Global Ecology and Conservation 6 (2016) 232–241

Contents lists available at ScienceDirect

Global Ecology and Conservation

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

Original research article

Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (*Shinisaurus crocodilurus*) from Vietnam

Mona van Schingen [a,b,*,1], Thomas Ziegler [a,b], Markus Boner [c], Bruno Streit [d], Truong Quang Nguyen [b,e], Vicki Crook [f], Stefan Ziegler [d,g,1]

[a] *Cologne Zoo, Riehler Str. 173, 50735 Cologne, Germany*
[b] *Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Straße 47b, 50674 Cologne, Germany*
[c] *Agroisolab GmbH, Prof.-Rehm-Str. 6, 52428 Jülich, Germany*
[d] *Department of Ecology and Evolution, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany*
[e] *Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Viet Nam*
[f] *TRAFFIC International, The David Attenborough Building, Pembroke Street, Cambridge CB2 3QZ, UK*
[g] *WWF Germany, Reinhardtstr. 18, 10117 Berlin, Germany*

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 10 March 2016
Accepted 10 March 2016
Available online 1 April 2016

Keywords:
Stable isotopes
CITES
Forensic tool
Wildlife crime
Shinisaurus crocodilurus

ABSTRACT

The international wildlife trade in allegedly "captive-bred" specimens has globally increased during recent years, while the legal origin of respective animals frequently remains doubtful. Worldwide, authorities experience strong challenges to effectively control the international trade in CITES-listed species and are struggling to uncover fraudulent claims of "captive-breeding". Forensic analytical methods are being considered as potential tools to investigate wildlife crime. The present case study is the first of its kind in reptiles that investigates the application of $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios to discriminate between captive and wild crocodile lizards from Vietnam. The CITES-listed crocodile lizard *Shinisaurus crocodilurus* is listed as endangered on the IUCN Red List mainly due to habitat loss and unsustainable exploitation for the international pet trade. Our results revealed significant differences in the composition of the two tested isotope systems between captive and wild individuals. Isotope values of skin samples from captive specimens were significantly enriched in ^{13}C and ^{15}N as compared to specimens from the wild. We also used the weighted *k*-Nearest Neighbor classifier to assign simulated samples back to their alleged place of origin and demonstrated that captive bred individuals could be distinguished with a high degree of accuracy from specimens that were not born in captivity. We conclude that isotope analysis appears to be highly attractive as a forensic tool to reduce laundering of wild caught lizards via breeding farms, but acknowledge that this potential might be limited to range restricted or ecologically specialist species.

* Corresponding author at: Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Straße 47b, 50674 Cologne, Germany. Tel.: +49 173 7603789.
E-mail address: mschinge@smail.uni-koeln.de (M. van Schingen).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.gecco.2016.03.004

1. Introduction

Recorded international trade in CITES-listed reptile species from Southeast Asia between 1998 and 2007 accounted for at least 17.5 million animals, but real levels of trade are expected to be significantly higher (Nijman, 2010). Approximately 20% of the recorded trade volume is expected to have derived from captive-breeding or ranching facilities, which have been promoted because they may reduce harvesting pressure on wild populations and simultaneously support local livelihoods (UNEP-WCMC, 2014). Trade in specimens labeled as captive bred has significantly increased in recent years (UNEP-WCMC, 2014), but concerns have also been expressed by some Parties of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) over possible fraudulent captive-breeding claims as well as inaccurate reporting of trade in wild versus ranched or captive-bred specimens (CITES, 2014). Parties to CITES have acknowledged that illegal harvest and trade in wild-taken reptile specimens through captive breeding facilities undermines the rules of the Convention and may result in the trade becoming detrimental to wild populations. The CITES Secretariat has, therefore, commissioned a study on methodologies to differentiate between wild and captive-bred CITES-listed snakes in the trade, including its parts and derivatives (CITES, 2013).

One of the forensic methodologies of interest is stable isotope analysis, which potentially can play an important role in the investigation of wildlife crime through identification and profiling of tissue samples (Moncada et al., 2012; Voigt et al., 2012). The quantitative measurement of stable isotope ratios in metabolically inert tissue samples has potential for accurately determining the origin of respective organism due to the fact that the isotopic composition of certain elements, such as carbon and nitrogen, of a consumer reflects its diet and contains information on its respective local food web (Ehleringer and Matheson, 2007; Fry, 2006). In theory, this property can also be applied for the differentiation between wild and captive animals. Wild specimens generally feed on various prey taxa, which again might have lived on diverse isotopic sources namely different plant species and parts, reflecting a complex food web or certain geographic region. By contrast, captive animals are usually kept under a controlled feeding regime with few prey species, which are often grown on a constant isotopic source. First practical evidence for the successful applicability was found by Kays and Feranec (2011) to distinguish captive from wild wolves, whereas Ewersen and Ziegler (2014) used stable isotope analysis to discern morphometric misidentifications of Neolithic wolves and dogs.

The endangered crocodile lizard, *Shinisaurus crocodilurus*, is one of the species, which is mainly threatened by habitat loss and unsustainable exploitation of remaining and already heavily diminished wild populations, which are restricted to isolated sites in northern Vietnam andsouthern China (e.g., Auliya et al., submitted for publication; Huang et al., 2008; Nguyen et al., 2014 and van Schingen et al., 2014b, 2015a). The inclusion in CITES Appendix II in 1990 caused an almost entire shift of the international trade in crocodile lizards to allegedly captive bred specimens (van Schingen et al., 2015a). However, evidence suggests that illegal domestic and international trade in wild crocodile lizards is ongoing, which is contributing to declines of effective population sizes to about 100 individuals in Vietnam and 950 in China, while some subpopulations have already been extirpated (Huang et al., 2008; van Schingen et al., 2014b, 2015a). A lack of law enforcement capacity and skills results in limited trade controls for this species, the problem being further exacerbated by the majority of trade having shifted to online platforms in recent years. Furthermore, there is no scientific methodology in place that enables enforcement officers or investigators to determine for certain whether specimens claimed as being captive-bred were in fact reproduced in controlled conditions, and not simply taken from the wild (van Schingen et al., 2015a).

We herewith provide the first case study on lizards testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals of the crocodile lizard from Vietnam. Scales are made of alpha and beta keratin and are metabolically inert since they were formed from the epidermis. The advantage of isotope analysis is the provision of a constant and long term signal on the species' diet, which makes it rather impossible for fraudulent captive-breeding farms to rapidly adapt isotopic signatures of target species via alteration of food (Rosenblatt and Heithaus, 2013; Warne et al., 2010). Therefore, we expected pronounced differences in the isotopic signatures between captive-bred and wild crocodile lizards, as well as differences in variance of isotopic signatures in wild compared to captive specimens. We aim to aid developing a conservation tool, which can be globally applied by e.g. exporting or importing CITES Parties to detect cases of mis-declaration of relevant specimens, if the legal origin is ambiguous. We also assess the accuracy of the methodology and evaluate its forensic potential and limitations while taking into account the effect of two environmental parameters (elevation and pollution) on isotopic signatures of wild individuals.

2. Methods

2.1. Study area

The study area encompassed all known *S. crocodilurus* localities in Vietnam, namely Tay Yen Tu Nature Reserve (NR) in Bac Giang Province as well as Yen Tu NR and Dong Son-Ky Thuong NR in Quang Ninh Province (Hecht et al., 2013; Le and Ziegler, 2003 and van Schingen et al., 2014a). The three sites are located within distances of 10–40 km of each other. All three NRs are part of the last remaining contiguous evergreen tropical broadleaf rainforest in northeast Vietnam, which has been extensively cleared in the recent past (Tordoff et al., 2000; BirdLife International, 2013). The dominant rock type in the region is granite, while Dong Son-Ky Thuong NR is situated in the border region to limestone dominated area. Furthermore, sampling sites were situated along an elevation gradient increasing from sites in Dong Son-Ky Thuong NR to Yen Tu NR (see