comprehensive database of more geographic regions and taxa, the use of SIA as a wildlife forensic tool can be expanded globally and support the trade regulation of a variety of organisms.

CRediT authorship contribution statement

Yik-Hei Sung: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, visualization, writing – original draft, writing – review and editing; **Jia Huan Liew:** investigation, methodology, writing – original draft, writing – review and editing; **Wing Sing Chan:** data curation, investigation, writing – review and editing; **Amy Wing Lam FOK:** data curation, investigation, writing – review and editing; **Julia Leung:** data curation, investigation, writing – review and editing; **Ho Fung WONG:** data curation, investigation, writing – review and editing; **David M BAKER:** conceptualization, methodology, writing – review and editing; **Timothy C. Bonebrake:** conceptualization, methodology, project administration, writing – review and editing; **Caroline DINGLE:** conceptualization, funding acquisition, methodology, writing – review and editing; **David DUDGEON:** funding acquisition, investigation, writing – review and editing; **Nancy E. Karraker:** funding acquisition, methodology, writing – review and editing; **Anthony LAU:** conceptualization, funding acquisition, writing – review and editing; **Violaine A. Colon:** investigation, methodology, writing – review and editing; **Ioannis MAGOURAS:** investigation, methodology, writing – review and editing; **Gary ADES:** investigation, project administration, writing – review and editing; **Paul CROW:** investigation, project administration, writing – review and editing; **Liz ROSE-JEFFREYS:** investigation, project administration, writing – review and editing; **Ricky SPENCER:** funding acquisition, writing – review and editing; **Jonathan J. FONG:** conceptualization, funding acquisition, investigation, project

administration, writing – original draft, writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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