No place like home: forensic provenancing of alien wildlife species using stable isotopes

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Research aim

We will develop and validate stable isotope methods for determining the provenance of at-large alien specimens, with the goal of differentiating between individuals released from captivity from those free-living in the wild.

Australia and the illegal pet trade

The illegal wildlife trade facilitates the global transport of live alien species, posing significant risks to global biodiversity[1]. Intentionally and accidentally released animals can introduce disease, compete with native fauna, and establish novel invasive populations[2]. It is crucial to identify vertebrate pest incursions to allow for early, cost-effective intervention. Novel surveillance tools are an important first step.

Here, we explore the application of stable isotope methods to distinguish between captive and at-large exotic vertebrate specimens resulting from differences in their natural and domestic diets.

Red-eared slider turtles

Red-eared slider turtles (*Trachemys scripta elegans*) are the most highly traded turtle species worldwide. In Australia, illegally-kept sliders are long-lived (50-70 years) and can be released into the wild, either accidentally or when they outlive their captivity. After release, sliders have the potential to establish wild populations, posing a risk to native turtle populations[3]. Red-eared slider populations are established on every continent except Antarctica[3].

Figure 1: Red-eared sliders are attractive as juveniles, but quickly grow large and become aggressive. As a consequence, pets are often dumped into local waterways and wetlands.

Methods

- Five turtles seized from pet stores or found in a wild-state were used for analysis.

Table 1: Red-eared slider turtle samples

Turtle	Status	Age	Location
A	Captive	Adult	QLD
B	Wild	Juvenile	VIC
C	Captive	Adult	VIC
D	Wild	Adult	NSW
E	Wild	Adult	NSW

- Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were measured from outer keratin layers (scute) of the carapace of five turtles of assumed captive and wild-state origins.
- In one individual (A), loose keratin plates easily peeled off and were analysed as 'old' keratin. In all other specimens (B through D) 'new' keratin was scraped directly from the carapace bone.
- Keratin was sampled from nine locations on a single scute (growth plate) in all individuals to assess variability within individuals.
- Additional sampling was performed on individual A to assess variability over multiple scutes and on individual B, a juvenile with visible growth pattern.

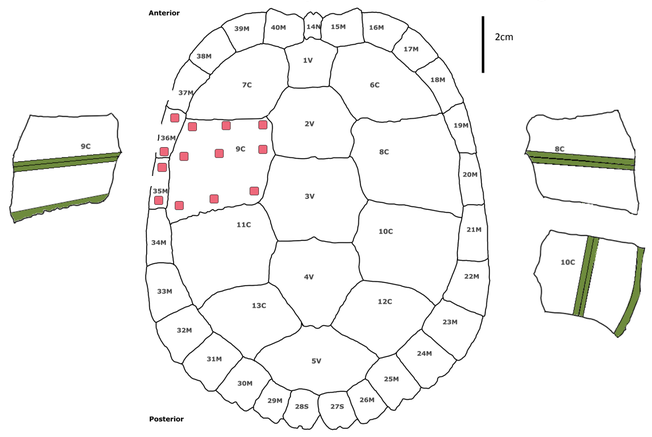

Figure 2: Sampling method map for scutes on turtle A. Isotope ratios were measured for old growth (green) on multiple scutes which peeled off the carapace of turtle A, then as spots for new growth (red) on the same scute across all turtles.

Results

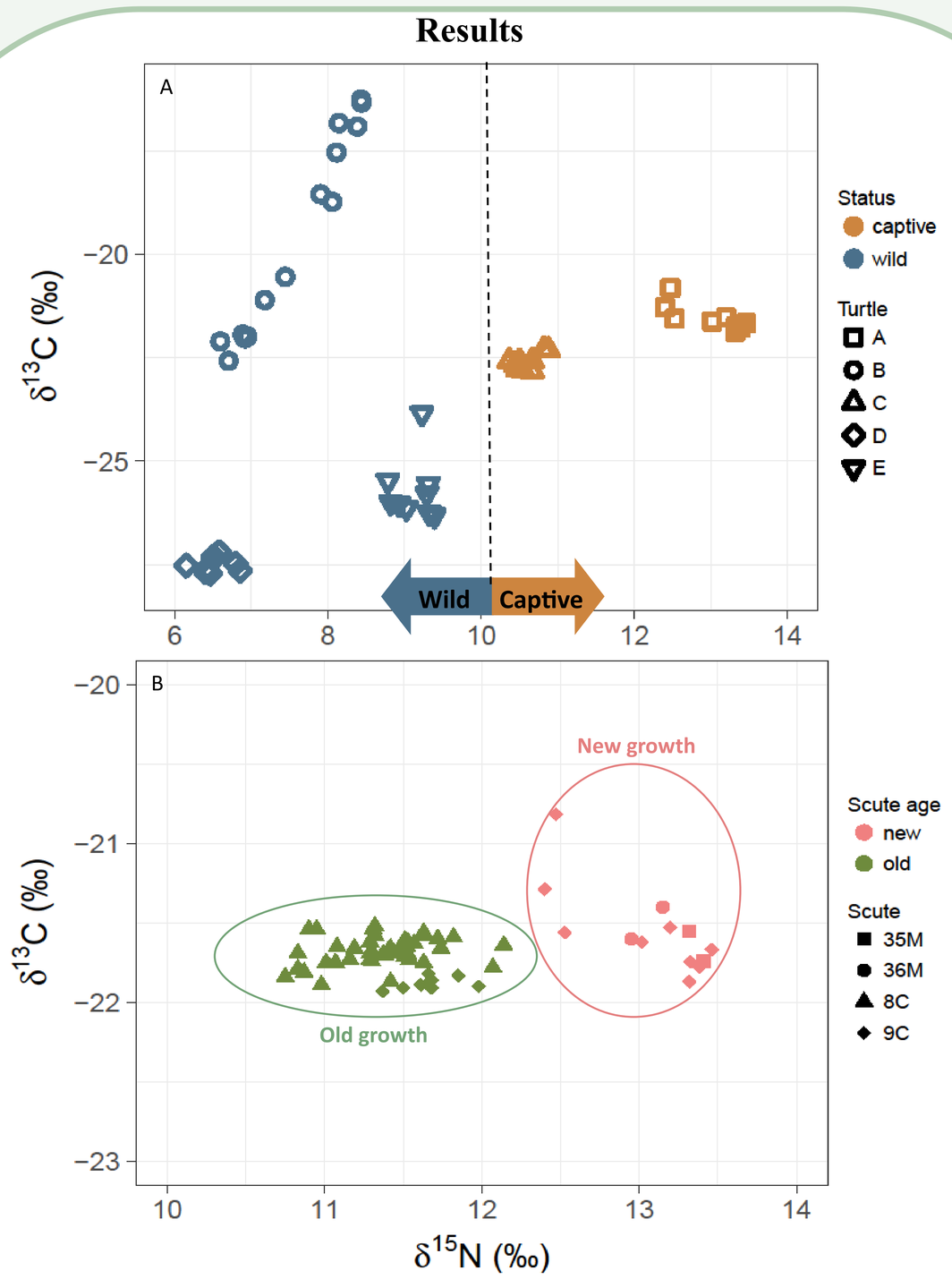

Figure 3: (A) $\delta^{15}N$ vs $\delta^{13}C$ for new growth keratin from the same scute of five individuals (9C).
(B) $\delta^{15}N$ vs $\delta^{13}C$ for keratin from turtle A. Old growth is loose keratin scutes, and new is attached to bone.

Differences between wild and captive turtles are observable in nitrogen isotopes, and to a lesser degree in carbon isotope ratios; suggesting differences in diets (Fig. 3A). The omnivorous diet and opportunistic behavior of red-eared sliders suggests that differences between isotopic composition of captive and wild turtles should be influenced by resource availability[4]. Higher nitrogen isotope ratios in captive animals is likely due to greater opportunity for carnivory through their provided diet, while wild turtles are more reliant on plant material[4].

Variability across scutes of individual adult turtles is minimal (Fig. 3A), however old and new growth is distinguishable by nitrogen isotope ratios (Fig 3B).

The variance in juvenile turtle B may be due to changes in the diet as the juvenile ages and reduces its dependence on plant material[5]. Alternatively, it may be due to a 'memory' effect, whereby the original hatchling isotope ratios have not been completely replaced by new growth[6].

Conclusion

- Differences in diet and environmental history between captive and at-large specimens lead to distinguishable isotope differences.
- Minimal variation within the new-growth scutes of specimens will allow for the creation of a repeatable and cost-effective method for identification of environmental history.
- Stable isotopes for identifying vertebrate pest incursions is promising.

Future research

We are currently investigating the further application of isotope and trace element analyses, including:

- Oxygen ($\delta^{18}O$) analysis to identify changes in water source.
- Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICPMS) of bone and toenail tissue.
- Review the $\delta^{15}N$ and $\delta^{13}C$ of potential captive food sources.

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