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Serpent's source: Determining the source and geographic origin of traded python skins using isotopic and elemental markers

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ABSTRACT

Commercial production systems for wildlife increasingly involve closed-cycle captive breeding, in which effective regulation requires methods for verifying the provenance of stock. We compared the isotopic and elemental compositions of skin from wild and captive-bred pythons raised under different diet regimes in Indonesia and Viet Nam to examine the efficacy of using these techniques as a means of determining the source and origin of skins entering international trade. We found significant differences in both isotopic and elemental markers between wild and captive-bred snakes, as well as those from different geographic origins. Combinations of both techniques were able to discriminate between diet treatments and geographic origins with up to 100% accuracy. Moreover, our experimental manipulation of python diets confirmed that the application of specific diet regimes (or the addition of known elemental markers) for captive-bred snakes can create signatures specific to those animals, vastly improving the efficacy of these methods. Our study strongly suggests that the analysis of isotope ratios and elemental markers offers a powerful tool for verifying the provenance of reptile skins entering trade – but these methodologies will be most applicable (and cost-effective) for species with small populations of genuine conservation concern, rather than for large volume trade in species for which there is little conservation risk.

1. Introduction

The international trade in wildlife is receiving increasing attention as overexploitation drives declines in the wild populations of many species (Broad et al., 2003; Biggs et al., 2016; UNODC, 2016). To combat this, captive breeding has been promoted to relieve harvesting pressure on wild animal populations (Lyons and Natusch, 2011; Nogueira and Nogueira-Filho, 2011; Challender and MacMillan, 2014) and, in several cases, this approach has been successful. For example, captive breeding and ranching of crocodilians for international trade has offered a meaningful substitute for wild-caught specimens (Hutton and Webb, 2003; MacGregor, 2006). In other cases, however, captive breeding facilities have been implicated in the laundering and export of wild-caught specimens under the guise of being captive-bred (Brooks et al., 2010; Lyons and Natusch, 2011). Such illicit activity undermines the rule of law and makes it challenging to accurately determine the true number of wild specimens entering trade and, thus, makes it difficult to undertake effective sustainability assessments.

The international trade in wildlife is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The Parties to CITES have recognised the laundering of wild specimens through captive-breeding facilities and have dedicated significant time and resources to tackling this problem (e.g., see TRAFFIC, 2013; Lyons et al., 2016; Lyons and Natusch, 2015). To facilitate such efforts, there is a need for the increased involvement of forensic science to combat wildlife crime (UNODC, 2016). A novel approach to establish the provenance of specimens entering trade involves the forensic application of stable isotope and elemental markers naturally present in the tissue of wild and captive-bred specimens.

The diets and environments of animals living at different sites or being raised within different production systems (e.g., wild versus captive-bred) vary greatly. For example, specimens born into and raised in captivity are typically fed a regular and uniform diet, whereas the diets and feeding frequency of wild individuals are highly variable, and fluctuate according to environment (e.g., seasonal resource availability) and physiology (e.g., diet changes over ontogeny) (Hobson, 1999;

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Bowen et al., 2005; Fry, 2006; Satterfield and Finney, 2002; Lyons and Natusch, 2015). For these reasons, the ratios of stable isotopes and the concentrations of many elements (e.g., heavy metals) accumulate at different rates within tissues, which can manifest as a specific isotopic or elemental "signature" indicative of the source or geographic origin of a particular specimen (Fry, 2006). Isotopic and elemental signatures have been successfully used to differentiate between wild and captive-bred mink (Hammershøj et al., 2005), between wild and farmed crustaceans, fish, and sea turtles (Moncada et al., 1998; Dempson and Power, 2004; Carter et al., 2015), and have recently been tested as a means to determine the origin of traded elephant ivory (Ziegler et al., 2016) and the source of Vietnamese crocodile lizards (M. van Schingen pers. comm. 2016).

Worldwide, reptiles are the wildlife group most commonly represented in illegal wildlife seizures (UNODC, 2016). Reptile trade is dominated by the trade in skins (primarily snakes, crocodilians and large lizards), with tens of millions of specimens traded annually to meet demand for exotic leathers (Luxmoore and Groombridge, 1990; Jenkins and Broad, 1994). Two of the most commercially important reptile species are the Burmese python (*Python bivittatus*) and reticulated python (*Python reticulatus*). Annual trade in skins of these species is estimated at a combined total of 500,000 specimens, with the value of their export, transformation, and product sales, estimated to be worth $US 1 billion (Kasterine et al., 2012). Both species are sourced either from the wild in Indonesia and Malaysia or from captive breeding farms in China, Thailand and Viet Nam (Kasterine et al., 2012; Natusch and Lyons, 2014; Natusch et al., 2016a). There is evidence of significant illegal trade in python skins (Kasterine et al., 2012; Natusch et al., 2016a). Specifically, concerns exist that those countries breeding snakes in captivity may be supplementing breeding efforts with wild-caught specimens, or that skins are being smuggled from one country to another (Kasterine et al., 2012; Natusch and Lyons, 2014; Natusch et al., 2016a).

Reticulated pythons remain common throughout their range, but Burmese pythons are considered vulnerable due to habitat loss and overexploitation (IUCN, 2012). Although both species are in no immediate risk of extinction, an accurate knowledge of the geographic origin and source (wild or captive-bred) of skins entering trade is critical for certifying export legality and confirming ongoing sustainability. Beyond these species, development of techniques to verify the source and origin of reptile skins in trade may prove vital for conserving other taxa at far greater risk of overexploitation. To test the efficacy of using isotopic and elemental markers for regulating the trade in reptile skins, we collected tissue samples from wild and captive-bred Burmese and reticulated pythons, as well as experimentally reared Burmese pythons under different diet regimes. We addressed three central questions: can isotopic and elemental markers be used to determine (1) the source of reptile skins (i.e., differentiate between wild and captive-bred specimens); (2) the geographic origin of reptile skins; and (3) if manipulation of those markers can create specific signatures useful for future forensic examinations of reptile skins entering trade.

2. Materials and methods

2.1. Compliance statement

We stress that no snakes were harmed for the purpose of our study; we merely utilised an existing trade. Our data were gathered from snakes bred for a commercial industry, which employs humane methods of killing reptiles for the skin trade (by brain destruction; Swiss Federal Veterinary Office, 2013). We collected all skin samples from dead snakes. All fieldwork was carried out with relevant permissions and permits from the Ministry of Research and Technology of the Republic of Indonesia (278/SIP/FRP/SM/IX/2014 and 311/SIP/FRP/SM/X/2014) and the Administration of Forestry of the Socialist Republic of Viet Nam (114/TCLN-CTVN). The Animal Ethics Screening Committee of the University of Witwatersrand, South Africa, approved our experimental procedures (approval number: 2014/17/B). All tissue samples were exported and imported with permits issued by the CITES Management Authorities of Indonesia (62/SATS/BKSDA-08/VIII/2015 and 13,223/IV/SATS-LN/2015), Viet Nam (14VN1221S; 15VN0041S and 15VN2302S), and Australia (PWS2014-AU-001227; PWS2015-AU-001940 and PWS2015-AU-001819). The Australian Department of Agriculture issued quarantine permits (IP15001981).

2.2. Tissue collection protocol

Most snake skins destined for international markets are sold raw (air-dried). Snakes are killed then skinned, and the skin is scraped free of any connective tissue. Skins are then stretched and nailed to wooden boards where they are left for one day to dry. Once dry, the skins are folded and can be stored in this state for several months (sometimes years) before being exported. In this condition, skins are designated biological material until they are tanned to make leather. We conducted all isotopic and elemental analyses on samples of raw (air-dried and untanned) skins (sensu Fig. 1). In some cases, we collected the entire skins of dead snakes, but where this was not possible (when skins were used for commercial purposes) we collected 50 × 50 mm samples from the posterior section (near the vent) of each skin. Because isotopic enrichment depends on tissue turnover rates that vary with the type of tissue examined, we performed initial isotopic measurements on six samples taken along the entire length of six snake skins (Lorrain et al., 2002; Bodin et al., 2006). Measurements taken on those skins showed them to be isotopically homogeneous within the limits of the overall standard deviations (see below). Future testing was limited to skin pieces taken from the posterior section of the skin.

2.3. Tissue collection from captive pythons

We collected 50 × 50 mm skin samples from captive-bred pythons from two different sources. The first were captive-bred reticulated pythons (*Python reticulatus*) reared on a diet of wild-caught rats (*Rattus argentiventer*) at a commercial breeding facility in Ho Chi Minh City, Viet Nam. The second were captive-bred Burmese pythons (*Python bivittatus*) raised in experimental treatments within another captive breeding facility in Ho Chi Minh City (see below). The second facility is one of Viet Nam's leading python farms, and has been breeding pythons for commercial purposes for >10 years.

2.4. Experimental design

To test the influence of diet on the isotopic and elemental compositions of python skins, we raised 70 Burmese pythons under different diet treatments at a python farm in Ho Chi Minh City, Viet Nam. We collected neonate pythons directly from eggs taken from females bred at the facility. For each python we maintained a photographic database of the skin patterns on a dorsolateral section of skin immediately posterior to the head. This allowed later identification of individuals; we did not use other forms of marking such as passive integrated transponder tags or cauterised scales to prevent damage to the skins, which were to be sold commercially at the end of the study. We divided snakes into five experimental diets, each comprising seven males and seven females (14 snakes in total); we randomized eggs among treatments to ensure each group included specimens from several different clutches (to avoid maternal influences on our results). On completion of the study, sample sizes differed among treatments because a small number of snakes died from common respiratory infections (see Natusch and Lyons, 2014).

Captive Burmese pythons in Viet Nam are fed predominantly on a diet of wild-caught rats and/or sausages made from reconstituted waste protein from other industries (e.g., pork, chicken, fish; Natusch and Lyons, 2014; Aust et al., 2016; Fig. 1). Sausages are a common food item used in some farms and are made at the facility. Sausages