

## MONITORING LIZARDS Using stable isotope analysis to determine the origin of monitor lizard *Varanus* spp. skins

MARCH 2016

Vicki Crook, Louisa Musing and Stefan Ziegler



#### TRAFFIC REPORT

TRAFFIC, the wildlife trade monitoring network, is the leading non-governmental organization working globally on trade in wild animals and plants in the context of both biodiversity conservation and sustainable development. TRAFFIC is a strategic alliance of WWF and IUCN.

Reproduction of material appearing in this report requires written permission from the publisher.

The designations of geographical entities in this publication, and the presentation of the material, do not imply the expression of any opinion whatsoever on the part of TRAFFIC or its supporting organizations concerning the legal status of any country, territory, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The views of the authors expressed in this publication are those of the writers and do not necessarily reflect those of TRAFFIC, WWF, IUCN.

© TRAFFIC 2016. Copyright of material published in this report is vested in TRAFFIC. ISBN: 978-1-85850-405-6

UK Registered Charity No. 1076722 **Suggested citation (Part 1):** Crook, V. and Musing, L. (2016). *Monitoring Lizards. Part I – Trade data analysis – International trade in monitor lizards (Varanus spp.)*. Final report for BfN funded project. TRAFFIC, UK. **Suggested citation (Part 2):** Ziegler, S. (2016). *Monitoring Lizards Part 2 – Pilot study testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals.* Final report for BfN funded project. TRAFFIC, UK.

Front cover photograph: Spiny-tailed Monitor Varanus acanthurus Credit: Frupus; http:/bit.ly/2aQqmCb Creative Commons CC BY-NC 2.0

## MONITORING LIZARDS

# Using stable isotope analysis to determine the origin of monitor lizard *Varanus* spp. skins

Vicki Crook, Louisa Musing and Stefan Ziegler



Spiny-tailed Monitor Varanus acanthurus

Project Partners: TRAFFIC, Stefan Ziegler, Agroisolab, Cologne Zoo and Stuttgart State Museum of Natural History.



Supported by the German Federal Agency for Nature Conservation and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety

# TABLE OF CONTENTS

# MONITORING LIZARDS: Using stable isotope analysis to determine the origin of monitor lizard *Varanus* spp. skins

Summary and Recommendations	iii
Zusammenfassung und Empfehlungen	v
PART 1: Trade data analysis:International trade in monitor lizards Varanus	spp.
Introduction	1
Varanus spp. trade analysis	2
Overview of Varanus trade, 1990-2014	2
Trade of Varanus skins and small leather products, 1990–2014	5
Trade of live Varanus, 1990–2014	8
Conclusion	14
References	15

# PART 2: Pilot study testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals

19
20
20
20
21
21
23
23
24
26
30
32
33

# Summary and recommendations

Quantitative measurement of stable isotope ratios in metabolically inert tissue samples has been identified as a potential tool for wildlife crime investigations that can be applied to differentiate between wild and captive-sourced animals. Targeted collection for international trade is regarded as one of the greatest threats to the survival of many species, particularly with regards to the commercial trade of reptiles as relatively little information on the status of most wild populations exists. In an effort to meet demand and reduce pressure on wild populations, several countries have encouraged the establishment of captive breeding facilities, however there are reports of fraudulent claims of captive-breeding, which undermine the trade regulations in place, such as those under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Concerns over high levels of exploitation and trade in an increasing number of monitor lizards, *Varanus* spp., have been reported in recent years. An overview of trade in *Varanus* species over the past 25 years (1990-2014), based on CITES trade data, is presented in this report (Part I). Globally, the largest quantities of *Varanus* specimens are traded as skins and leather products for the leather industry. This trade is predominantly made up of wild sourced specimens, with no significant changes in reported sourcing to captive-breeding facilities in recent years. The second most traded commodity of *Varanus* is live specimens, and for a number of *Varanus* species, trade data show a shift to reported captive-born or captive-bred sourcing in the last few decades. The majority of these shifts appear to have occurred for *Varanus* species native to and exported from Indonesia.

The trade data analysis complements the pilot study carried out over six months in 2015-2016, testing the applicability of isotopic markers in the epidermis to discriminate between captive and wild individuals using various monitor lizard species and the Crocodile Lizard *Shinisaurus crocodilurus* (Part II). For the pilot study three different isotopic datasets of lepidosaurian reptiles were generated and tested for their potential to predict specimen provenance. The results showed that:

- Wild specimens of species with vast distribution ranges, such as *Varanus salvator*, show considerable variation in isotope ratios, and therefore provenance is difficult to establish using isotope analysis.
- Under quasi-controlled feeding of specimens in captivity, isotopic ranges are smaller than those in wild populations. This result strongly supports the potential for development of a reference framework of breeding farms against which specimens of ambiguous origin can be cross-checked.
- An isotopic <sup>15</sup>N marker (glycine) is still detectable in shed epidermal fragments of monitor lizards more than three months after the application and thus, has potential for specific forensic applications, such as trade chain analysis.
- Marking juvenile and sub-adult specimens with enriched <sup>15</sup>N glycine as chemical imprint of legal captive-bred origin might not be effective due to the rapid epidermal renewal cycle.

The trade data and isotope analyses carried out as part of this pilot project both provide useful information in order to focus ongoing and future isotope research on species and regions of the world where sourcing claims may require further verification in the future.

Based on the pilot study isotope analysis results, it is recommended that a follow on project looks into several breeding farms of reptiles in international trade for which an isotopic reference framework could be established. Of particular interest would be to assess whether the isotopic profiles of specimens kept in such facilities remain constant over time. Stable isotope analysis can then be used for geographic assignments within a probability context and exclude unlikely areas of provenance.

Based on the trade data analysed, priority species for further testing could include live wild-taken (W) and captive-born (F) and/or captive-bred (C) specimens of a number of Varanus species for which high numbers of C and F specimens were identified in trade over the last decade, such as *V. timorensis, V. prasinus, V. indicus, V. beccarii* and *V. rudicollis.* Furthermore, it would be essential to compare isotopic signatures of W, F and C specimens that have been collected/bred in Indonesia and elsewhere (potentially from/in areas of close proximity or similar habitat conditions) in order to establish whether variations in isotope sources are significant enough to differentiate specimens in such cases.

It would be beneficial to carry out some additional background research to establish the precise focus of such a follow on project, covering the following aspects:

- Review trade data with a specific aim of identifying CITES-listed reptile species (not only *Varanus*) with restricted ranges, traded as live specimens for the pet trade and for which a move to captive-bred sources has been reported; where many species are identified, those for which the EU appears to be an important end market, should be prioritised.
- Identify bona fide breeding farms, zoos and herpetological associations for potential future collaboration, and collate any readily available information on breeding practices and feeding regimes (such as procuring food from the wild) used for the species identified in the trade review, above.
- Review results of other recent/ongoing isotope research projects focusing on verifying the origin of reptile specimens (including skins), such as those on pythons and tortoises, and take into consideration any lessons learnt from these.
- Consider the feasibility of testing species which have slower growth/renewal rates, such as tortoises, and may therefore be more suitable for marking than *Varanus*.

# Zusammenfassung und Empfehlungen

Die quantitative Messung von stabilen Isotopenverhältnissen in metabolisch inerten Gewebeproben wurde als mögliches Artenschutzinstrument identifiziert, um zwischen Individuen zu unterscheiden, die aus der Wildnis oder aus Nachzuchten in Gefangenschaft stammen. Die gezielte Sammlung für den internationalen Handel gilt als eine der größten Bedrohungen für das Überleben vieler wildlebender Tierarten, vor allem im Hinblick auf den kommerziellen Handel von Reptilien, da über den Status der meisten Wildpopulationen relativ wenig Informationen vorhanden ist. In ihrem Bemühen, die Nachfrage zu befriedigen und den Druck auf Wildpopulationen zu verringern haben mehrere Länder die Einrichtung von Zuchtanlagen gefördert, aber es gibt Berichte zu falsch deklarierten Nachzuchten, die die Handelsvorschriften untergraben, wie sie im Rahmen des Übereinkommens über den internationalen Handel mit gefährdeten Arten freilebender Tier- und Pflanzenarten (CITES) bestehen.

In den letzten Jahren wurde vermehrt über eine Zunahme der Nutzung und des Handels mit Waranen, *Varanus* spp., berichtet. Eine Übersicht über den Handel mit *Varanus* Arten im Laufe der letzten 25 Jahre (1990-2014), auf Basis von CITES Handelsdaten, wird in diesem Bericht (Teil I) dargestellt. Weltweit die größten Mengen von Waranprodukten werden als Häute und Lederprodukte für die Lederwarenindustrie gehandelt. Der Handel wird überwiegend aus wildgefangenen Exemplaren bedient, wobei keine signifikanten Veränderungen bei den gemeldeten Herkunftsangaben aus Nachzuchten in Gefangenschaft über die letzten Jahre beobachtet werden kann. Das zweitmeist gehandelte Waranprodukt sind lebende Exemplare, und für eine Reihe von Arten zeigen die Handelsdaten der letzten Jahrzehnte eine Verschiebung zu in Gefangenschaft geboren oder in Gefangenschaft nachgezüchteten Exemplaren. Die meisten dieser Veränderungen gelten für *Varanus* Arten, die in Indonesien heimisch und von dort exportiert werden.

Die Fachdatenanalyse ergänzt eine Pilotstudie, die über einen Zeitraum von sechs Monaten im Zeitraum 2015-2016 durchgeführt wurde, und welche die Unterscheidung von wilden und nachgezüchteten Individuen an Hand von Isotopenmarkierungen in der Epidermis verschiedener Waran-Arten sowie der Krokodilschwanzechse (*Shinisaurus crocodilurus*) geprüft hat (Teil II). Im Rahmen dieser Studie (Teil II) wurden drei verschiedene Datensätze von Schuppenechsen generiert und deren Potenzial für die Herkunftsuntersuchung untersucht. Die Ergebnisse der Isotopenuntersuchungen sind die Folgenden:

- Individuen aus der Wildnis von Arten mit großen Verbreitungsgebieten, wie *Varanus salvator*, zeichnen sich durch eine erhebliche Variabilität der Isotopenmuster aus, so dass sich die Herkunftsbestimmung über Isotopenuntersuchungen schwierig gestaltet.
- Unter quasi-kontrollierten Fütterungsbedingungen von Individuen in Gefangenschaft sind die Isotopenbereiche enger und Isotopenmuster homogener als bei Wildpopulationen. Dieses

Ergebnis unterstützt die Entwicklung eines Referenzrahmens von Nachzuchteinrichtungen, mit dem Individuen zweifelhafter Herkunft abgeglichen werden können.

- Der Isotopenmarker <sup>15</sup>N (Glycin) kann noch nach drei Monaten in gehäuteten Epidermisschuppen nachgewiesen werden und eignet sich daher für bestimmte forensische Anwendungen, wie beispielsweise der Untersuchung von Marktketten.
- Die Markierung von juvenilen und subadulten Individuen mit angereichertem <sup>15</sup>N als chemischen Abdruck von legalen Nachzuchten scheint bedingt durch die schnellen Häutungszyklen der Epidermis wenig geeignet zu sein.

Sowohl die Handelsdaten als auch die Isotopenanalysen, die im Rahmen dieses Projekts durchgeführt wurden, liefern nützliche Informationen, um laufende und künftige Isotopenforschung auf Arten und Regionen der Welt zu konzentrieren, in denen die Frage der verifizierten Herkunft zunehmend an Bedeutung erlangt.

Auf der Grundlage der Studie wird empfohlen, ein Pilotprojekt zu starten, das mehrere Zuchtfarmen von Schuppenechsen enthält, für die ein Isotopen-Referenzrahmen festgelegt werden sollte. Von besonderem Interesse wäre zu prüfen, ob die Isotopenprofile von Proben aus solchen Einrichtungen im Laufe der Zeit konstant bleiben. An Hand der stabilen Isotopenanalyse können dann geografische Zuordnungen mit einer bestimmten Wahrscheinlichkeit angegeben und unwahrscheinliche Herkunftsgebiete ausgeschlossen werden.

Basierend auf der Analyse der Handelsdaten, sollten weitere Untersuchungen durchgeführt werden an Wildfängen (W), in Gefangenschaft geborenen (F) und/oder in Gefangenschaft gezüchteten (C) Individuen von *Varanus* Arten, für die eine hohe Zahl von C und F Individuen im Handel ist (*V. timorensis*, *V. prasinus*, *V. indicus*, *V. beccarii* und *V. rudicollis*). Darüber hinaus wäre es wichtig, Isotopensignaturen von W, F und C Individuen zu vergleichen, die aus Indonesien stammen bzw. dort gezüchtet wurden um festzustellen, ob genügend deutliche Variationen in den Isotopensignaturen auftreten, um die vermuteten Herkunftsgebiete zu unterscheiden.

Es wäre vorteilhaft, weitere Hintergrundinformationen zu recherchieren, um den Fokus eines solchen Folgevorhabens genau zu definieren und dabei die folgenden Aspekte abzudecken:

- Überprüfung der Handelsdaten mit dem Ziel, CITES gelistete Reptilienarten (nicht nur *Varanus* spp.) mit kleinen Verbreitungsarealen zu identifizieren, die lebend gehandelt und für die in den letzten Jahren vermehrt Nachzuchten gehandelt werden. Ein Fokus sollte auf Arten liegen, die in die EU importiert werden.
- Identifizierung von bona fide Zuchtfarmen, Zoos und herpetologischen Verbänden für eine mögliche zukünftige Zusammenarbeit; des Weiteren die Sammlung von verfügbaren Informationen über Zuchtmethoden und Futterwirtschaft (wie Beschaffung der Nahrung aus der Natur) für die identifizierten gehandelten Arten, siehe oben.

- Überprüfung und Anwendung der Ergebnisse von anderen aktuellen / laufenden Isotopen Forschungsprojekten (Pythons und Schildkröten), deren Schwerpunkt ebenfalls auf der Herkunftsbestimmung von Reptilien (einschließlich Häute) liegt.
- Überprüfung der Machbarkeit der Isotopenmarkierung auf Reptilienarten, die ein langsameres Wachstum / Erneuerungsraten der Epidermis haben, wie zum Beispiel Schildkröten.

# PART 1: Trade data analysis – International trade in monitor lizards (*Varanus* spp.)

Vicki Crook and Louisa Musing



Nile Monitor Lizard Varanus niloticus

Project Partners: TRAFFIC, Stefan Ziegler, Agroisolab, Cologne Zoo and Stuttgart State Museum of Natural History.



Supported by the German Federal Agency for Nature Conservation and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety

TRAFFIC Report: Monitoring Lizards: Using stable isotope analysis to determine the origin of monitor lizard Varanus spp. skins

## INTRODUCTION

Targeted collection for international trade is regarded as one of the greatest threats to the survival of many species. The commercial trade of reptiles is of particular concern as there is relatively little information available on the status of most wild populations. A recent study (Böhm, *et al.*, 2013) concluded that, worldwide, almost 20% of reptile species are threatened with extinction and that for a similar proportion there is not enough information to make an assessment.

Multiple species and vast numbers of reptiles are traded domestically and internationally for a variety of reasons including as skins, meat, ingredients in traditional medicine and live for the pet trade. In an effort to meet demand and reduce pressure on wild populations, several countries have encouraged the establishment of captive breeding facilities. However, severe reservations have been expressed about the conservation impact these facilities are having due to reports that many animals traded as captive-bred have in fact been sourced illegally from the wild (Outhwaite *et al.*, 2015; Outhwaite *et al.*, in prep; CITES, 2013).

Fraudulent claims of captive-breeding or ranching undermine trade regulations in place to protect species, such as listing of species in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). CITES Parties have recognized the importance of developing mechanisms to help combat this phenomenon, which include tools to help CITES authorities and law enforcement officers accurately identify cases of fraudulent source declarations (CITES, 2013). Isotope analysis has been proposed as a potential tool for this purpose (Outhwaite *et al.*, in prep, Lyons and Natusch, 2015).

Concerns over high levels of exploitation and trade in an increasing number of *Varanus* species have been reported in recent years (Koch *et al.*, 2013; Outhwaite *et al.*, in prep; Pernetta, 2009). Currently, 73 species of *Varanus* including 21 subspecies are recognized (Koch *et al.* 2010; Welton *et al.* 2014). Twenty-two *Varanus* species have been evaluated for the IUCN Red List: *V. mabitang* is listed as Endangered, *V. komodoensis* and *V. olivaceus* as Vulnerable, and *V. nuchalis* as Near Threatened, with remainder listed as either Least Concern or Data Deficient. *Varanus* species face a number of potential threats including habitat destruction, human consumption, traditional medicine, and collection for the global leather industry and pet trade (Schlaepfer *et al.* 2005; Bennet *et al.* 2010). Owing to concerns that international trade may have a detrimental impact on species survival, the entire genus *Varanus* was listed in CITES Appendix II in 1975, apart from *V. bengalensis, V. flavescens, V. griseus, V. komodoensis* and *V. nebulosus* which were included in Appendix I.

As for many reptiles, this group of species is also affected by concerns over fraudulent claims of captivebreeding. Detailed, recent trade analyses for these species are, however, limited. An overview of trade in *Varanus* species over the past 25 years, based on CITES trade data, is presented in this report (Part I). The trade data analysis complements the pilot study carried out over six months in 2015–2016, testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals using the Crocodile Lizard and various monitor lizard species. The pilot study methods and results are presented in Part II.

#### Varanus spp. trade analysis

Trade data for all species of the genus *Varanus* for years 1990 to 2014 were extracted from the CITES Trade Database managed by UNEP-WCMC<sup>1</sup> in January 2016. More than 98% of reported exporterand importer-reported quantities for this 25 year period were number of specimens (i.e. the unit was blank) and 99% of exporter- and importer-reported quantities were for commercial purposes (purpose code T or blank). The analysis therefore focused on trade in specimens for commercial purposes. Comparative tabulations, which compare the imports and exports reported by individual CITES Parties submitted to the UNEP-WCMC CITES Trade Database, were used. While trade records should be identical, in practice these often differ between the importing and the exporting country. This is often caused by the fact that reporting is typically based on export permits issued rather than the export permits actually utilized. For these reasons, both exporter- and importer-reported quantities were considered in the analyses and as data were generally comparable across the years, exporter-reported quantities were also examined to highlight apparent gaps in trade reported by exporters. The analysis differentiated exports and re-exports and also examined trade in specimens reported with different source codes<sup>2</sup>, where appropriate.

#### Overview of Varanus trade, 1990-2014

Over the last 25 years (1990–2014), nearly 55 million specimens of *Varanus* species were reportedly traded internationally; ~ 20 million specimens exported and ~35 million specimens re-exported (Table 1). Fifty-four species of *Varanus* were reported in trade over this 25 year period, with *V. salvator*, *V. niloticus* and *V. exanthematicus* as the most prevalent species, respectively, for both exports and re-exports representing 99% of all specimens traded. Specimens recorded to the genus level only (*Varanus* spp.) made up no more than 0.1% of all reported trade.

In terms of commodities traded, *Varanus* specimens are subject to two main markets; the leather industry (e.g. skins and small leather products) and the pet trade (Table 1). The principal commodities exported between 1990 and 2014 were skins (~90%) and live specimens (6%), while re-exports were predominantly of small leather products (~45%) and skins (~41%) (Table 1).

<sup>&</sup>lt;sup>1</sup> United Nations Environment Programme – World Conservation Monitoring Centre: <u>http://trade.cites.org/</u>

<sup>&</sup>lt;sup>2</sup> Source codes include: **C**: Animals bred in captivity in accordance with *Resolution Conf. 10.16 (Rev.*); **D**: Appendix-I animals bred in captivity for commercial purposes; **F**: Animals born in captivity (F1 or subsequent generations that do not fulfil the definition of 'bred in captivity' in Resolution Conf. 10.16 (Rev.); **I**: Confiscated or seized specimens; **O**: Pre-Convention specimens; **R**: Specimens originating from a ranching operation; **U**: Source unknown; **W**: Specimens taken from the wild. Source codes I, O, U, W and those that were left blank were assumed to be specimens taken from the wild.

While the number of specimens traded for the leather industry is significant, there has been a general decline in trade over the past two decades. Exports of both skins and small leather products gradually decreased from  $\sim$ 2 million specimens in 1990 to  $\sim$ 500 000 specimens in 2012<sup>3</sup>, representing an overall decline of  $\sim$ 75% (Figure 1).

On the other hand, there has been a gradual overall increase in exports of live specimens over the last 25 years, averaging ~ 50 000 specimens per annum (Figure 2). In terms of re-exports of live specimens, a relatively low number were re-exported until 2008, averaging ~1900 specimens per annum between 1990 and 2008. Annual numbers of live specimens re-exported then increased to ~38 000 between 2009 and 2011, however in 2012 a total of 170 554 specimens were re-exported (Figure 2). This significant increase is attributable to Viet Nam re-exporting 164 500 specimens of *V. salvator* that reportedly originated from Lao People's Democratic Republic (PDR) and were destined for China (Lao PDR, however, did not report the export of these specimens to Viet Nam).

Table 1. Number of *Varanus* specimens exported and re-exported, by principal commodity, according to exporter-reported quantities, 1990–2014. Source: UNEP-WCMC CITES Trade Database

Exports		<b>Re-exports</b>			
Commodity	Number of specimens	Percentage (%)	Commodity	Number of specimens	Percentage (%)
			Leather products		
Skins	18 242 540	89.8	(small)	15 382 324	44.5
Live	1 281 473	6.3	Skins	14 022 920	40.6
Leather products					
(small)	713 836	3.5	Skin pieces	2 349 247	6.8
Skin scraps	33 600	0.2	Leather items	2 100 602	6.1
Leather items	10 519	0.1	Live	333 357	1.0
Other	22 575	0.1	Other	346 199	1.0
Total	20 304 543	100	Total	34 534 649	100

<sup>&</sup>lt;sup>3</sup> Data for 2013 and 2014 are incomplete - some CITES Parties have yet to submit their reports for 2013 and 2014 or submitted after the deadline, and therefore quantities for these years are likely to be underestimates.

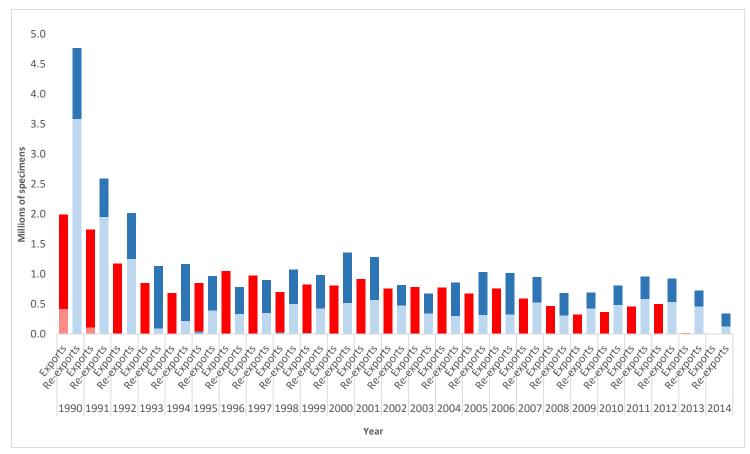


Figure 1. Number of *Varanus* skins and small leather products specimens exported and re-exported, according to exporter-reported quantities, 1990–2014. Source: UNEP-WCMC CITES Trade Database. *Darker* shades - skins, lighter shades - small leather products.

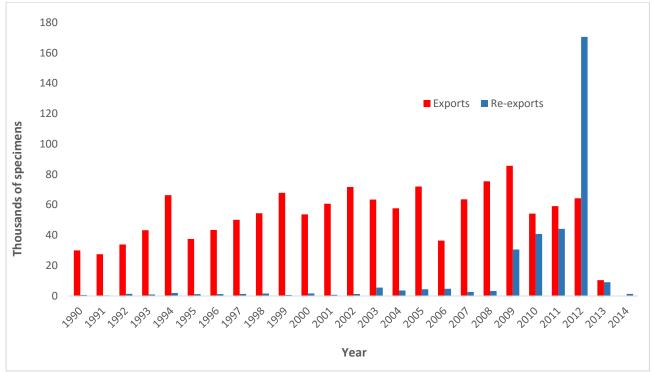


Figure 2. Number of live *Varanus* specimens exported and re-exported, according to exporter-reported quantities, 1990–2014. Source: UNEP-WCMC CITES Trade Database.

# Trade of *Varanus* skins and small leather products, 1990–2014

Approximately 18 million skin specimens of six species of *Varanus* and ~700 000 small leather products of four *Varanus* species were exported internationally for commercial purposes between 1990 and 2014. In terms of re-exports, ~14 million skin specimens of five *Varanus* species and ~15 million small leather products of eight *Varanus* species were traded internationally. Two species – *V. salvator* and *V. niloticus* – made up 99% of this trade and these two species remained the top species in trade throughout this period (Table 2).

Table 2. Number of Varanus skins and small leather products exported and re-exported, by species, according				
to exporter-reported quantities, 1990-2014. Source: UNEP-WCMC CITES Trade Database. LPS - small				
leather products				

	Exports		<b>Re-exports</b>	
Species	Skins	LPS	Skins	LPS
Varanus salvator	14 241 447	652 016	11 392 843	4 688 743
Varanus niloticus	3 980 965	58 581	2 530 605	10 675 946
Other	20 128	3 239	99 472	17 636
Total	18 242 540	713 836	14 022 920	15 382 324

In terms of the source of exports and re-exports of *Varanus* skins and small leather products in trade between 1990 and 2014, 99% were reportedly sourced from the wild (Source code W, hereafter referred to as W). One exception to this was the reported export of 6 000 skins from Malaysia to Singapore in 2003 coming from captive-bred sources. In the same year, Singapore re-exported ~2 000 "captive-bred" (Source code C, hereafter referred to as C) specimens to Macau that reportedly originated from Malaysia. Over the last 25 years, the United States of America (hereafter USA) also re-exported ~12 500 small leather products of captive-bred (C) source, ~7 500 of which had reportedly originated in Indonesia.

Seventeen countries/territories were involved in exporting *Varanus* skins between 1990 and 2014, according to exporter-reported data, with the top five countries accounting for 96% of total specimens traded. Indonesia was the principal exporter, exporting over half (~10 million) of all *Varanus* skins over the past 25 years, with the second highest trading Party, Malaysia, exporting ~3 million (Table 3). The third, fourth and fifth most important exporters were all African countries – Sudan, Mali and Chad – and together exported nearly 20% of all skin specimens over the past 25 years. In more recent years – between 2003 and 2012 – these five countries remained the top exporters of *Varanus* skins, with Indonesia and Malaysia exporting ~5 million specimens (~87%).

In terms of re-exports, 42 countries/territories were involved with the top five trading nearly 80% of all skins (Table 3). Singapore, known to be a major re-exporter of reptile products (Nijman, 2010) was the main re-exporter of *Varanus* skins (accounting for ~9 million, 65% of total re-exports) between 1990 and 2014 with 94% of skins re-exported from Singapore reportedly originating in Indonesia and Malaysia. EU Member States re-exported ~2.5 million *Varanus* skins (~17% of the total) with France and Spain together exporting ~1.3 million skins. The principal re-exporters remained the same in recent years.

Exports			Re-exports		
Exporter	Number of specimens	Percentage (%)	Exporter	Number of specimens	Percentage (%)
Indonesia	10 220 658	56	Singapore	9 181 344	65
Malaysia	3 715 173	20	France	911 016	6
Sudan	1 659 471	9	Spain	442 639	3
Mali	1 081 188	6	USA	441 828	3
Chad	785 362	4	Hong Kong	402 059	3
Other	780 688	4	Other	2 644 034	19
Total	18 242 540	100	Total	14 022 920	100

Table 3. Number of *Varanus* skins exported and re-exported, by country/territory, according to exporterreported quantities, 1990-2014. Source: UNEP-WCMC CITES Trade Database.

The countries/territories involved in exporting *Varanus* skins were similar when comparing exporterand importer-reported quantities, however, there were some differences in re-exporters. According to importer-reported quantities, Singapore remained the top re-exporter, and while France and Hong Kong also remained among the top five re-exporters, accounting for 4% and 3% respectively, Spain and the USA were replaced by the United Kingdom (hereafter UK, 8%) and Italy (4%).

Fifty countries/territories were involved in importing the ~18 million *Varanus* skin exports with Singapore (40%), Japan (16%), France (8%), Mexico (5%) and the USA (5%) as the top five importers. Of note is that whilst Singapore, France and the USA were countries mainly involved in importing exports of *Varanus* skins and then re-exporting them (Table 3), Japan and Mexico were not. Furthermore, Egypt was also a major importer of *Varanus* skins accounting for 5%. In terms of importers of *Varanus* skin re-exports, 79 countries/territories imported the ~14 million specimens and the proportions imported per country/territory were more evenly spread; Japan (23%), Mexico (18%), USA (11%), Hong Kong (9%) and Italy (5%).

Twenty-six countries/territories exported small leather products of *Varanus* species between 1990 and 2014 according to exporter-reported data. The top five exporters made up 98% of all trade. Thailand was the principal exporter of small leather products over the last 25 years (nearly 60%, ~420 000 specimens, Table 4). Indonesia and China were each responsible for another ~15% of exports, with Senegal and Madagascar together responsible for less than 10%. Of note is that when looking at trade trends in more recent years, between 2003 and 2012 Thailand did not report the export of any specimens, while Indonesia and Senegal were the top exporting countries of *Varanus* small leather products, respectively, accounting for ~97% of the ~61 000 specimens exported. Principal trading countries/territories over the last 25 years differed slightly when comparing exporter- and importer-reported quantities<sup>4</sup>. According to importer-reported quantities, Thailand was still the principal exporter, but China, Senegal and Madagascar were replaced by Mali (10%) and Sudan (7%) in the top five.

<sup>&</sup>lt;sup>4</sup>According to importer-reported quantities, the exporter of 25% of all exported specimens, was reported as "various countries" (code XV).

Between 1990 and 2014, 59 countries/territories re-exported 15 million small leather products, according to exporter-reported quantities. The majority of specimens were re-exported from Europe – France (19%, 2.9 million specimens), Switzerland (16%, 2.5 million), Italy (13%, 1.9 million) and Germany (5%, 840 000) (Table 4). France, Italy and Switzerland are known to process large quantities of reptile products, including tanning, cutting and manufacturing (Webb *et al.*, 2012). In more recent years – between 2003 and 2012 – 4 million small leather products were re-exported with European countries continuing to play important roles: Italy (re-exporting 20% of specimens), Switzerland (13%), and France (10%). Mexico and Hong Kong were also important re-exporters over the past 10 years, accounting for 15% and 11% respectively.

When comparing re-exports of small leather products reported by exporters and importers, there were a number of differences. According to importer data, a total of 112 countries/territories were involved, and Hong Kong was the main re-exporter trading 4.9 million specimens, while Switzerland and France accounted for 18% and 10% respectively. Germany was replaced by Austria (~1.6 million specimens). Mauritius accounted for similar number of specimens (~1.4 million specimens) according to both exporter- and importer-reported quantities.

Exports			<b>Re-exports</b>		
	Number of	Percentage		Number of	Percentage
Exporter	specimens	(%)	Exporter	specimens	(%)
Thailand	420 080	59	France	2 910 377	19
Indonesia	111 687	16	Switzerland	2 529 220	16
China	103 759	14	Italy	1 954 654	13
Senegal	44 733	6	Mauritius	1 478 463	10
Madagascar	21 212	3	Germany	842 710	5
Other	12 365	2	Other	5 666 900	37
Total	713 836	100	Total	15 382 324	100

 Table 4. Number of Varanus small leather products exported and re-exported, by country/territory, according to exporter-reported quantities, 1990-2014. Source: UNEP-WCMC CITES Trade Database.

Fifty-five countries/territories imported *Varanus* small leather products between 1990 and 2014, while 199 countries/territories were involved in importing the re-exports. For both, the same top five countries/territories were involved; the USA, Hong Kong, France, Switzerland and Japan. Of note is that a high proportion of importing countries/territories, for both the exports and re-exports of *Varanus* small leather products, were recorded as "unknown" or "various" (codes XX and XV, respectively). This is likely due to the manufacture of small leather products from a number of different specimens that had been imported from different countries/territories.

### Trade of live Varanus, 1990–2014

Approximately 1.5 million specimens of 49 species of *Varanus* were traded internationally for commercial purposes between 1990 and 2014; ~1.3 million specimens of these were exports and 300 000 were re-exports. Further analysis focuses on exports only.

Nearly 90% of all live *Varanus* exports were made up of three species; *V. exanthematicus*, *V. salvator* and *V. niloticus* (Figure 3). *Varanus exanthematicus* and *V. niloticus* are native to Africa; the distribution of *V. exanthematicus* extends throughout sub-Saharan Africa while the range of *V. niloticus* is distrusted across central and southern Africa. *Varanus salvator* is native to South and Southeast Asia. *Varanus exanthematicus* made up the highest proportion in recent years; ~250 000 specimens between 2004 and 2013 compared with ~190 000 of *V. salvator* and 65 000 of *V. niloticus*. The drop in total 2006 exports as shown in Figure 3 can be attributed to a lack of reported exports of *V. exanthematicus* by exporting countries/territories. According to importer data, however, this species was traded in 2006 in similar quantities to preceding years (~30 000 specimens annually).

Forty-six countries/territories exported live specimens of *Varanus* over the 25 year period, according to exporter-reported quantities, of which the top five exporters accounted for 92% of reported exports (Table 5). The top exporter was Ghana (27% of live specimens), followed closely by Indonesia (18%), Benin (16%), Togo (16%) and Malaysia (15%). The principal exporters remained the same in recent years.

Between 1990 and 2014, 88 countries/territories imported live specimens of *Varanus* according to exporter-reported quantities, of which the top five importers accounted for 83%. The USA was the top importer (59% of live specimens), followed by Hong Kong, Japan, the UK and Germany (Table 5). EU Member States accounted for ~15% of live *Varanus* imports: aside from the UK and Germany, France and Spain were the other main EU Member State importers. The principal importers remained the same in recent years.

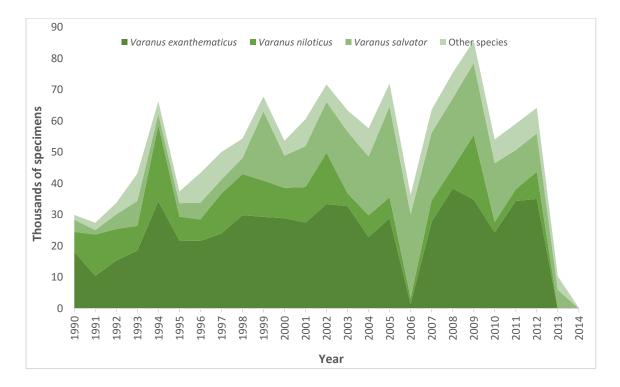


Figure 3. Number of live *Varanus* specimens exported, by species, according to exporter-reported quantities, 1990–2014. Source: UNEP-WCMC CITES Trade Database.

	Number of	Percentage		Number of	Percentage
Exporter	specimens	(%)	Importer	specimens	(%)
Ghana	347 499	27	USA	760 708	59
Indonesia	228 946	18	Hong Kong	171 328	13
Benin	205 911	16	Japan	51 165	4
Togo	199 440	16	UK	44 854	4
Malaysia	197 651	15	Germany	39 053	3
Other	102 026	8	Other	214 365	17
Total	1 281 473	100	Total	1 281 473	100

 Table 5. Number of live Varanus specimens traded, by main exporter and importer, according to exporter 

 reported quantities, 1990-2014. Source: UNEP-WCMC CITES Trade Database

Live *Varanus* specimens traded between 1990 and 2014 were reportedly sourced predominantly from the wild (W) (75%). The trade in wild specimens (W) remained relatively constant across this period averaging ~38 000 specimens per year. The remainder comprised of ranched specimens (21%), and specimens reported as captive-born or captive-bred (2%). Trade in captive-born (Source code F, hereafter referred to as F) and captive-bred (C) specimens commenced in the late 1990s and from 2004 onwards has been in the region of 5 000 specimens per year (Figure 4).

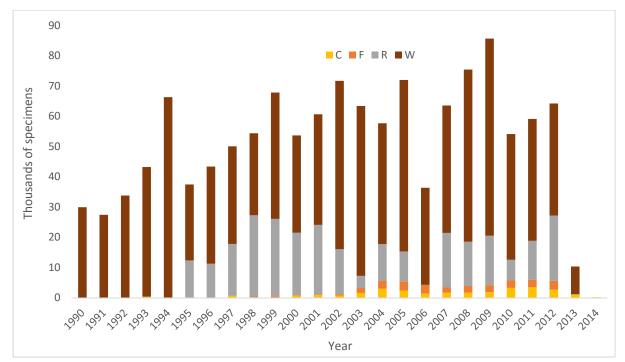


Figure 4. Live Varanus specimens exported, by source, according to exporter-reported quantities, 1990 and 2014. Source: UNEP-WCMC CITES Trade Database. C – captive-bred, F – captive-born, R – ranched, W – wild.

The principal live *Varanus* exporters (Benin, Ghana, Indonesia, Malaysia and Togo) were all involved in exporting *Varanus* specimens from various sources (Table 6). Indonesia, Benin and Togo exported the majority of specimens of non-wild source between 1990 and 2014 – Benin and Togo together exported 99% of all ranched specimens and Indonesia exported 85% of all captive-born (F) and captive-bred (C) specimens.

	Source				
Exporter	С	F	R	W	Total
Ghana			1 330	346 169	347 499
Indonesia	21 366	25 476		182 104	228 946
Benin	400		166 467	39 044	205 911
Togo	374		99 455	99 611	199 440
Malaysia	865			196 786	197 651
Other					
countries/territories	5 214	176	570	96 066	102 026
Total	28 219	25 652	267 822	959 780	1 281 473

 Table 6. Live Varanus specimens exported, by country/territory and source, according to exporter-reported

 quantities, 1990-2014. Source: UNEP-WCMC CITES Trade Database

Between 1998 and 2002, Indonesia's annual exports of live captive-born (F) *Varanus* specimens averaged 271 specimens per year. However, in 2003 1 611 specimens were exported representing an increase in trade of 264% between 2002 and 2003 with specimens mainly destined for the USA (49%) and France (22%) (Figure 5). Between 2004 and 2012, annual exports of captive-born (F) specimens continued to average 200 per annum. It must be noted that Indonesia has yet to submit its reports to

UNEP-WCMC for 2013 and 2014, hence the lack of data for these years, however according to importer-reported quantities, 2 241 specimens and 1 259 specimens were imported from Indonesia in 2013 and 2014, respectively.

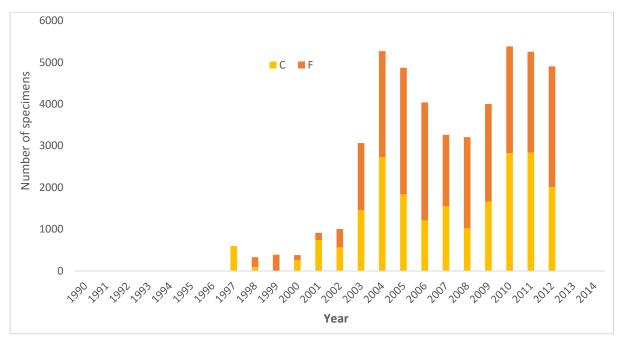


Figure 5. Indonesia's reported exports of captive-born (F) and captive-bred (C) live *Varanus* specimens, 1990–2014. Source: UNEP-WCMC CITES Trade Database

A similar pattern was observed for specimens reported as captive-bred (C). Before 2003, Indonesia had not exceeded an annual export rate of ~448 live captive-bred (C) *Varanus* specimens. However, in 2003, Indonesia reported exports of 1452 captive-bred (C) specimens, representing a 158% increase in trade between 2002 and 2003; these were destined for the USA (49%), France (23%), Japan (19%) and Taiwan (6%). While Indonesia's overall exports of wild specimens (W) continued over the 25 year analysis period, there was a steady decline in reported exports of wild specimens (W) from 1996 onwards, coinciding with the start of Indonesia's reported exports of captive-born (F) and captive-bred (C) specimens (Figure 6 and 7).

Over the past 10 years (2003-2012), Indonesia reported exporting over 40 000 captive-born (F) and captive-bred (C) specimens. These were composed mainly of *V. timorensis*, *V. salvator*, *V. rudicollis*, *V. melinus*, *V. prasinus*, and *V. beccarii* (Table 7).

Table 7. Live *Varanus* specimens exported by Indonesia, by species and source, according to exporterreported quantities, 2003-2012. Source: UNEP-WCMC CITES Trade Database. *C- captive-bred*, *F – captiveborn*.

Species	С	F	Total
Varanus timorensis	9 111		9 111
Varanus salvator	818	4 744	5 562
Varanus rudicollis	96	3 816	3 912
Varanus melinus	165	3 538	3 703
Varanus prasinus	3 673		3 673
Varanus beccarii	1 058	1 958	3 016
Varanus macraei	713	2 134	2 847
Varanus panoptes	141	2 390	2 531
Varanus indicus	2 363		2 363
Varanus dumerilii	65	1 693	1 758
Other species	920	3 847	4 767
Total	19 123	24 120	43 243

Seventy-two per cent of all captive-bred (C) specimens exported by Indonesia since 1997 were of one species, *V. timorensis. Varanus timorensis* is a protected species in Indonesia (Koch *et al.*, 2013) and only captive-bred (C) specimens are permitted for export. Site visits and interviews conducted in 2006 in Indonesia concluded that most facilities reporting exports of captive-bred (C) *V. timorensis* specimens for commercial purposes were not capable of producing such quantities (Nijman and Shepherd, 2009). According to the researchers, it was believed all specimens traded at that time were in fact were wild caught (Nijman and Shepherd, 2009). Such scrutiny of the legitimacy of Indonesia's reported exports of captive-bred and captive-born specimens has been previously intimated. Evidence of Indonesian breeding farms used to illegally launder green pythons has been confirmed (Lyons and Natusch 2011), and investigations of the captive-breeding facilities of Tokay Geckos in Indonesia have raised doubts to the logistical and financial ability of these facilities to produce the quantity of specimens they claim to harvest (Nijman and Shepherd, 2015).

Similar changes in source of specimens in trade from Indonesia have also occurred for other *Varanus* species. For example, trade of *V. rudicollis* from Indonesia was dominated by wild-caught (W) specimens until 1999. In 1999 Indonesia reduced its annual export quota to 900 live wild-caught specimens (from 1800 in 1998 and 2250 in 1997). The quota reduction coincided with the start, and then significant increase, in reported exports of captive-born (F) specimens of *V. rudicollis* (Figure 6).

Trade data also show an increase in trade of captive-bred (C) specimen *V. prasinus* from Indonesia in the past 20 years. *Varanus prasinus* is a protected species in Indonesia and only captive-bred (C) specimens are permitted for export. Importer reported data appeared more complete for this species and was therefore used for further analysis. In the 1990s, the majority of *V. prasinus* specimens imported from Indonesia (~40 specimens per year) were reported to be from wild sources (W). Reported trade in captive-bred (C) specimens commenced in 1997, and from 2004 onwards, there was a sudden increase in trade of captive-bred (C) specimens (~300 per year). These changes in trade patterns coincided with

Indonesia setting export quotas for live wild-caught (W) *V. prasinus*<sup>5</sup> (between 1997 and 2004 only) and imports of wild specimens of *V. prasinus* from Indonesia were suspended to the EU between 1997 and 2010 (Species Plus, 2016<sup>6</sup>) (Figure 7). Based on the effort required to breed *V. prasinus* compared to the ease of harvesting wild individuals, serious concerns have been raised suggesting that these captive-bred (C) specimens may in fact have been taken from the wild, (Nijman and Shepherd, 2009).

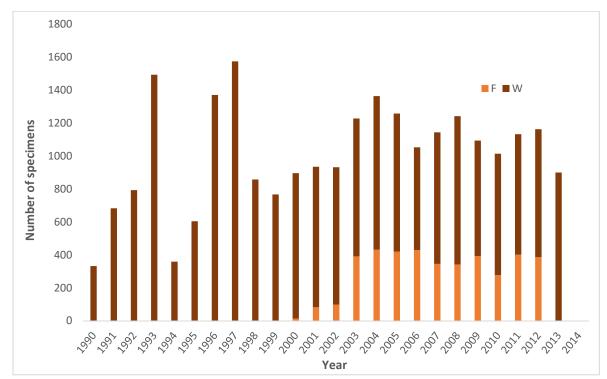


Figure 6. Indonesia's reported exports of live captive-born (F) and wild (W) specimens of *V. rudicollis*, according to exporter-reported quantities, 1990–2014. Source: UNEP-WCMC CITES Trade Database

<sup>&</sup>lt;sup>5</sup> Prior to CITES CoP15 in 2010, *V. prasinus* and *V. beccarii* were considered a single species (called *V. prasinus*). In 2010, this taxon was split into two distinct species; *V. prasinus* and *V. beccarii*.

<sup>&</sup>lt;sup>6</sup> Species Plus website available at: <u>http://www.speciesplus.net/</u>

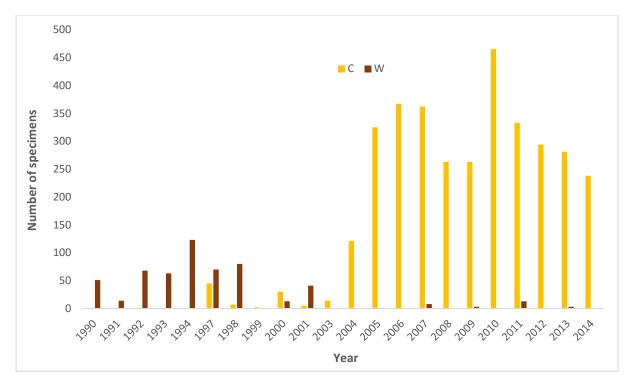


Figure 7. Indonesia's exports of wild (W) and captive-bred (C) live specimens of *V. prasinus*, according to importer-reported quantities, 1990–2014. Note: Until 2010 this included *V. beccarii*. Source: UNEP-WCMC CITES Trade Database.

## CONCLUSION

Based on CITES trade data compiled for the last 25 years (1990–2014), *Varanus* specimens have been subject to international trade for two main commodity markets, the leather industry and the live animal trade. Irrespective of the commodity, three species of *Varanus, V. exanthematicus, V. niloticus V. salvator,* were consistently the top traded species making up more than ~90%. While trade in commodities for the leather industry—comprising mainly skins and small leather products—has involved significant numbers of *Varanus* specimens, a general decline in trade over the past two decades is evident. This has not been the case for live trade with records showing a gradual increase in both exports and re-exports of *Varanus* specimens.

Approximately 55 million various *Varanus* specimens were both exported and re-exported over this 25 year period of which ~85% was made up of skins and small leather products and 7% of live trade. Countries/territories in Southeast and East Asia, Africa, Europe and the Americas, respectively, were involved in exporting and re-exporting skins and small leather products. The primary importers, in order of importance, included countries/territories from Southeast and East Asia, Europe and the Americas. For the live trade, countries/territories in Africa and Southeast Asia exported ~90% of specimens that were primarily destined for North America, East Asia and Europe, respectively.

In terms of the sourcing of *Varanus* specimens, 99% of *Varanus* skins and small leather products, and 75% of live specimens were taken from the wild. However, when looking at the sourcing of live

specimens of certain taxa since the early 2000s, there has been distinct shift of exported specimens taken from the wild to reportedly being captive-bred or captive-born. Indonesia was the main country involved in this change in source of specimens in the trade, with data showing sudden increases in the number of specimens reported to be captive-bred (C) and captive-born (F). In some cases, these sudden increases coincided with Indonesia reducing their export quotas for live wild-caught specimens as well as import suspensions being imposed by the EU on wild-caught *Varanus* specimens.

### REFERENCES

Bennett, D., Gaulke, M., Pianka, E.R., Somaweera, R. and Sweet, S.S. 2010. *Varanus salvator*. The IUCN Red List of Threatened Species. Version 2014.3. <<u>www.iucnredlist.org</u>>. Downloaded on 6 January 2015.

Böhm, M., Collin, B., Baillie, J.E.M and 90 authors. 2013. The conservation of the world's reptiles. *Biological Conservation*, 157, 372–385.

CITES, 2013. Snake trade and conservation management (Serpentes spp.). CoP16, Decisions 16.102 to 16.108, Bangkok.

Koch, A., Auliya, M. and Ziegler, T., 2010. Updated checklist of the living monitor lizards of the world (Squamata: Varanidae). *Bonn Zoological Bulletin* 57 (2), 127–136.

Koch, A., Ziegler, T., Bohme, B. and Auliya, M. 2013. Pressing problems: Distribution, threats, and conservation status of the monitor lizards (Varanidae: *Varanus* spp.) of Southeast Asia and the Indo-Australian archipelago. *Herpetological Conservation and Biology* 8 (Monograph 3), 1–62.

Lyons, J. and Natusch, D. 2015. *Methodologies for differentiating between wild and captive-bred CITES-listed snakes*. IUCN-SSC Boa and Python Specialist Group. Presented at the 28<sup>th</sup> meeting of the CITES Animals Committee, 30 August-3 September 2015

Nijman, V. and Shepherd, C.R. 2009. *Wildlife trade from ASEAN to the EU: issues with the trade in captive-bred reptiles from Indonesia*. TRAFFIC Europe for the European Commission, Brussels, Belgium Nijman, V. 2010. An overview of international wildlife trade from Southeast Asia. *Biodiversity and Conservation* 19:1101–1114.

Outhwaite, W., Mundy, V., Kecse-Nagy, K and Crook, V. 2015. *Concerns regarding trade in specimens claimed to be derived from captive breeding or ranching – Assessment of select examples.* Report commissioned by the CITES Secretariat.

Outhwaite, W., Chng, S. C. L, and Kecse-Nagy, K. in prep. Overview of conservation status and trade issues of selected South-East Asian reptile and stingray species used by the skin industry – Focus on Indonesia and Thailand. TRAFFIC, UK, Cambridge.

Pernetta, A. P. 2009. Monitoring the Trade: Using the CITES Database to Examine the Global Trade in Live Monitor Lizards (*Varanus* spp.). *Boawak*, 3(2), pp. 37–45

Schlaepfer, M. A., Hoover, C. and Dodd Jr, K, 2005. *Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations.* Bioscience 55 (3), 256–264.

Webb, G.J.W., Manolis, C. and Jenkins, R.W.G. 2012. Improving international systems for trade in reptile skins based on sustainable use. Report prepared for the United Nations Conference on Trade and Development (UNCTAD) BioTrade Initiative.

Welton, L.J., Wood Jr, P.L., Oaks, J.R., Siler, C.D. and Brown, R.M. 2014. Fossil-calibrated phylogeny and historical biogeography of Southeast Asian water monitors (*Varanus salvator* Complex). *Molecular Phylogenetics and Evolution* 74, 29–37.

16 TRAFFIC Report: Monitoring Lizards: Using stable isotope analysis to determine the origin of monitor lizard Varanus spp. skins

PART 2: Pilot study testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals

Stefan Ziegler



Water Monitor Lizard Varanus salvator

Project Partners: TRAFFIC, Stefan Ziegler, Agroisolab, Cologne Zoo and Stuttgart State Museum of Natural History.



Supported by the German Federal Agency for Nature Conservation and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety

18 TRAFFIC Report: Monitoring Lizards: Using stable isotope analysis to determine the origin of monitor lizard Varanus spp. skins

### INTRODUCTION

Quantitative measurements of stable isotopes have been used extensively in the fields of biology, ecology, geology as well as in forensic investigation and identification (Hobson, 1999; Ehleringer and Matheson, 2007). The use of natural abundance of isotope variation as geographic tracers to determine the provenance of food commodities has been established for materials that are naturally less variable isotopically, such as beef, dairy products and beverages but also for products and derivatives from species with distinct distribution areas, such as honey (Kelly *et al.*, 2005 and references therein).

Isotopes are incorporated directly from the diet into animal tissues while undergoing certain changes in isotope ratios (so-called fractionation; Urey, 1947). Stable isotope analysis investigates intrinsic chemical tissue signatures that provide information on local food webs, climate and other environmental parameters (Tieszen and Boutton, 1988). The quantitative measurement of stable isotope ratios in metabolically inert tissue samples has the potential to accurately determine the origin of a specimen, as the isotopic composition of certain elements, such as carbon and nitrogen, reflect an organism's diet and contains information on its respective local food web (Ehleringer and Matheson, 2007; Fry, 2006).

Stable isotope analysis has been identified as a potential tool for the investigation of wildlife crime (Moncada *et al.*, 2012; Voigt *et al.*, 2012) and in theory, it can be applied to differentiate between wild and captive-sourced animals. Wild specimens generally feed on a variety of taxa, which in turn may have consumed different taxa. These diverse isotopic sources, together, can indicate the presence of a specific complex food web or certain geographic region. By contrast, captive animals are usually kept under a controlled feeding regime of a few select taxa, which are generally in contact with less variable isotopic sources. Isotopic analysis has already successfully been used to distinguish captive and wild wolves (Kays and Feranec, 2011) and has being proposed as a potential method to distinguish wild-taken and captive-bred specimens in trade (Lyons and Natusch, 2015; Outhwaite *et al.*, in prep).

This report (Part II) provides the methods and results from a pilot study testing the applicability of isotopic markers in scales and faeces to discriminate between captive and wild individuals, using the Crocodile Lizard *Shinisaurus crocodilurus* and various Monitor lizard *Varanus* species (*V. acanthurus, V. macraei, V. melinus* and *V. salvator*) as test case species. The study was comprised of three components:

- (i) analysis of historical museum samples of wild-taken *V. salvator*, in order to evaluate the isotopic variation of a species with a wide distributional range;
- (ii) analysis of skin samples taken from live wild, "semi-captive" and captive specimens of the restricted range Crocodile Lizard *Shinisaurus chinensis* from the north of Vietnam in order to determine the isotopic variation of a reptile species with restricted range distribution and whether wild and captive specimens can be differentiated in these cases; and

(iii) analysis of shed skin fragments and faeces from live V. acanthurus, V. macraei and V. melinus individuals undergoing feeding experiments with an <sup>15</sup>N enriched marker under controlled conditions at Cologne Zoo, in order to examine the time lag for isotopic signals to appear and establish whether specimens could potentially be "marked" as captive-bred.

### METHODS

#### Historical samples of Varanus salvator

In order to evaluate the isotopic variation of a species with a vast distribution range, small fragments from historical *Varanus salvator* specimens (ten individuals) held at the State Museum of Natural History, Stuttgart were extracted and tested. The samples were originally collected from Indonesia between 1832 and 1892 and had been stored in ethanol since then. The exact provenance of sampled material was not known, but geographic locations, such as the name of islands where they were collected was curated (Appendix I). Small fragments (approx. 1cm<sup>2</sup>) of the skin were taken from the ventral section of the body of each individual, and one individual was also sampled from the dorsal section. The skin fragments were stored in polyethylene bags until analysis.

#### Recent samples of Shinisaurus crocodilurus

In order to determine the isotopic variation of wild and captive specimens of a reptile species with restricted range distribution, 21 skin samples (Appendix II) of the endangered Crocodile Lizard *Shinisaurus crocodilurus*, were analysed. The remaining and already heavily diminished wild populations of *S. crocodilurus* (threatened by habitat loss and unsustainable exploitation), are restricted to isolated sites in northern Vietnam and southern China (van Schingen *et al.*, 2014).

Samples were taken from:

- 10 wild specimens from all the three known occurrence sites of *S. crocodilurus* in Vietnam
- three "semi-captive" specimens originating from sites in Tay Yen Tu Nature Reserve, that had been kept for at least three years at the Me Linh Station for Biodiversity<sup>7</sup>, Vinh Phuc Province, in the north of Vietnam.
- eight "captive" (captive-bred) specimens, namely offspring born at the Me Linh Station.

Small tissue parts of the tail tip (~ 0.5 cm) were taken from each individual and subsequently stored in 70% ethanol. Since this tissue is capable of regeneration, this is a harmless sampling method used for lizards (Comas *et al.*, 2014).

<sup>&</sup>lt;sup>7</sup> A conservation breeding programme facility established between the Institute for Ecology and Biological Resources (IEBR) and the Cologne Zoo (Ziegler *et al.* 2015; Ziegler and Nguyen 2015).

#### Feeding experiments of Varanus spp.

The third test involved controlled feeding experiments, examining the time lag for isotopic signals to appear in samples of shed epidermal skin fragments and faeces and to establish if specimens could potentially be "marked" as captive-bred in this way. The test group was composed of ten specimens of three monitor lizard species (*Varanus acanthurus*, n=4; *V. macraei*, n=2; *V. melinus*, n=4), weighing between 50 g and 250 g, held individually in different facilities at the terrarium of the Cologne Zoo (see Appendix III for specimen identification numbers used). They were fed twice with dead new born mice on 22<sup>nd</sup> and 29<sup>th</sup> September 2015 consecutively. The mice had each been injected with 50 to 150 µl liquid <sup>15</sup>N enriched isotopic marker (glycine). In addition to the test group (n=10), two specimens of *V. acanthurus* (R193, R689) composed the control group. The test and control groups each contained one adult specimen (R195 and R193 respectively)—all other specimens were juveniles or sub-adults. After the specimens were fed with the injected mice, a normal feeding regime (insects and mice) was resumed. After the isotopic marking, the enclosures of all individuals were inspected and screened for shed skin fragments on a daily basis. If skin fragments were visible, the pieces were removed and stored in polyethylene bags until analysis (Appendix III). In total, 215 skin samples were collected between October 2015 and January 2016.

#### Isotope analysis

All scale and faeces samples were analysed at the accredited (DIN EN ISO/IEC 17025:2005) Agroisolab Facility for Stable Isotope Research in Jülich, Germany between April 2015 and February 2016. Samples were dried and cut into small aliquots with a scalpel. As the museum and *Shinisaurus* samples had been stored in ethanol, these were vacuum dried (10mbar) for 48 hours to separate the ethanol from the samples.

Sub-samples of 1-4.5 mg were loaded into 4 x 6 mm tin capsules for carbon and nitrogen isotopic measurements by a Nu Horizon<sup>\*</sup> continuous flow isotope ratio mass spectrometer. Results were reported relative to the Vienna Pee Dee Belemnite ( $\delta^{13}$ C) and atmospheric N<sub>2</sub> ( $\delta^{15}$ N), respectively and measured isotopic ratios (R) were expressed in  $\delta$  units in the conventional permil notation, where  $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ . After every tenth sample the calibrated laboratory standard (leucine) was also measured. The laboratory standard was calibrated against a set of international standards (carbon: IAEA-CH-6, IAEA-CH-7; nitrogen: IAEA-N-1, IAEA-N-2).

In order to assess the precision of the analyses, at least two replicate measurements for each sample were performed, when sufficient material was available. Analytical uncertainties, based on these replicate analyses were typically in the range of 0.1‰ ( $\delta^{13}$ C,  $\delta^{15}$ N) and corresponding relative errors were 0.4% ( $\delta^{13}$ C) and 1.4% ( $\delta^{15}$ N). Statistical analyses were conducted using the R software environment for statistical computing and graphics.

The *S. crocodilurus* samples were also subject to an assignment simulation. According to the origin of the classifier (i) wild, (ii) semi-captive, or (iii) captive, multi-isotope testing was applied. The rationale for the model was that samples with small Euclidian distance have an identical source of origin. The

weighted *k*-Nearest Neighbour Classifier was used (Hechenbichler and Schliep, 2004) to assign the vector to the classifier whose summed kernel densities is maximized among its *k* nearest neighbours. In order to address the problem that the limited sample size reduces natural variation, the mean and standard deviation (sd) were calculated for each classifier to simulate 100 isotopic ratios per class and the data were randomly subdivided into a training t (n = 200) and test set (n = 100). Lowest misclassification was achieved at k = 4. The test group was composed of randomly selected samples of wild (n=35), semi-captive (n=33) and captive (n=32) origin. Accuracy of the model was defined as the proportion of correctly assigned samples divided by the number of total samples.

### RESULTS

#### Historical samples of Varanus salvator

Mean  $\delta^{13}$ C was -19.98‰ (sd: 2.66), mean  $\delta^{15}$ N was 10.16‰ (sd: 1.99) and mean  $\delta^{34}$ S was 6.48‰ (sd: 3.52). Isotope values were divided by region.  $\delta^{15}$ N values were significantly higher in Java than in Sumatra (ANOVA; *F*-value: 7.01, *P* = 0.021, Fig. 8). This trend was not established for  $\delta^{13}$ C or  $\delta^{34}$ S, however.

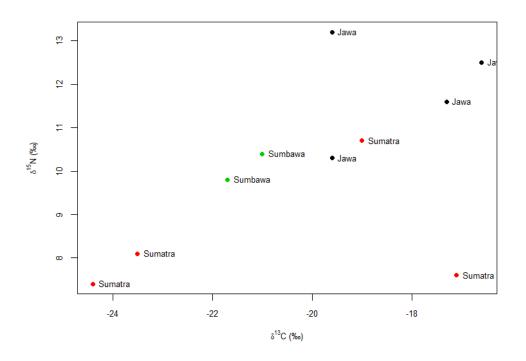


Figure. 8. Bivariate plot of isotope ratios ( $\delta^{13}$ C and  $\delta^{15}$ N) of skin samples from *Varanus salvator* with sites of origin

There is also statistical evidence that age class expressed as (i) juvenile, (ii) subadult, and (iii) adult has an isotopic effect. Juvenile specimens of *V. salvator* had significantly more positive  $\delta^{13}$ C values than adult specimens (Fig. 9), whereas this trend could not be detected for  $\delta^{15}$ N and  $\delta^{34}$ S. However, the number of samples was fairly limited so these statistical trends identified should be regarded with caution.

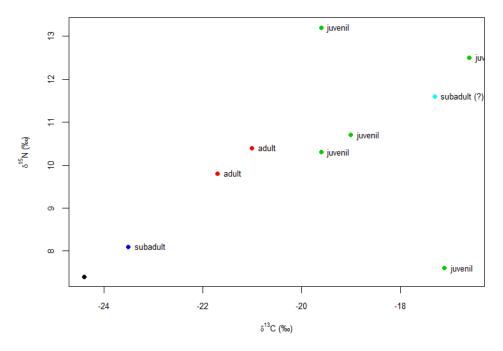


Figure 9: Bivariate plot of isotope ratios ( $\delta^{13}$ C and  $\delta^{15}$ N) of skin samples from *Varanus salvator* with presumed age category. \**Note: Juvenil = juvenile*.

#### Recent samples of *Shinisaurus crocodilurus*

The origin of the *S. crocodilurus* samples had a strong effect on the isotopic ratios (Fig. 10). The mean  $\delta^{13}$ C values of wild and captive specimens were -24.6‰ and -23.7‰, whereas the mean  $\delta^{15}$ N values of wild and captive specimens were 5.9‰ and 8.9‰, respectively. Mean isotope values of skin samples from captive specimens were significantly enriched in <sup>13</sup>C (t-test; t = 3.92, d.f. = 10.23, p-value = 0.003) and <sup>15</sup>N (t-test; t = 10.45, d.f. = 15.87, p-value < 0.001) as compared to specimens from the wild. Means of specimens in the semi-captive category tended to be near the means of the captive group ( $\delta^{13}$ C: -23.8‰;  $\delta^{15}$ N: 8.2‰). The standard deviation in both tested isotopic systems was lowest in the captive specimens ( $\delta^{13}$ C: 0.17‰;  $\delta^{15}$ N: 0.57‰). Standard deviation of the wild group was 0.72‰ for  $\delta^{13}$ C and 0.66‰ for  $\delta^{15}$ N. The semi-captive group showed the largest standard deviation (sd  $\delta^{13}$ C: 1.62‰; sd  $\delta^{15}$ N: 1.19‰), which could be primarily attributed to sample SC12 from this group which differed from other samples of that group by more than 2.2‰ in both isotope ratios.

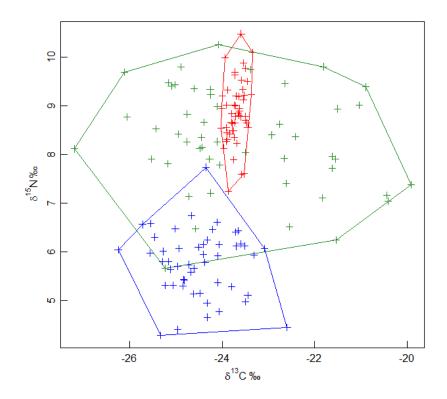


Figure 10. Bivariate plot of simulated data (n = 300) with convex hull for each classifier (red - captive origin; green – semi-captive origin; blue – wild origin).

Accuracy for the weighted *k*-NN rule differed depending on the source of origin (Table 8) and was highest among the captive group, with almost 98% correct assignments. The percentage of correct assignments was lower in the semi-captive (89%) and wild (91%) specimens. However, differentiation between the captive and wild group was 100% since no samples from the captive group were assigned to the wild population and vice versa. Six samples (17%) from the wild group were assigned to the semi-captives, while the test statistics assigned three semi-captives (9%) to the wild population.

Table 8: Results of weighted k-NN validation of test data (n = 100; k = 4). Accuracy = proportion of correctly assigned samples divided by the number of total samples. Origin as classifiers (no. of captive samples = 32; no. of semi-captive samples = 33; no. of wild samples = 35).

	Assigned to			Accuracy
	Captive	Semi-captive	Wild	
Captive	31	1	0	98%
Semi-captive	1	29	3	89%
Wild	0	6	29	91%

## Feeding experiments of Varanus spp.

The control group showed that the carbon source of the general feeding regime was constant over time (standard deviation < 0.39‰ for  $\delta^{13}$ C). The standard variation of the nitrogen source, however, varied between 0.47‰ and 2.3‰ for  $\delta^{15}$ N. Specimen R193 in the control group showed a maximum  $\delta^{15}$ N value of 15.2‰, which was around 50% higher than the combined mean in the control group (Fig. 11). Although no detailed information was available about the previous feeding regime, such  $\delta^{15}$ N variation in zoo specimens is possibly linked to prey taxa (beetle larvae, crickets) which fed on industrially produced powder and pellets, such as fish and poultry.

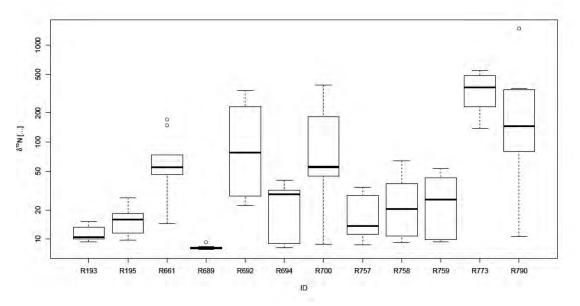
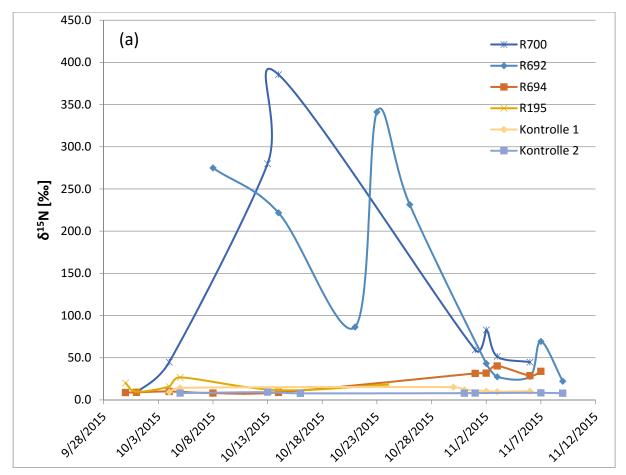
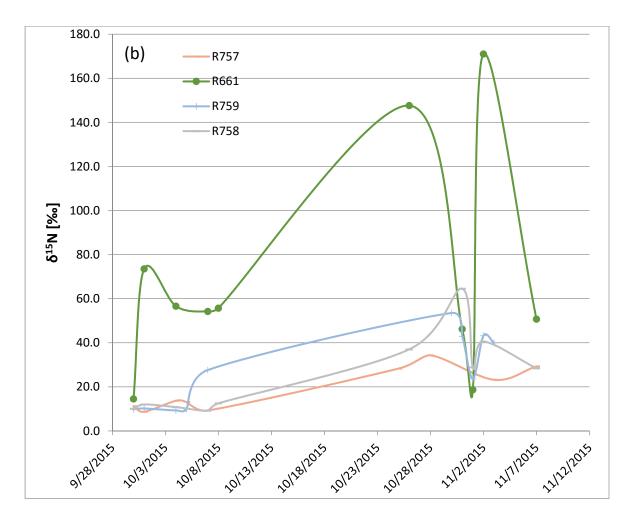


Figure 11: Boxplot of  $\delta^{15}N$  values over time of specimens included in the feeding experiment, including the control group (specimens R193 and R689). Y-axis on logarithmic scale.

In 70% of the specimens taking part in the feeding experiments, the <sup>15</sup>N enriched signal was detected in shed skin fragments within two weeks of feeding with the isotopic marker. A similar pattern was detected in all three species that took part in the feeding experiment (Fig, 12a, b, c). The signal was particularly prominent in juvenile and sub-adult specimens, weighing between 50 g and 90 g, for which shedding usually takes place more frequently. Enriched <sup>15</sup>N signals were particular evident in test specimens R692 and R700 (*V. acanthurus;* Fig. 12a) and R773 and R790 (*V. macrei;* Fig. 12c) with values up to 4000 percent above the combined  $\delta^{15}$ N mean of the control group (Fig.11).



\*Note: Kontrolle = control



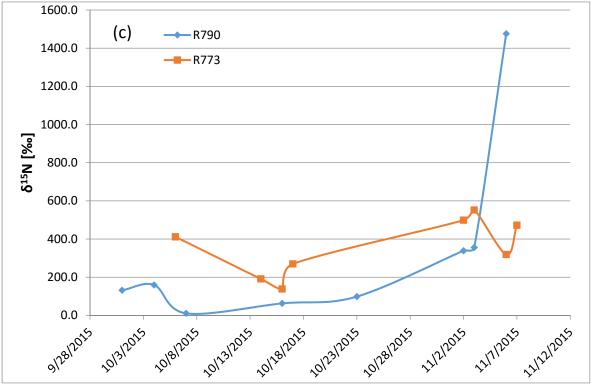


Figure 12a, b, c:  $\delta^{15}$ N values over time of test and control specimens by species (a) *V. acanthurus*, (b) *V. melinus*, (c) *V. macrei*.

Detection of the <sup>15</sup>N marker in the heavier sub-adult specimens of *V. melinus* (around 250 g) was delayed (with the exception of R661) and detected almost three weeks after feeding (Fig. 12b). Samples from juvenile specimens from *V. acanthurus* and *V. macrei* also demonstrated a pattern that  $\delta^{15}$ N values were recurrent. This was somewhat extended in *V. melinus*.

In nourished reptiles, the exogenous nitrogen from prey items, predominantly in the form of proteins, is digested and absorbed into circulation where it forms a relatively labile nitrogen pool. Once in circulation, individual amino acids can be mobilized for tissue growth if needed. Skin samples from one adult specimen of *V. acanthurus* (R195) only showed an increase in  $\delta^{15}$ N, with a maximum value of 26.7‰. It is likely that this specimen only replaced a few epidermal cells, which could be linked to regenerating epidermis rather than cyclical epidermal renewal.

The widest range of skin samples were available for specimen R700—collected over a period of 119 days, from beginning of October 2015 to end of January 2016 (Fig. 13). The analysis suggest that the protein incorporated into scales which were shed in January 2016 came from a nitrogen source pool whose isotopic signature was altered by the <sup>15</sup>N marking with glycine in September 2015. This would translate into a shedding cycle of about three month for this juvenile/sub-adult specimen. The relationship between  $\delta^{15}$ N and days is significant (ANOVA; F-value: 2.504, P = 0.0386), with a polynomial regression as shown in Fig. 13.

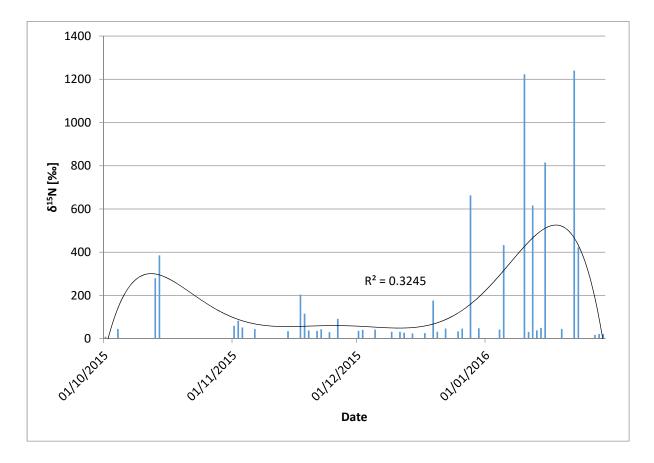


Figure 13. Variation of  $\delta^{15}$ N values over time for specimen R700 (*V. acanthurus*) with polynomial adrenaline.

The ratios of  $\delta^{15}$ N from skin and faeces samples collected on identical dates are shown in Fig. 14. Although the number of samples was limited and therefore no significant statistical relation could be established (ANOVA; *F*-value: 63.3, *P* = 0.0796), it appears that  $\delta^{15}$ N values increased quicker in faeces than in skin tissue. Higher <sup>15</sup>N content in faeces can be expected because diet-tissue equilibrium cannot yet be assumed since the <sup>15</sup>N enriched isotopic marker has only been applied twice at the onset of the feeding experiment.

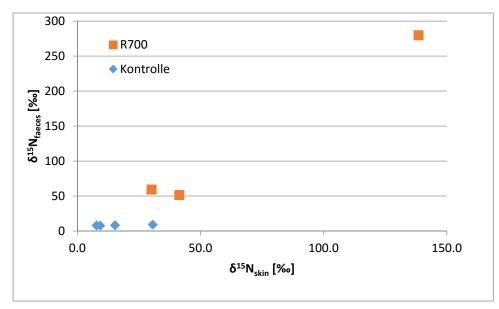


Figure 14:  $\delta^{15}$ N values over time in skin and faeces samples from one test and one control specimen. \**Note: Kontrolle = control.* 

## DISCUSSION AND CONCLUSION

Species with vast distribution ranges, such as *Varanus salvator*, show considerable variation in isotope ratios, and therefore provenance is difficult to establish using isotope analysis. This is further complicated by the fact that different age classes show different isotopic signatures. Thus, building up reference databases for the provenance of wild specimens with vast distribution ranges does not appear to be useful for law enforcement.

Under quasi-controlled feeding regimes of specimens in captivity, isotopic ranges are smaller than those in wild populations. This result strongly supports the potential for developing a reference framework of breeding farms against which specimens of ambiguous origin can be cross-checked. Stable isotope analysis can then be used for geographic assignments to one of the referenced breeding farms within a probability context and exclude unlikely areas of provenance. Such a reference framework would help determine the origin of captive-born and captive-bred species regardless of their range.

Such a reference framework may even work for lepidosauria reptiles originally taken from the wild and that were kept in captivity for several years as has been shown in this pilot study for *S. crocodilurus* where no sample from the captive group was assigned to the wild population and vice versa. An

explanation might be the presence of metabolically inert keratin tissue in the tip of the tail that is not shed during progressive epidermal renewal.

The isotopic <sup>15</sup>N marker (glycine) was still detectable in shed epidermal fragments of monitor lizards more than three months after the application and could be used for determining the origin of the specimens. This technique is particularly useful for specific forensic applications, such as trade chain analysis.

Marking juvenile and sub-adult specimens with enriched <sup>15</sup>N glycine as a chemical imprint of legal captive-bred origin might not be effective due to the rapid epidermal renewal cycle, with the consequence that the markers are detectable in the epidermis within two to three weeks after application already. Therefore, wild-caught individuals could still be easily laundered through breeding farms. However, marking might be more promising for other reptile species which do not shed their epidermis periodically, such as tortoises.

The results of the pilot study have shown that isotopic fingerprinting (based on carbon and nitrogen) is suitable for discriminating reptile specimens that are kept in captivity from wild populations. This is due to the fact that isotopic variances of  $\delta^{13}$ C and  $\delta^{15}$ N are usually smaller in captive specimens which have more homogenous diets than specimens living in wild. However, isotopic differences may be reduced in situations where breeding farms procure food locally from the wild, and captive-breeding occurs in areas within the species' native range. It would be important to take this into consideration for future research. For facilities that are geographically separated from a species native range, water isotopes (hydrogen and oxygen) may also be useful to distinguish origin.

## REFERENCES

Comas, M., Escoriza, D. and Moreno-Rueda, G. 2014. Stable isotope analysis reveals variation in trophic niche depending on altitude in an endemic alpine gecko. *Applied Ecology* 15, 362–369.

Ehleringer, J.R. and Matheson, S.M. 2007. Stable isotopes and courts. Utah Law Review 2, 385-442.

Fry, B. 2006. Stable isotope ecology. Springer.

Hechenbichler K. and Schliep, K.P. 2004. Weighted k-Nearest-Neighbor Techniques and Ordinal Classification. DiscussionPaper399,SFB386,Ludwig-MaximiliansUniversityMunich(http://www.stat.uni-muenchen.de/sfb386/papers/dsp/paper399.ps)

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314-326.

Kays, R. and Feranec, R.S. 2011. Using stable carbon isotopes to distinguish wild from captive wolves. *Northeastern Naturalist* 18(3), 253–264

Kelly, S., Heaton, K. and Hoogewerff, J. 2005. Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends in Food Science & Technology* 16, 555–567.

Lyons, J. and Natusch, D. 2015. *Methodologies for differentiating between wild and captive-bred CITES-listed snakes*. IUCN-SSC Boa and Python Specialist Group. Presented at the 28<sup>th</sup> meeting of the CITES Animals Committee, 30 August–3 September 2015

Moncada, F.G., Hawkes, L.A., Fish, M.R., Godley, B.J., Manolis, S.C., Medina, Y., Nodarse, G. and Webb, G.J.W. 2012. Patterns of dispersal of hawksbill turtles from the Cuban shelf inform scale of conservation and management. *Biological Conservation*, 148, 191–199.

Outhwaite, W., Chng, S. C. L, and Kecse-Nagy, K. in prep. Overview of conservation status and trade issues of selected South-East Asian reptile and stingray species used by the skin industry – Focus on Indonesia and Thailand. TRAFFIC, UK, Cambridge.

Tieszen, L.L. and Boutton, T.W. 1988. Stable carbon isotopes in terrestrial ecosystems research, in: Rundel, P.W., Ehleringer, J.E., Nagy, K.A. (Eds.), Stable Isotopes in Ecological Research. Springer, Heidelberg, pp 167–195.

Urey, H.C. 1947. The Thermodynamic Properties of Isotopic Substances. J. Chem. Soc., 562-581.

van Schingen, M., Pham, C.T., Thi, H.A., Bernardes, M., Hecht, V.L., Nguyen, T.Q., Bonkowski, M. and Ziegler, T. 2014. Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures. *Revue Suisse de Zoologie* 121(3), 1–15.

Voigt, C.C., Popa-Lisseanu, A.G., Niermann, I. and Kramer-Schadt, S. 2012. The catchment area of wind farms for European bats: A plea for international regulations. *Biological Conservation* 153, 80–86.

Ziegler, T., Rauhaus, A., Tran, D.T., Pham, C.T., van Schingen, M., Dang, P.H., Le, M.D. and Nguyen, T.Q. 2015. Die Amphibien- und Reptilienfauna der Me Linh Biodiversitätsstation in Nordvietnam. *Sauria* 37(4), 11–44.

Ziegler, T. and Nguyen. T.Q. 2015. Neues von den Forschungs- und Naturschutzprojekten in Vietnam und Laos. Zeitschrift des Kölner Zoos 58(2), 79–108.

## APPENDICES

**Appendix I**: Results of the stable isotopes analysis of 10 skin/scale samples from *Varanus salvator* from Indonesia. Isotope ratios are expressed as mean and standard deviation (sd). Stable isotope ratios (R) are expressed in  $\delta$  units in the conventional permil notation where  $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ .

Region	Locality	Age class	Sampling year	ID	δ <sup>13</sup> C	sd	$\delta^{15}N$	sd	δ <sup>34</sup> S	sd
Sumatra	Padang	juvenile	1892	1455	-19.0	0.3	10.7	0.4		
Java	-	juvenile	1831	10142	-19.6	0.3	10.3	0.1		
Sumatra	Padang	juvenile	1892	1465a	-17.1	0.2	7.6	0.1		
Sumatra	Padang	subadult	1892	1465b	-23.5	0.4	8.1	0.2		
Sumatra	Padang		1892	1465c	-24.4	0.4	7.4	0.2	7.6	0.6
Java	-	juvenile	1860	1457a	-19.6	0.2	13.2	0.1	7.6	0.4
Java		juvenile	1860	1457b	-16.6	0.1	12.5	0.1	-0.6	0.5
Java		subadult (?)	1860	1457c	-17.3	0.1	11.6	0.1	7.2	0.3
Sumbawa		adult	1873	4463 , distal	-21.0	0.4	10.4	0.3	8.9	0.6
Sumbawa		adult	1873	4463 , prox.	-21.7	0.1	9.8	0.1	8.2	0.3

**Appendix II**: Isotopic ratios of nitrogen and carbon in wild, semicaptive and captive *Shinisaurus crocodilurus* from Vietnam. Isotope ratios are expressed as mean and standard deviation (sd). Stable isotope ratios (R) are expressed in  $\delta$  units in the conventional permil notation where  $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ .

Sample	Class	Origin	δ <sup>13</sup> C	sd	$\delta^{15}N$	sd
SC1	Wild	Tay Yen Tu	-25.6	0.1	6.3	0.1
SC2	Wild	Tay Yen Tu	-24.7	0.1	6.3	0.1
SC3	Wild	Tay Yen Tu	-24.9	0.1	6.3	0.1
SC4	Wild	Tay Yen Tu	-24.6	0.1	4.5	0.1
SC5	Wild	Yen Tu	-23.5	0.1	6.6	0.1
SC6	Wild	Tay Yen Tu	-24.0	0.1	6.2	0.2
SC7	Wild	Yen Tu	-23.8	0.1	6.2	0.2
SC8	Wild	Yen Tu	-24.3	0.2	5.5	0.1
SC9	Wild	Tay Yen Tu	-25.6	0.1	5.3	0.1
SC10	Wild	Dong Son-Ky Thuong	-25.1	0.1	5.3	0.1
SC11	Semicaptive	Me Linh Station	-24.6	0.1	7.4	0.2
SC12	Semicaptive	Me Linh Station	-21.9	0.1	9.6	0.2
SC13	Captive	Me Linh Station	-23.8	0.3	9.3	0.2
SC14	Captive	Me Linh Station	-23.6	0.1	9.3	0.1
SC15	Captive	Me Linh Station	-23.9	0.2	8.7	0.1
SC16	Captive	Me Linh Station	-23.7	0.1	9.7	0.1
SC17	Captive	Me Linh Station	-23.4	0.1	8.7	0.1
SC18	Captive	Me Linh Station	-23.6	0.3	8.3	0.1
SC19	Captive	Me Linh Station	-23.9	0.4	9.2	0.1
SC20	Captive	Me Linh Station	-23.6	0.1	8.0	0.5
SC21	Semicaptive	Me Linh Station	-24.8	0.1	7.7	0.1

Appendix III: Isotopic ratios of nitrogen and carbon in specimens of Varanus spp. that took part in the feeding experiments at Cologne
Zoo. Isotope ratios are expressed as mean and standard deviation (sd). Stable isotope ratios (R) are expressed in $\delta$ units in the
conventional permil notation where $\delta = [(R_{sample}/R_{standard}) - 1] \ge 1000$ .

Species	Sex	ZIMS ID	Weight	Isotopic ma	arker applied	Sampling date	δ <sup>13</sup> C	sd	$\delta^{15}N$	sd
				C	on					
				22/09/2015	29/09/2015					
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	01/10/2015	-22.6	0.1	8.9	0.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	04/10/2015	-22.7	0.1	44.9	9.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	13/10/2015	-22.9	0.2	279.9	28.9
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	14/10/2015	-23.2	0.2	385.5	13.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	01/11/2015	-23.2	0.1	59.3	1.8
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	02/11/2015	-23.0	0.1	83.0	48.6
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	03/11/2015	-22.5	0.3	51.5	1.9
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	06/11/2015	-22.4	0.1	44.7	0.1
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	14/11/2015	-22.6	0.1	34.8	1.3
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	17/11/2015	-23.0	0.2	203.8	244.3
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	18/11/2015	-22.8	0.2	115.8	118.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	19/11/2015	-22.7	0.2	37.9	0.0
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	21/11/2015	-22.9	0.1	36.0	4.6
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	22/11/2015	-22.9	0.1	43.5	0.0
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	24/11/2015	-22.7	0.1	30.2	1.6
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	26/11/2015	-22.6	0.1	91.5	12.6
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	01/12/2015	-22.8	0.2	35.8	1.6
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	02/12/2015	-22.7	0.2	41.0	11.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	05/12/2015	-22.7	0.1	41.8	10.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	09/12/2015	-22.7	0.1	31.8	1.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	11/12/2015	-22.9	0.1	31.5	0.5
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 μl	12/12/2015	-22.8	0.1	27.0	1.8
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 μl	14/12/2015	-22.8	0.1	24.2	3.6

V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	17/12/2015	-22.2	0.2	25.8	2.9
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	19/12/2015	-22.8	0.2	176.5	180.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	20/12/2015	-22.6	0.2	31.9	0.7
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	22/12/2015	-22.7	0.1	46.9	19.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	25/12/2015	-22.7	0.1	33.5	3.0
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	26/12/2015	-22.8	0.1	46.3	5.5
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	28/12/2015	-23.2	0.3	662.8	789.0
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	30/12/2015	-22.9	0.4	48.3	20.1
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	04/01/2016	-22.6	0.1	42.3	8.7
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	05/01/2016	-23.2	0.1	432.7	541.5
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	10/01/2016	-23.4	0.1	1223.0	18.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	11/01/2016	-22.7	0.7	31.4	1.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 μl	2 x 50 µl	12/01/2016	-23.2	0.7	616.1	818.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	13/01/2016	-22.6	0.1	38.4	3.4
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	14/01/2016	-22.9	0.1	49.6	0.1
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	15/01/2016	-23.0	0.3	815.2	1083.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	19/01/2016	-22.8	0.1	45.2	0.8
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	22/01/2016	-23.4	0.1	1240.1	0.2
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	23/01/2016	-22.8	0.8	422.1	552.8
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	27/01/2016	-23.3	0.1	17.2	1.8
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	28/01/2016	-22.4	0.0	22.0	0.3
V. acanthurus	1,0	R700	ca. 80 g	2 x 50 µl	2 x 50 µl	29/01/2016	-22.4	0.1	22.1	0.5
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	08/10/2015	-22.7	0.1	274.9	99.9
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	14/10/2015	-22.8	0.1	221.7	29.3
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	21/10/2015	-22.3	0.2	86.4	28.6
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	23/10/2015	-23.0	0.2	341.2	158.3
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	26/10/2015	-22.8	0.2	231.5	6.1
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	02/11/2015	-22.9	0.1	43.2	0.6
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	03/11/2015	-22.9	0.1	27.4	0.7
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 μl	2 x 50 µl	06/11/2015	-23.0	0.1	27.9	1.0

V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 μl	07/11/2015	-23.0	0.1	69.3	43.5
V. acanthurus	0,1	R692	ca. 80 g	2 x 50 µl	2 x 50 µl	09/11/2015	-22.4	0.1	22.2	0.1
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	30/09/2015	-22.7	0.1	8.7	0.1
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	01/10/2015	-22.9	0.1	9.0	0.1
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	04/10/2015	-22.7	0.1	10.0	1.3
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	08/10/2015	-22.8	0.1	8.1	0.1
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	14/10/2015	-22.8	0.1	8.9	1.3
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	01/11/2015	-23.1	0.3	31.4	5.5
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	02/11/2015	-23.1	0.2	31.7	7.1
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	03/11/2015	-22.7	0.2	40.2	11.7
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	06/11/2015	-22.8	0.1	28.8	5.0
V. acanthurus	0,1	R694	ca. 80 g	2 x 50 µl	2 x 50 µl	07/11/2015	-23.1	0.1	33.9	0.1
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	01/10/2015	-22.6	0.1	131.6	28.7
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	04/10/2015	-22.3	0.1	159.3	63.3
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	07/10/2015	-22.6	0.1	10.6	0.1
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	16/10/2015	-22.6	0.1	62.6	0.1
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	23/10/2015	-22.8	0.1	98.4	8.5
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	02/11/2015	-21.6	0.2	338.8	90.7
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	03/11/2015	-21.8	0.1	356.1	0.1
V. acanthurus	0,0,1	R790	50-90 g	2 x 50 µl	2 x 50 µl	06/11/2015	-21.9	0.1	1476.1	0.1
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	30/09/2015	-23.4	0.1	20.0	8.3
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	01/10/2015	-23.5	0.2	9.8	0.3
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	04/10/2015	-23.5	0.4	15.9	5.5
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	05/10/2015	-23.3	0.2	26.7	2.0
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	14/10/2015	-23.9	0.2	11.6	1.1
V. acanthurus	1,0	R195	ca. 180 g	3 x 50 µl	3 x 50 µl	24/10/2015	-23.0	0.1	18.2	0.1
V. macraei	0,0,1	R773	50-90 g	1 x 50 µl	2 x 50 µl	06/10/2015	-22.7	0.1	411.8	40.9
V. macraei	0,0,1	R773	50-90 g	1 x 50 µl	2 x 50 μl	14/10/2015	-22.8	0.1	191.0	54.4
V. macraei	0,0,1	R773	50-90 g	1 x 50 µl	2 x 50 μl	16/10/2015	-22.7	0.2	138.4	112.5
V. macraei	0,0,1	R773	50-90 g	1 x 50 µl	2 x 50 μl	17/10/2015	-22.8	0.1	269.5	0.1

V. macraei	0,0,1	R773	50-90 g	1 x 50 μl	2 x 50 μl	02/11/2015	-22.0	0.1	499.0	4.2
V. macraei	0,0,1	R773	50-90 g	1 x 50 μl	2 x 50 μl	03/11/2015	-22.1	0.1	552.0	0.1
V. macraei	0,0,1	R773	50-90 g	1 x 50 μl	2 x 50 μl	06/11/2015	-22.2	0.1	319.2	0.1
V. macraei	0,0,1	R773	50-90 g	1 x 50 μl	2 x 50 μl	07/11/2015	-22.3	0.1	472.1	0.1
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	30/09/2015	-23.5	0.1	14.5	5.1
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	01/10/2015	-23.5	0.1	73.5	6.6
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	04/10/2015	-23.7	0.1	56.6	16.9
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	05/10/2015	-23.7	0.1	54.2	0.1
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	07/10/2015	-23.4	0.1	55.7	4.4
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	02/11/2015	-23.7	0.1	147.6	13.7
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	03/11/2015	-23.7	0.1	46.2	25.0
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 μl	06/11/2015	-23.7	0.1	18.7	2.0
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 µl	07/11/2015	-23.7	0.1	171.0	0.1
V. melinus	0,0,1	R661	ca. 250 g	2 x 50 μl	2 x 50 µl	09/11/2015	-23.6	0.1	50.7	0.1
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	30/09/2015	-23.6	0.1	9.8	0.2
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	01/10/2015	-23.5	0.1	10.2	0.7
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	04/10/2015	-23.5	0.1	9.3	0.1
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	05/10/2015	-23.6	0.1	9.9	0.9
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	07/10/2015	-23.6	0.1	27.6	26.6
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	30/10/2015	-23.5	0.1	53.5	12.3
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 μl	2 x 50 µl	31/10/2015	-23.4	0.1	42.9	0.1
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 µl	2 x 50 µl	01/11/2015	-23.5	0.1	23.6	0.1
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 µl	2 x 50 µl	02/11/2015	-23.7	0.1	43.2	0.1
V. melinus	0,0,1	R759	ca. 250 g	2 x 50 µl	2 x 50 µl	03/11/2015	-23.6	0.1	39.9	0.1
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	30/09/2015	-24.1	0.4	11.2	2.9
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	01/10/2015	-24.0	0.1	8.7	0.3
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	04/10/2015	-23.8	0.2	13.7	1.6
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	05/10/2015	-23.8	0.1	13.1	0.1
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	07/10/2015	-23.6	0.1	9.3	0.1
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 µl	2 x 50 µl	25/10/2015	-24.0	0.1	28.3	0.1

V. melinus	0,0,1	R757	ca. 250 g	2 x 50 μl	2 x 50 μl	28/10/2015	-23.9	0.1	34.3	0.1
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 μl	2 x 50 µl	03/11/2015	-23.8	0.3	23.2	11.0
V. melinus	0,0,1	R757	ca. 250 g	2 x 50 μl	2 x 50 µl	07/11/2015	-23.7	0.2	29.3	16.2
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 μl	30/09/2015	-24.0	0.1	10.1	0.1
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 μl	01/10/2015	-23.8	0.1	12.0	1.1
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 μl	04/10/2015	-24.0	0.2	10.8	2.6
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	07/10/2015	-24.1	0.6	9.2	1.1
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	08/10/2015	-23.7	0.2	12.5	5.1
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	26/10/2015	-23.9	0.1	37.0	4.3
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	31/10/2015	-23.7	0.1	64.5	4.4
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	01/11/2015	-24.1	0.2	28.8	0.2
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	02/11/2015	-24.0	0.1	40.5	5.6
V. melinus	0,0,1	R758	ca. 250 g	2 x 50 μl	2 x 50 µl	07/11/2015	-23.8	0.1	28.4	0.9
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	alte Probe	-23.8	0.1	9.4	0.2
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	alte Probe	-23.3	0.1	11.1	0.3
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	alte Probe	-23.3	0.1	11.1	0.1
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	04/10/2015	-23.4	0.1	9.4	0.6
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	05/10/2015	-23.6	0.1	14.3	0.5
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	30/10/2015	-23.6	0.1	15.2	0.1
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	31/10/2015	-23.4	0.1	12.2	1.7
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	02/11/2015	-23.4	0.1	10.5	1.3
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	03/11/2015	-23.0	0.1	9.7	0.1
V. acanthurus	0,1	R193	ca. 130 g	Control	Control	06/11/2015	-22.9	0.1	10.4	0.2
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	05/10/2015	-22.6	0.1	8.1	0.1
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	13/10/2015	-22.8	0.1	9.2	0.2
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	16/10/2015	-22.6	0.1	7.8	0.1
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	31/10/2015	-22.5	0.1	8.0	0.4
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	01/11/2015	-22.9	0.3	8.1	0.3
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	07/11/2015	-22.4	0.1	8.4	0.1
V. acanthurus	1,0	R689	ca. 80 g	Control	Control	09/11/2015	-21.7	0.1	7.9	0.4

TRAFFIC, the wildlife trade monitoring network, is the leading non-governmental organization working globally on trade in wild animals and plants in the context of both biodiversity conservation and sustainable development.

For further information contact: (Part 1) Vicki Crook: vicki.crook@traffic.org (Part 2) Stefan Ziegler: stefan.ziegler@wwf.de

Website: www.traffic.org

UK Registered Charity No. 1076722, Registered Limited Company No. 3785518

