ARTICLES

Biawak, 12(2), pp. 73–83

Testing the Applicability of ^{15}N Isotopic Marker in Skin Tissue to Distinguish Between Captive and Wild Monitor Lizards

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Abstract - In an effort to meet the demand for the trade and to reduce pressure on wild populations of monitor lizards (*Varanus* spp.), several countries have encouraged the establishment of captive breeding facilities. The fear has been expressed, however, that these facilities are not able to produce the quantity of specimens they claim and therefore supplement their supply with wild specimens. Thus, reliable methods are required for verifying the declaration of origin. Stable isotope analyses have been discussed as a potential method to discriminate wild from captive specimens. We herein designed a controlled feeding study and marked ten specimens of three *Varanus* species (*V. acanthurus, V. macraei,* and *V. melinus*) at the Cologne Zoo in Germany with ^{15}N enriched glycine in order to examine the time lag for the isotopic signal to appear in renewed epidermis. We found that the ^{15}N enriched marker was detected within two to five weeks after exposition and conclude that ^{15}N isotopic signature in keratin skin tissue is likely to change quickly after the introduction of novel diets. Thus, distinguishing captive-bred from wild origin based on $\delta^{15}N$ values in shed skin material might not be effective due to permanent, rapid epidermal renewal and therefore of limited forensic relevance. Thus, the study of isotopic signals from tissue samples including several layers and tissues could be an alternative approach. Furthermore, diet shift studies relating to isotopic incorporation rates as well as reconsidering the examination of site-specific markers are recommended. Additional methods need to be tested for suitability to identify the origin of monitor lizards being declared as captive-bred, which can prevent the laundering of wild-caught individuals through breeding farms.

Introduction

Monitor lizards 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; Bennett *et al*., 2010). Concerns over high levels of exploitation and trade in an increasing number of *Varanus* species

have been reported throughout the last decade (Pernetta, 2009; Koch *et al*., 2013; Crook & Musing, 2016). Owing to concerns that international trade may have a detrimental impact on species survival, the entire genus *Varanus* was listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1975, apart from *V. bengalensis, V. flavescens, V. griseus, V. komodoensis* and *V. nebulosus* which were included in Appendix I. Between 1990 and 2014, nearly 55 million specimens of *Varanus* species were reportedly legally traded internationally of which approximately 20 million specimens were exported and approximately 35 million specimens were re-exported (Crook & Musing, 2016). The principal commodities exported during this period were skins (~90%) and live specimens (6%). For some of the commercially used species CITES export quotas are in place in some countries of origin in order to limit the international trade to a sustainable level. However, in some cases concerns have been expressed that current quotas may not reflect the actual conservation status of respective species or do not sufficiently consider recent taxonomic changes and potential unrecognized lineages (Koch *et al*., 2010; Setyawatiningsih *et al.*, 2015; Shaney *et al*., 2017; Welton *et al*., 2014). Several species which are internationally sold for especially high prices in the pet trade are known to have extremely limited distribution ranges, assuming that even small offtake might be detrimental to wild populations (*e.g.*, Bennett, 2015; Koch *et al.*, 2013).

In an effort to still meet the international demand for leather products and pets by reducing the pressure on wild populations, several countries have encouraged the establishment of captive breeding facilities. Between 2003 and 2012, Indonesia reported exports of over 40,000 captive-hatched (F) and captive-bred (C) *Varanus* specimens (Crook & Musing, 2016). However, severe reservations have been expressed about the conservation impact of these facilities, due to reports that many animals traded as captive-bred have in fact been sourced illegally from the wild (CITES, 2013; Outhwaite *et al.*, 2015; Bennett, 2015). Evidence of breeding farms used to illegally launder green tree pythons (*Morelia viridis*) has been confirmed (Lyons & Natusch, 2011), and investigations of the captive-breeding facilities of tokay geckos (*Gekko gecko*) have raised doubts to the logistical and financial ability of these facilities to produce the quantity of specimens they claim to harvest (Nijman & Shepherd, 2009, 2015).

Fraudulent claims of captive-breeding or ranching undermine trade regulations in place to protect species. Parties to CITES have recognized the importance of developing court-proof mechanisms to help combat this phenomenon, which include tools to help law enforcement authorities to accurately identify cases of fraudulent source declarations (CITES, 2013; TRAFFIC, 2013). Furthermore, a new CITES resolution (Res. Conf. 17.7) to review trade in animal specimens reported as produced in captivity for species-country-combinations of concern has been recently established (CITES, 2017).

Forensic analytical methods can play an important role in conserving and managing wild populations as well as in the investigation of wildlife crime through identification and profiling of tissue samples (Voigt *et al.*, 2012). Stable isotope analysis has been proposed as a potential tool to differentiate between wild and captive-sourced animals (Natusch *et al*., 2017). The quantitative measurement of stable isotope ratios in metabolically inert tissue samples has potential for accurately determining the origin of respective organisms due to the fact that the isotopic composition of certain elements, such as carbon and nitrogen, of a consumer reflects its diet and contains information on its respective local food web and its ecosystem (Ehleringer & Matheson, 2007). Wild specimens generally feed on a variety of available prey organisms, which in turn may have consumed numerous different taxa. These diverse isotopic sources, together, can indicate the presence of a specific complex food web or certain geographic region (Fry, 2006). By contrast, captive animals are usually kept under a controlled feeding regime of a few selected food sources, which generally have been in contact with less variable isotopic sources (Peterson & Fry, 1987; Ewersen *et al*., 2018).

Isotopic analyses of carbon and nitrogen have already successfully been applied to distinguish wild caught from captive-bred crocodile lizards (*Shinisaurus crocodilurus*) (van Schingen *et al*., 2016), farmed and wild salmon (Dempson & Power, 2004), Australian prawns (Carter *et al*., 2015) and bream (Rojas *et al*., 2007). Isotopic analyses have also been used to distinguish captive and wild mammals such as wolves (Kays & Feranec, 2011), and farm-bred vs. wild mink (Hammershøj *et al*., 2007). Dittrich *et al.* (2017) indicated isotopic composition to be a useful tool to distinguish between intensively farmed and naturally grown frogs. They assumed that the little isotopic variation in frog legs from the same supplier indicates specimens to have been farmed, while stronger isotopic variability and higher $\delta^{15}N$ composition might be an indication that frogs have been caught in the wild.

Here, we present the results of a controlled feeding study in three different small- to medium-sized *Varanus* species kept at the Cologne Zoo: the arid-adapted ridge-tailed monitor (*V. acanthurus*) which reaches about 70 cm in total length (TL) and is distributed over the tropical and subtropical portions of Western Australia, the Northern Territory, northwest Queensland, and associated offshore islands (Dryden, 2004); the blue-speckled tree monitor (*V. macraei*), an arboreal species with a prehensile tail that can grow up to 110 cm TL, from Batanta, a small island close to the northwest coast of the Bird's head Peninsula, Irian Jaya, New Guinea (Böhme & Jacobs, 2004; Ziegler *et al*., 2010); and the quince monitor (*V. melinus*), a tropical lowland forest dweller reaching up to 128 cm TL, from the Sula Archipelago, Moluccas, Indonesia (Ziegler & Böhme, 2004; Ziegler *et al*., 2010). The study was designed in order to examine the time lag for isotopic signals to appear in cyclically renewed *Varanus* scales. This information should help to assess the loophole through which wild-caught individuals could potentially be laundered in breeding farms.

Methods

Feeding study

Ten specimens of three monitor lizard species (*Varanus acanthurus,* n = 4; *V. macraei,* n = 2; *V. melinus,* n = 4), weighing between 50 and 250 g, participated in a controlled feeding study. The animals were kept separately in different enclosures in the Terrarium Section of the Cologne Zoo (see Figs. 1-3 and Table 1 for details on the specimens). In June and August 2015, samples of shed skin fragments were collected from all specimens (Fig. 4). The specimens were fed twice with 1-3 dead new born mice, depending on the target specimen's weight, consecutively on 22 and 29 September 2015. The dead mice had each been injected with 50 µl of liquid ^{15}N-enriched isotopic marker (glycine). After the specimens were fed the injected mice, a normal feeding regime was resumed: all monitor lizards were fed two times a week with invertebrates (*V. acanthurus* mainly with crickets, *V. melinus* and *V. macraei* with crickets, locusts and occasionally earthworms and *Zophobas* larvae). At maximum once a week, all species were provided with vertebrates of adequate sizes (*V. acanthurus* mainly newborn mice, *V. macraei* 1-3 week-old mice, *V. melinus* young to adult mice or small rats, and occasionally chicks or fish). In addition to the test group (n = 10), two specimens of *V. acanthurus* composed the control group (Table 1). After the isotopic marker was applied, the enclosures of all individuals were inspected and screened for shed skin fragments on a daily basis. To facilitate the collection of skin samples, the specimens were kept on relatively uniform ground substrate during the sampling period, such as sand for *V. acanthurus*, and pine bark or a mixture of coconut fibre and plant soil for *V. macraei* and *V. melinus*, respectively. Other furnishings that provided hiding and climbing opportunities and water bowls remained inside the terraria. The skin fragments (Fig. 5) were removed and stored in labelled polyethylene bags until analysis. If detected, pieces of loose shed skin which were still attached to the animals were carefully removed with tweezers. Samples were collected between October 2015 and March 2016.

Isotope analysis

All samples were analysed at the accredited (DIN EN ISO/IEC 17025:2005) Agroisolab Facility for

Fig. 1. Adult *Varanus acanthurus* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 2. Adult *V. macraei* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 3. Subadult *V. melinus* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 4. Samples of shed skin fragments were collected from all studied specimens, either directly as in this *V. acanthurus*, or from the ground of their enclosures. Photographed by **Anna Rauhaus**.

Stable Isotope Research in Jülich, Germany. Samples were dried and cut into small aliquots with a scalpel. 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 HorizonR continuous flow isotope ratio mass spectrometer. Results were reported relative to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N2 ($\delta^{15}N$), respectively, and measured isotopic ratios (R) were expressed in δ units in the conventional permil notation, where:

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x\ 1000$$

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 % ($\delta^{15}N$). Mean weekly isotope values (the first week began at 4 October 2015) were calculated in order to avoid the risk of point estimates (Jackson *et al.*, 2011). Variation of the normal feeding regime was controlled by measuring $\delta^{13}C$ of the collected skin fragments and showed no significant differences over time.

Results

It was found that the $\delta^{13}C$ value of all specimens varied slightly during the study period but the standard deviation never exceeded 0.4‰ (Fig. 6, Tables 2–4). The $\delta^{15}N$ value of the two control specimens varied more throughout the study period, with a standard deviation

Fig. 5. Shed skin fragments of *V. acanthurus* (left), *V. melinus* (middle), and *V. macraei* (right). Photographed by **Anna Rauhaus**.

Table 1. List of specimens included in the controlled feeding study. D = Dosage of liquid ^{15}N isotopic marker. *= received in 2007 as adult. Specimens were weighed in September 2015.

ID	Species	Sex	Hatch date	Origin	Weight	D 22.9.	D 29.9.
R193	*Varanus acanthurus*	f	unknown*	Berlin Zoo	ca. 130 g	control	
R689	*Varanus acanthurus*	m	07/2014	Cologne Zoo	ca. 80 g	control	
R700	*Varanus acanthurus*	m	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R692	*Varanus acanthurus*	f	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R694	*Varanus acanthurus*	f	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R195	*Varanus acanthurus*	m	06/2008	Cologne Zoo	ca. 180 g	3 x 50 µl	3 x 50 µl
R790	*Varanus macraei*	f	07/2014	Cologne Zoo	50-90 g	2 x 50 µl	2 x 50 µl
R773	*Varanus macraei*	f	11/2014	Cologne Zoo	50-90 g	1 x 50 µl	2 x 50 µl
R661	*Varanus melinus*	m	06/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R759	*Varanus melinus*	?	05/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R757	*Varanus melinus*	m	08/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R758	*Varanus melinus*	f	08/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl

of 2.17‰, and variation was particularly prominent in R193 (Table 2).

The maximum $\delta^{15}N$ value detected amongst the control specimens (R193, R689) was 15.2‰ (Table 2), therefore a $\delta^{15}N$ value < 20‰ was assumed as "glycine marker not visible" for *Varanus acanthurus* and the two other tested *Varanus* species. In case the $\delta^{15}N$ value exceeded this threshold, we expected the "glycine marker being detected", correspondingly. In seven of the ten specimens taking part in the feeding study, the ^{15}N enriched signal was detected in shed skin fragments within two weeks after the onset of the study (Fig. 7, Tables 2–4). In all individuals the signal was visible at the latest five weeks after the application of the isotope marker. One specimen (R195) did exceed the isotopic marker detection threshold, but only once (Table 2).

The peak was particularly prominent in juvenile and sub-adult specimens, weighing between 50 and 90 g, for which shedding usually takes place more frequently (Arnold, 1986). However, no statistical significance (t-test; t = -2.12, d.f. = 4.07, p = 0.1003) could be detected for the mean weekly $\delta^{15}N$ values of the two weight classes: (i) 80–90g; (ii) >180g. In three specimens (R661, R692, R790), the isotopic signal was detected within the first week after the start of the feeding study (Fig. 7). From three individuals (R661, R700, R758), samples were collected over a period of 25 weeks. Even after this timespan, the signal could still be detected in *V. acanthurus* (R700), whereas two specimens of *V. melinus* (R661, R758) evidently showed shed skin fragments without glycine marker after 14 weeks (Fig. 7). The low values were followed by peak $\delta^{15}N$ values in week 16 in all three specimens (R661, R700, R758). Specimen R661 developed a skin disease sometime between week 16 and 25, which may have altered the moulting cycle to some extent.

Discussion

This study was a pilot project to offer a basis for additional studies to distinguish between captive- and wild-bred monitor lizards. We could show that the carbon source of the general feeding regime was relatively constant over time (standard deviation: < 0.4‰ for $\delta^{13}C$, Fig. 6, Table 2). The standard variation of the nitrogen source in the control specimens, however, varied between 0.4‰ and 2.17‰ for $\delta^{15}N$ (Table 2). Such $\delta^{15}N$ variation in zoo specimens is possibly linked to the trophic position and reflects prey taxa (beetle larvae, crickets), which are fed, amongst others, on industrially produced powder and pellets, such as fish flakes and wheat germ but also on fresh apples and carrots. However, this variation of the ^{15}N source potentially masks isotopic differences between captive and wild diets, which lie in the range of 2‰ to 3‰, as it has been established for the crocodile lizard (*Shinisaurus crocodilurus*) (van Schingen *et al.*, 2016). In *S. crocodilurus* the tail tip was analyzed, which includes several skin layers and tissues and might offer a broader overview over the diet than only the outermost layer as was applied in this study.

In contrast to humans, which have a continuous production of skin cells, squamates produce new skin cyclically (Alibardi, 2000). Thus, we assumed that enriched $\delta^{15}N$ values from the feeding study would

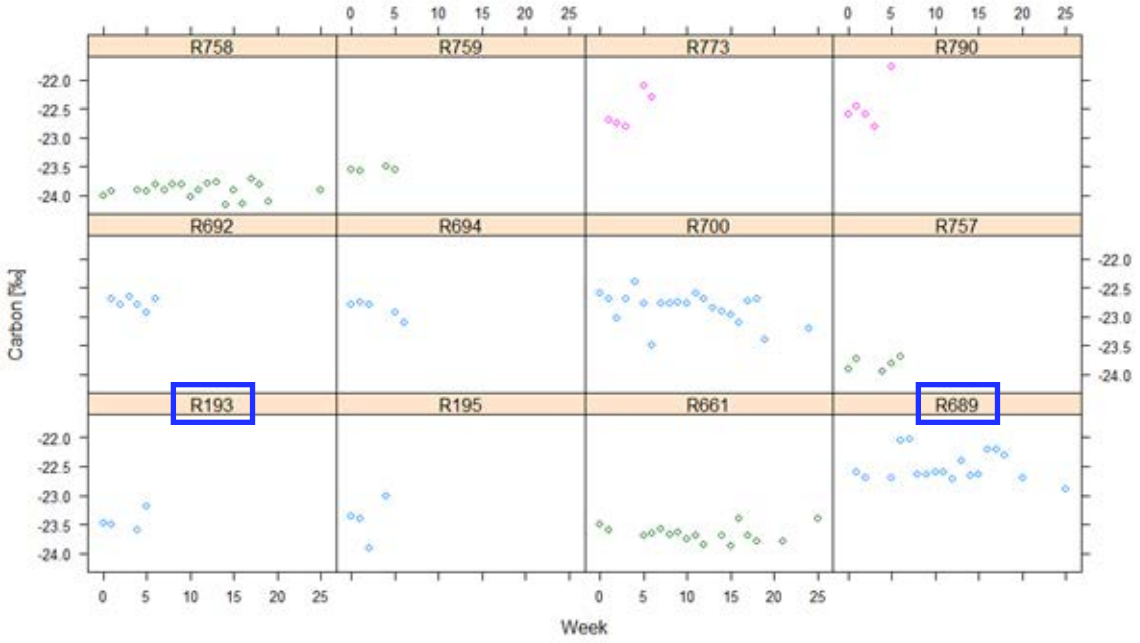

Fig. 6. $\delta^{13}C$ values over time of specimens, including the control group (R193 and R689), separated by species (blue *V. acanthurus*, pink *V. macraei* and green *V. melinus*). The y-axis shows the $\delta^{13}C$ values in permille. The x-axis gives the time in weeks. Each box represents one individual. The IDs of the individuals are shown on the rose x-axis (for details see Table 1). The control specimens (R193 and R689) are blue-rimmed.

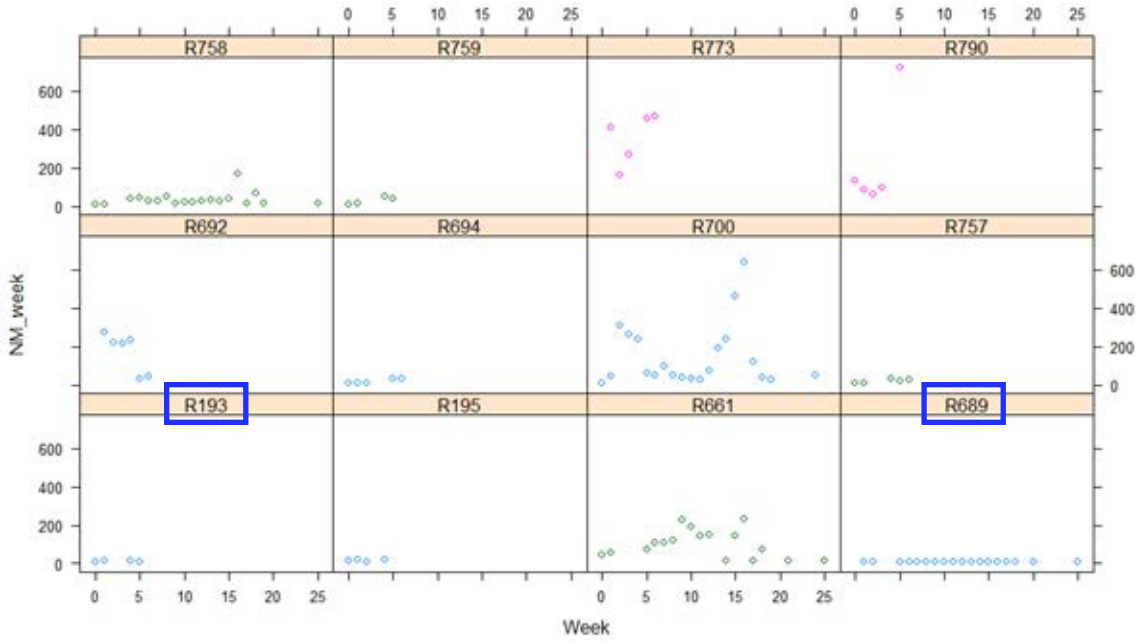

Fig. 7. $\delta^{15}N$ values over time of specimens, including the control group (R193 and R689), separated by species (blue *V. acanthurus*, pink *V. macraei* and green *V. melinus*). The y-axis shows the $\delta^{15}N$ values in permille. The x-axis gives the time in weeks. Each box represents one individual. The IDs of the individuals are shown on the rose x-axis (for details see Table 1). The control specimens (R193 and R689) are blue-rimmed.

Table 2. **δ**^{13}C and **δ**^{15}N isotope values for all *Varanus acanthurus* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015. * = control specimens.

	*R193		R195		*R689		R692		R694		R700	
Week	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
0	-23.47	10.53	-23.37	12.47					-22.8	8.85	-22.6	8.9
1	-23.5	11.85	-23.4	21.3	-22.6	8.1	-22.7	274.9	-22.75	9.05	-22.7	44.9
2			-23.9	11.6	-22.7	8.5	-22.8	221.7	-22.8	8.9	-23.03	309.2
3							-22.65	213.8			-22.7	264.25
4	-23.6	15.2	-23	18.2			-22.8	231.5			-22.4	240.5
5	-23.18	10.7			-22.7	8.05	-22.93	32.83	-22.93	33.03	-22.78	59.63
6					-22.05	8.15	-22.7	45.75	-23.1	33.9	-23.5	51.3
7					-22.03	7.27					-22.78	98.08
8					-22.63	7.23					-22.78	50.3
9					-22.63	7.47					-22.75	38.4
10					-22.6	7.2					-22.77	35.03
11					-22.6	6.95					-22.6	25.67
12					-22.72	7.12					-22.7	72.2
13					-22.4	7.5					-22.85	194.35
14					-22.65	7.45					-22.9	237.5
15					-22.63	7.23					-22.97	462.28
16					-22.2	7.65					-23.1	642.65
17					-22.2	7.35					-22.73	120.85
18					-22.3	7.35					-22.7	38.9
19											-23.4	27
20					-22.7	7.2						
24											-23.2	48.1
25					-22.9	7.4						
Mean	-23.44	12.07	-23.42	15.89	-22.51	7.51	-22.76	170.08	-22.88	18.75	-22.85	146.19
SD	0.18	2.17	0.37	4.64	0.25	0.42	0.1	103.57	0.14	13.44	0.27	164.85

only be detectable until the start of the next moulting process. However, we found that the ^{15}N enriched marker was detectable in all specimens within a few weeks of, or even days after exposition to the isotope marker. This indicates that the *Varanus* species tested appear to shed at least some parts of the skin constantly as it has already been demonstrated for the genital skin of lacertid lizards (in den Bosch, 2001). Most of the specimens which showed traces of the isotopic marker in their skin were still relatively young, implying that they are still shedding their skin more frequently. We also observed that *V. acanthurus* shed rather large skin pieces at a time over a period of a few days, whereas *V. melinus* and especially *V. macraei* mostly shed much smaller epidermal pieces or individual scales without a dedicated onset of a shedding cycle.

Depending on the amount of time skin fragments are laying in the enclosures, decomposition processes are assumed to alter the isotopic composition of the skin, which may contribute to a broader isotopic variation in the samples. Since animals shed their skin in pieces and some old skin parts may remain attached to the animals longer than others, one cannot assure that the chronological order in which single skin pieces were collected correspond to the relative age of the skin per se. Thus, the temporal time axis has to be interpreted with caution. This phenomenon also has forensic relevance: distinguishing captive-bred from wild origin based on δ^{15}N values might not be effective due to the rapid epidermal renewal cycle, with the consequence that new signals are detectable in the epidermis within

Table 3. **δ**^{13}C and **δ**^{15}N isotope values for all *V. macraei* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015.

	R773		R790	
Week	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
0			-22.6	131.6
1	-22.7	411.8	-22.45	84.95
2	-22.75	164.7	-22.6	62.6
3	-22.8	269.5	-22.8	98.4
5	-22.1	456.73	-21.77	723.67
6	-22.3	472.1		
Mean	-22.53	354.97	-22.44	220.24
SD	0.31	133.05	0.4	282.53

Table 4. **δ**^{13}C and **δ**^{15}N isotope values for all *Varanus melinus* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015.

	R661		R757		R758		R759	
Week	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**
0	-23.5	44	-23.9	9	-24	10.07	-23.55	10
1	-23.6	55.5	-23.73	12.03	-23.93	10.83	-23.57	15.6
4			-23.95	31.3	-23.9	37	-23.5	53.5
5	-23.7	70.83	-23.8	23.2	-23.93	44.6	-23.55	37.4
6	-23.65	110.85	-23.7	29.3	-23.8	28.4		
7	-23.57	111.63			-23.9	27.5		
8	-23.67	121.57			-23.8	51.45		
9	-23.63	226.37			-23.8	18.05		
10	-23.75	191.2			-24.03	20.3		
11	-23.7	141.57			-23.9	24.4		
12	-23.85	152.05			-23.78	25.53		
13					-23.77	32.3		
14	-23.7	14.6			-24.15	26.55		
15	-23.87	144.9			-23.9	37.03		
16	-23.4	234.7			-24.13	168.57		
17	-23.7	13.3			-23.7	14		
18	-23.8	75.5			-23.8	71.1		
19					-24.1	17.5		
21	-23.8	15.2						
25	-23.4	16.2			-23.9	17.3		
Mean	-23.66	102.35	-23.82	20.97	-23.91	35.92	-23.54	29.13
SD	0.14	72.93	0.11	10.05	0.13	35.47	0.03	20.09

two to three weeks after exposition to new dietary regimes and food webs. ^{15}N isotopes are incorporated directly from the diet into the tissues of the lizards (Urey, 1947). Therefore, wild-caught individuals could readily be laundered undetected through breeding farms at least if cyclically renewed skin material is investigated by isotope testing.

We also found a δ^{15}N peak in sampled skin fragments from week 16 (Fig. 7), suggesting that shed skin material was formed and deposited immediately after the application of ^{15}N enriched glycine in September 2015. This would translate into a moulting cycle of approximately four months for the *Varanus* specimens tested in this study. Our results confirm that ^{15}N isotopic signature in keratin skin tissue is likely to change quickly after the introduction of novel diets. Thus, breeders could theoretically manipulate isotope ratios within *Varanus* spp. collected from the wild to create signatures indicative of captive provenance, thus masking their "wild-type" isotopic label (Natusch *et al.*, 2017). However, our results also demonstrated that through an intentional addition of "isotopically labelled" materials (*e.g.*, glycine) to the diet of captive specimens, the creation of site-specific (or farm-specific) signatures can be created (Ziegler, 2016). Our results have shown that the ^{15}N pool in *Varanus* sp. only gradually declines over time and can still be detected with confidence after 16 weeks. This result is particularly promising for developing a reference framework of breeding farms against which particularly valuable specimens can be cross-checked along the trade chain.

However, isotopic markers were shown to rapidly produce great variance and fluctuations in isotopic signatures of varanid skin over short time periods. Due to the great variance it can be assumed that it would be impossible to differentiate wild and captive specimens that had recently been fed with isotopic markers. Thus, we assume that isotopic markers as a label for captive

specimens in certain breeding facilities are not suitable to differentiate between captive and wild animals unless the markers are used to track specific specimens. Furthermore, although ^{15}N markers are not very expensive and stable isotopic analysis of $\delta^{13}C$ and $\delta^{15}N$ can be obtained for less than $100 USD, the application of isotopic markers in large breeding farms would be labour-intensive and might pose implementation difficulties.

However, the variance of isotopic signatures has shown to be much smaller in captive specimens than in wild lizards, snakes and frogs (Dittrich *et al.*, 2017; Natusch *et al.*, 2017; van Schingen *et al.*, 2016). This approach, however, requires sufficient and georeferenced samples of both wild and captive specimens in order to form a sound judgement on the specimens' source. Adult and slow-growing ectotherms reportedly have lower incorporation rates than similarly sized adult endotherms (Cloyed *et al.*, 2015). The rate of isotopic incorporation differs among tissues within a species as well as among species with different metabolic demands, body sizes, growth rates and protein turnover (Cloyed *et al.*, 2015). While skin and blood plasma have a relatively fast isotopic incorporation rate and thus provide diet information integrated over short timescales (days or weeks) before collection, other tissues such as bone collagen incorporate isotopes very slowly and thus provide diet information integrated over much longer timescales (years). Warne *et al.* (2010) conducted a diet switch study (from C^3 based to C^4 based diet) in small prairie lizards (*Sceloporus undulatus*) and large collared lizards (*Crotaphytus collaris*) showing that carbon retention times in skin tissue last around 94 and 311 days, respectively. Lattanzio & Miles (2015) showed an isotopic turnover occurring after around 15.5 days in claw tissue of *Urosaurus ornatus* according to a diet shift study. Isotopic incorporation rates were shown to depend on the temperature, age and size of individuals (Warne *et al.*, 2010), namely that isotopic incorporation rates were shown to decline with body mass, while growth was found to substantially contribute to isotopic incorporation. A similar diet shift study for adult *Varanus* specimens of different sizes is suggested in order to get an estimate on the time span needed until such a change is visible in the skin tissue under normal conditions. Furthermore it could be investigated if, and to what extent isotopic signatures of specimens with different origins that are kept together in a group, (*i.e.*, in a zoo collection) differ from each other.

Our results have shown that there are limitations to this technique. In addition to stable isotope work, molecular approaches to track the provenance of certain species of reptiles have been developed recently and have demonstrated interesting insight into the broad-scale geographic structure of genetic diversity (Murray-Dickson *et al.*, 2017). It might be worthwhile to test the combination of both relatedness and habitat signatures. Although the overall accuracy of such a procedure remains to be determined, it is foreseeable that error probabilities can be decreased to levels acceptable for decision makers and law enforcement.

Acknowledgments - This publication partly presents the results of a project which was funded through the German Federal Agency for Nature Conservation (BfN) with grant no. Z 1.2-526 02/2015/R1 from the German Federal Ministry of Environment, Nature Conservation and Nuclear Safety (BMU). We cordially thank Theo Pagel, Cologne, for supporting this research, and Lorraine Heymann, Cologne, for collecting and labelling the skin samples of the specimens at Cologne Zoo`s Terrarium Section. Our thanks go to the team of Agroisolab for conducting the stable isotope analysis. We are grateful to Roland Melisch and Katalin Kecse-Nagy from TRAFFIC, the wildlife trade monitoring network, and Bruno Streit, Goethe University Frankfurt, as well as anonymous reviewers who gave valuable comments on the manuscript.

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Received: 18 May 2018; Accepted: 12 December 2018