Stable isotope measurements as analytical tools for the traceability of crocodile–derived products

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Abstract

Stable isotope measurements as analytical tools for the traceability of crocodile–derived products. In this preliminary study we examined the application of dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) to identify the origin of skins and meat derived from wild and farmed crocodiles. Traceability protocols can benefit from analytical techniques that are able to distinguish farmed or wild organisms. Scutes and muscle samples were obtained from wild and farmed crocodiles *Crocodylus acutus* (n = 14) and *C. moreletii* (n = 9). Isotopic values in scutes differed significantly between wild and farmed organisms, this difference being higher for $\delta^{15}N$ than for $\delta^{13}C$ values. When both isotopic values were integrated using a discriminant analysis, we observed a significant categorization. The isotopic values of muscle samples were very similar to those measured in scutes from the same individuals. In addition, two specimens of *C. acutus* were kept on a constant diet for 97 days to obtain reference isotopic values and tissues were compared. We also estimated the isotopic discrimination factors between tissues and the supplied diet.

Key words: Crocodilians, Natural biomarkers, Skin trade, Wildlife

Resumen

Mediciones de isótopos estables como herramientas analíticas para la trazabilidad de productos derivados de cocodrilos. En el presente estudio preliminar se examinó la aplicación de análisis isotópicos duales ($\delta^{13}C$ y $\delta^{15}N$) para determinar el origen de la piel y la carne de cocodrilos silvestres y criados en granjas. En algunos protocolos de trazabilidad, las técnicas analíticas que permiten distinguir los organismos silvestres de los criados pueden resultar de utilidad. Se recogieron muestras de escamas y músculo de cocodrilos silvestres y mantenidos en cautiverio. Las especies de las que se obtuvieron las muestras fueron *Crocodylus acutus* (n = 14) y *C. moreletii* (n = 9). Los valores isotópicos de las muestras de escamas fueron significativamente diferentes entre los animales silvestres y los criados en granjas, aunque la diferencia fue mayor con respecto a los valores de $\delta^{15}N$ que a los de $\delta^{13}C$. Al integrar ambos valores isotópicos en un análisis discriminante, se observó una significativa categorización de ambos grupos. Los valores isotópicos relativos a las muestras de músculo fueron muy similares a los determinados en escamas provenientes de los mismos individuos. Asimismo, a dos de los ejemplares de *C. acutus* se les proporcionó una dieta constante durante 97 días a fin de obtener valores isotópicos de referencia y poder hacer comparaciones entre diferentes tejidos. También se calcularon los factores de discriminación isotópica entre los tejidos y la dieta suministrada.

Palabras clave: Cocodrilianos, Biomarcadores naturales, Comercio de pieles, Fauna silvestre

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Introduction

It has been estimated that up to 19 % of reptile species are threatened with extinction (Böhm et al., 2013). This figure includes Crocodilian species, in particular those subject to poaching and habitat loss. Given the high market value of reptile skins, illegal captures of wild individuals still represent a generalized problem in several tropical countries (IUCN SSC Crocodile Specialist Group, 2021). In the case of Mexico, the natural populations of American crocodile (*Crocodylus acutus*) and Morelet's crocodile (*C. moreletii*) were severely threatened during the 1970s due to intensive extraction. Populations successfully recovered after the implementation of new legislation that supported long–term protection programs and the re–establishment of authorized crocodile farms. The latter drove important changes in the CITES status of wild Morelet's crocodile, which was positioned at a lower threat status in 2012 (CONABIO, 2021). In Mexico, although crocodile species are still under special protection (NOM–059–SEMARNAT–2010), the trade of these animals and their derived products is legal, although strongly regulated (CITES, 2020). Crocodile farms have an important role in local economies as they foster ecotourism, repopulation programs and habitat conservation through pilot development projects. Commercialization of crocodile–derived products (skin, meat and fat) is currently increasing, and it is estimated that an average of 1,500 skins are sold from Mexico to the international markets every year (CONABIO, 2016). Skins and meat are produced in certified farms that follow both the local knowledge and recently developed ranching protocols (Barrios and Cremieux, 2018). Despite these protocols, however, poaching and illegal commercialization of crocodiles and their products continue in several areas of the country. Traceability methods have been implemented to guarantee the legal provenance of crocodiles and derived products. However, most of these traceability systems are paper–based and prone to falsification and deliberate mislabeling. The fraudulent mislabeling of animal–derived products is a recurrent illegal practice in the sale of unauthorized products.

Several analytical techniques can support and reinforce the traceability process of animal derived products (Van Eeden, 2021). One of the most reliable techniques consists of the analysis of stable isotopes at natural abundance levels. The underlying principle in the use of stable isotopes as biomarkers is that the carbon and nitrogen stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of animal consumers reflect the isotopic values of the assimilated dietary components that contribute to tissue biosynthesis (Ramos and González–Solís, 2012). Under this assertion, stable isotope values can be applied to infer trophic relationships between diets and consumers. As nutrients flow within the organisms, the heavier isotopes of carbon and nitrogen (^{13}C and ^{15}N) tend to bioaccumulate in tissues due to a metabolic discrimination (Martínez del Rio and Wolf, 2005). The dietary nutrients and the metabolic pathways they take elicit distinctive isotopic values among animals and tissues.

Previous studies have employed isotopic measurements to infer the origin of animal–derived products. For example, Zhaxi et al. (2021) applied multi–element stable isotopes analysis to determine the geographical origin of chicken. In a similar approach, Liu et al. (2020) applied isotopic methods to discern the origin of wild, lake–farmed and pond–farmed carp.

Dietary attributes frequently confer specific isotopic values to wild and farmed animals and such values have been used as biomarkers to distinguish the origin of economically important animal species (Gamboa–Delgado et al., 2014; Wang et al., 2018). In the particular case of crocodilians, the nutrition of farmed crocodiles is based on the rather constant food regimes provided in contrast to wild animals, which have a more varied diet, such as crustaceans, insects, fish, birds, and mammals (Andreu and Quiroz, 2003). From an ecological point of view, farmed crocodiles occupy lower trophic positions than their wild counterparts due to the type of food they receive in captivity, frequently poultry and fish offal. As poultry, in turn, is fed on grain–based diets having characteristically less enriched $\delta^{13}C$ and $\delta^{15}N$ values, these isotopic values are reflected in poultry. It is therefore to be expected that the nutrients (and isotopes) composing the poultry tissue will be transferred to the crocodiles. The commercialized products derived from crocodiles are mainly skins and meat, both of which are composed of structural proteins with slow metabolic turnover rates. Their respective isotopic values can therefore reflect dietary characteristics over a long period of time (Rosenblatt and Heithaus, 2013).The aim of the present study was to determine the origin of crocodiles and their products by measuring the $\delta^{13}C$ and $\delta^{15}N$ values in samples of skin (scutes) and muscle obtained from two different crocodilian species, under the hypothesis that the natural $\delta^{13}C$ and $\delta^{15}N$ values in crocodile tissues differ between wild and farmed crocodiles. In addition, from the available data, we estimated discriminant functions in order to evaluate the use of isotopic values as discriminatory variables.

Material and methods

Species and sampling methods

In the present study, fourteen American crocodiles (*Crocodylus acutus*: 3 farmed, 11 wild) and nine Morelet's crocodiles (*C. moreletii*: 6 farmed, 3 wild) were sampled. The target tissues collected from both species were skin (caudal scutes) and muscle since skin and meat are the main commercialized products derived from crocodiles. Scutes were collected from wild crocodiles, from individuals held captive in rural households, and also from juvenile crocodiles raised on farms. In the present study, the latter two groups are referred to as farmed/captive crocodiles. After the animals were immobilized and total length was measured, two dorsal tail scutes were cut and preserved in 70 % ethanol solution. Sub–samples of preserved

Table 1. Mean carbon and nitrogen isotope values (‰) in scutes and muscle sampled from wild and farmed crocodiles (*C. moreletii* and *C. acutus*) (mean values ± SD): [a, b] significant differences in the mean isotopic values determined in wild and farmed crocodiles of the same species (horizontal comparisons).

*Tabla 1. Promedio de valores isotópicos de carbono y nitrógeno (‰) en muestras de escamas y músculo de cocodrilos silvestres y criados (*C. moreletii *y* C. acutus*) (valores promedio ± DE): [a, b] diferencias significativas en los valores isotópicos medios determinados en cocodrilos salvajes y de granja de la misma especie (comparaciones horizontales).*

		Origin/species			
		Wild		Farmed/captive	
Tissue	Mean value	*C. moreletii*	*C. acutus*	*C. moreletii*	*C. acutus*
Scutes	$\delta^{13}C$	–23.05 ± 0.92[a]	–25.56 ± 1.31[a]	–21.10 ± 1.31[b]	–22.21 ± 2.71[b]
	$\delta^{15}N$	13.68 ± 0.92[a]	12.59 ± 1.28[a]	7.80 ± 0.85[b]	8.20 ± 0.63[b]
Muscle	$\delta^{13}C$	–24.66 ± 0.48[a]	n/a	–20.87 ± 1.64[b]	–22.42 ± 0.67
	$\delta^{15}N$	14.45 ± 0.60[a]	n/a	7.48 ± 0.87[b]	7.84 ± 0.72

scutes from wild animals were also acquired from private and institutional herpetological collections. Muscle tissue was sampled from crocodile meat purchased from authorized farms, or retailers who had reliable information on the crocodile's origin (table 1s in supplementary material). In the case of wild animals, only three muscle samples were obtained, all three from preserved tissue of *C. moreletii* crocodiles.

Isotopic differences between crocodile tissues and isotopic discrimination factors

To further compare the isotopic values of scutes and muscle tissue and to determine the isotopic values of liver, dermis and whole blood, we kept two individuals of *C. acutus* (44 and 105 cm) under controlled conditions at a Wildlife Management Unit (SEMARNAT code: DGVS–PIMVS–CR–IN–1043). Crocodiles were donated by the crocodile farm 'La Palma' in San Blas, Nayarit Mexico. Upon arrival, they were fed a constant diet consisting of poultry meat and offal acquired from a supplier. We expected that the dietary isotopic values would eventually be reflected in the different tissues. This feeding period also allowed us to estimate the isotopic discrimination factors (isotopic difference between organism/tissues and diet, $\Delta^{15}N$ and $\Delta^{13}C$). After three months of rearing (97 days), animals were euthanized and dissected to obtain samples of representative organs and tissues (table 3). Dissected organs and tissues were rinsed in distilled water and preserved in 70 % ethanol until pre–treatment for stable isotope analysis. As sample preservation frequently includes solvent immersion (ethanol), tests were conducted to verify the conservation of stable isotope values in treated and untreated dry samples of scute derived from wild and farmed crocodiles.

Pretreatment of samples and stable isotope analysis

Small triplicate subsamples were oven dried (60 ºC, 24 h) and ground using mortar and pestle. As scutes are difficult to grind, dry scutes were frozen and cut into very small pieces before lipid extraction. Lipid extraction was performed following Beaudoin et al. (2001) by suspending the ground material in a 50:50 solution of chloroform–methanol for 12 h. Samples were then oven–dried, homogenized, and kept in desiccators. Tissue samples of 900 to 1,100 µg were packed in tin cups and organized in 96–well microplates. The procedures were carried out at the Escuela Nacional de Ingeniería Pesquera (Universidad Autónoma de Nayarit, México). Samples were analyzed at the Stable Isotope Facility (University of California–Davis) using a PDZ Europa Scientific Roboprep elemental analyzer coupled to a PDZ Europa Hydra 20/20 stable isotope ratio mass spectrometer. Repeated measurements of calibration standards indicated an instrument precision of 0.09 ‰ for $\delta^{15}N$ and 0.12 ‰ for $\delta^{13}C$.

Statistical analysis

After analysis, the isotopic signatures of the scute samples were classified according to their origin and production method. A canonical discriminant analysis (CDA) was applied to determine the classification power of the variables $\delta^{15}N$ and $\delta^{13}C$ to categorize scute samples collected from wild animals and those from farmed/captive animals. The CDA standardizes values after applying discriminant functions and provides an indicator of how well the grouped variables are separated. Wilks' lambda indicated the magnitude at which the dual stable isotope values contributed to discriminating between groups (origin). Discriminant functions were estimated for each species. The isoto-

Table 2. Discriminant function coefficients estimated from the $\delta^{15}N$ and $\delta^{13}C$ values determined in wild and farmed/captive crocodile scutes.

Tabla 2. Coeficientes de las funciones discriminantes estimadas a partir de los valores isotópicos $\delta^{15}N$ y $\delta^{13}C$ determinados en escamas de cocodrilos silvestres y criados en granja/cautivos.

Discriminant functions	*C. moreletii*	*C. acutus*
Wild	$-6.9\times\delta^{15}N - 17.5\times\delta^{13}C - 156.7$	$18.9\times\delta^{15}N - 13.6\times\delta^{13}C - 261.4$
Farmed/captive	$-18.4\times\delta^{15}N - 24.8\times\delta^{13}C - 192.1$	$13.8\times\delta^{15}N - 11.3\times\delta^{13}C - 184.4$

pic values for each element ($\delta^{15}N$ and $\delta^{13}C$) were also compared between wild and farmed/captive animals using Mann–Whitney tests. The isotopic values of newly hatched crocodiles were not included in the statistical analysis as these animals strongly reflected the isotopic values of the maternal nutrients. Moreover, as young animals have higher metabolic rates –which can in turn affect the isotopic values (Vander Zanden et al., 2015)– only juveniles and subadults were used for the statistical comparisons.

Results and discussion

$\delta^{13}C$ and $\delta^{15}N$ values in wild and farmed/captive animals

Table 1 shows mean $\delta^{13}C$ and $\delta^{15}N$ values of scute and muscle tissue from wild and farmed *C. moreletii* and *C. acutus*. Scutes collected from wild crocodiles (both species) showed a mean $\delta^{13}C$ value ± SD of –24.66 ± 1.88‰, while the value observed in farmed animals was –21.48 ± 1.79‰. Although the difference was small, it was statistically significant ($p < 0.011$). A higher difference was observed in scute $\delta^{15}N$ values ($p < 0.001$) between wild (12.87 ± 1.36‰) and farmed animals (8.24 ± 0.62‰). Dual isotopic values measured in scutes were similar in both species from the same type of habitat. The differences of $\delta^{13}C$ and $\delta^{15}N$ values in wild and farmed crocodiles can be attributed to the isotopic influence of their respective diets (Phillips, 2012). Scutes from wild crocodiles had higher $\delta^{15}N$ values than their farmed counterparts, while the opposite was observed for $\delta^{13}C$ values.

Muscle tissue samples from wild crocodiles showed a mean $\delta^{13}C$ value of –24.66 ± 0.48‰, while the respective estimated value observed in farmed animals was –21.32 ± 1.56‰. For $\delta^{15}N$ values, the corresponding values were 14.45 ± 0.60‰ and 7.66 ± 0.69‰. A statistical comparison between muscle tissue from wild and farmed/captive crocodiles was not feasible as only three samples were acquired from wild animals. However, the isotopic similarity between muscle tissue and scutes in the same individuals indicates that a significant difference might also be detected between the isotopic values of muscle derived from wild and farmed/captive crocodiles.

Isotopic values as discriminant variables

After analyzing the dual isotopic values in a discriminant analysis (table 2), we detected a significant difference (Wilks' lambda = 0.131, $p < 0.001$) between the two groups of data representing wild and farmed/captive crocodiles (fig. 1), indicating the significance of the independent variables $\delta^{13}C$ and $\delta^{15}N$, values to the discriminant function. Results from the CDA indicated that the use of both isotopic values effectively separated the groups of wild and farmed/captive crocodiles. When applied separately for each species, $\delta^{15}N$ values were more reliable (Wilks' lambda 0.088, $p < 0.001$ for *C. moreletii*; 0.167, $p < 0.003$ for *C. acutus*) than $\delta^{13}C$ values (Wilks' lambda 0.541, $p < 0.011$ for *C. moreletii*; 0.575, $p < 0.160$ for *C. acutus*) in identifying the origin of crocodiles and their products. $\delta^{15}N$ values were significantly higher in wild animals than in farmed animals. Such differences might be due to diet type given that farmed crocodiles derive most of their nutrients from poultry and fish offal (Barrios and Cremieux, 2018). In contrast, the natural diet of wild crocodiles is highly diverse (Perez–Higareda et al., 1989) and based on organisms belonging to higher trophic niches (Andreu and Quiroz, 2003). Such dietary characteristics were clearly reflected in the available isotopic data. We hypothesize that the isotopic values in tissue from farmed crocodiles differ from those measured in wild crocodiles because the $\delta^{13}C$ and $\delta^{15}N$ values of farmed animals are significantly influenced by the isotopic composition of the main feeding items they receive (poultry and fish offal). For example, poultry is fed on grain–based diets that have specific, rather depleted isotopic values that, in turn, are transferred to poultry. In a recent study, Woodborne et al. (2021) sampled different tissues from farmed *Crocodylus niloticus* fed exclusively on poultry. The isotopic values measured in poultry ($\delta^{13}C = -17.2$ and $\delta^{15}N = 3.0$‰) were similar to those determined in the poultry offal supplied in the present study ($\delta^{13}C = -21.6$ and $\delta^{15}N = 4.1$‰).

Isotopic differences among crocodile tissues and isotopic discrimination factors

After the 97 day feeding period, $\delta^{13}C$ values measured in sampled scutes closely resembled the isotopic

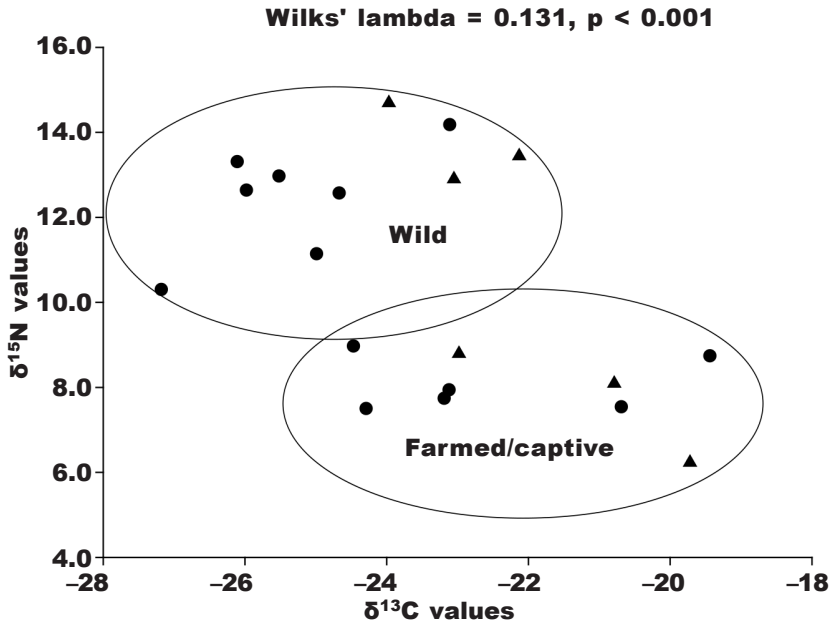

Fig. 1. Carbon and nitrogen stable isotope values of scutes sampled from wild and farmed crocodiles *C. moreletii* (triangles) and *C. acutus* (circles). Wilks' lambda indicates a significant categorization after applying a canonical discriminant analysis to the isotopic values.

Fig. 1. Valores isotópicos de carbono y nitrógeno en escamas obtenidas de cocodrilos C. moreletii *(triángulos) y* C. acutus *(círculos) silvestres y criados. El valor lambda de Wilks indica una categorización significativa después de aplicar un análisis canónico discriminante a los valores isotópicos.*

Table 3. $\delta^{13}C$ and $\delta^{15}N$ values (‰) measured in different tissues of *C. acutus* obtained from the crocodile farm 'La Palma' and kept in captivity for 97 days under a constant diet composed of poultry offal (mean values ± SD). $\Delta^{13}C$ and $\Delta^{15}N$ values (‰) indicate the isotopic differences between diet and tissues (isotopic discrimination factors).

Tabla 3. Valores $\delta^{13}C$ y $\delta^{15}N$ (‰) determinados en diferentes tejidos de C. acutus *obtenidos de la granja de cocodrilos "La Palma" y mantenidos en cautiverio durante 97 días bajo una dieta constante compuesta por despojos de aves de corral (valores medios ± DE). Los valores $\Delta^{13}C$ y $\Delta^{15}N$ (‰) indican la diferencia isotópica entre tejidos y la dieta (factores de discriminación isotópica).*

Tissue	$\delta^{13}C$	$\delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
Scutes	–20.77 ± 0.62	7.49 ± 0.70	1.36	3.40
Muscle	–22.59 ± 0.70	8.37 ± 0.73	–0.34	4.28
Liver	–8.24 ± 0.87	8.42 ± 0.43	3.40	5.07
Blood	–22.12 ± 0.31	8.06 ± 0.55	–0.48	3.97
Dermis	–19.75 ± 1.34	9.24 ± 0.36	1.89	5.15
Intestine	–21.12 ± 0.83	7.16 ± 0.30	0.52	3.07
Heart	–20.79 ± 0.28	7.70 ± 0.74	0.85	3.61
Scat	–18.39 ± 0.81	5.23 ± 0.92	3.25	1.14
Diet (poultry offal)	–21.64 ± 0.17	4.09 ± 0.23	–	–

values of diet (table 3). $\delta^{15}N$ values between diet and scutes had a difference of 3.4‰. However, it is important to consider that in the present study, not all tissues types obtained from the two captive individuals might have reached full isotopic equilibrium with the diet, in particular tissues having slow turnover rates. Tissues with higher metabolic rates have a faster turnover rate, and thus tend to reflect the isotopic values of the assimilated dietary components faster (MacAvoy et al., 2005). Previous studies on crocodilians have reported that the t_{50} or half time (the time it takes for half of the existing tissue to resemble the dietary isotopic signature after a diet shift) for muscle tissue was 43 days for nitrogen and 31 days for carbon (Caut, 2013). In contrast, Rosenblatt and Heithaus (2013) reported t_{50} values of up to 103 days for nitrogen and 147 days for carbon in scutes of American alligator (*Alligator mississippiensis*). In the present study, and based on the available growth and isotopic data, we estimated that the t_{50} values of the constituent carbon and nitrogen in scutes from the two *C. acutus* kept in captivity, were 55 and 49 days, respectively

The immersion of scutes in ethanol (up to six months) had a negligible effect on the isotopic values as compared with the values of dry, untreated scute samples. The isotopic data determined in the different tissues showed that $\delta^{13}C$ values were more isotopically enriched (^{13}C) in liver, while muscle tissue was isotopically depleted. Tissues, ordered by decreasing ^{13}C enrichment, were: liver > dermis > scutes > heart > intestine > blood > muscle. In contrast, the decreasing order of ^{15}N enrichment in tissues was: dermis > liver > muscle > blood > heart > scutes > intestine. The isotopic differences between scutes and muscle obtained from the two captive individuals were small (2‰ for $\delta^{13}C$ and 1‰ for $\delta^{15}N$ values). On the other hand, the isotopic discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) between the supplied diet and the values found in the different tissues were smaller for $\delta^{13}C$ (–0.48 to 3.40‰) and larger for $\delta^{15}N$ (1.14 to 5.15‰). These values are similar to $\Delta^{13}C$ and $\Delta^{15}N$ values reported for scutes and blood in *Alligator mississippiensis* (Rosenblatt and Heithaus, 2013). Woodborne et al. (2021) conducted a study in Nile crocodiles (*C. niloticus*) fed on poultry and the isotopic discrimination factors between scutes (keratin) and diet were narrow ($\Delta^{13}C$ = –0.9 and $\Delta^{15}N$ = +1.4‰) but they are comparable to the values estimated in the present study ($\Delta^{13}C$ = +1.3 and $\Delta^{15}N$ = +3.4‰).

Conclusion

In this preliminary study, the isotopic values of scutes sampled from wild and farmed/captive crocodiles differed significantly. It can be inferred from these findings that crocodile skins having isotopic values in the range of –19.7‰ to –23.3‰ for $\delta^{13}C$ values, and 7.5 to 9.0‰ for $\delta^{15}N$ values, could be products derived from farmed animals. In Mexico, as most crocodile farms grow *C. moreletii,* the above–mentioned values and the obtained discriminant functions can be relevant. However, the number of available samples was limited and additional research is needed to collect and analyze samples from other representative locations. The effect of post–harvest treatments applied to skins (drying, tanning) on the isotopic values also requires further study. From the two captive crocodiles (*C. acutus*), referential isotopic values were obtained from different tissues, which allowed us to estimate the isotopic discrimination factors between diet and types of tissue. Isotopic measurements can be used as viable, natural chemical markers to differentiate the provenance of skins, supporting their potential for future use in traceability protocols designed to regulate the trade of products derived from crocodiles.

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Supplementary material

Table 1s. $\delta^{13}C$ and $\delta^{15}N$ values (‰) measured in scutes and muscle sampled from wild and farmed crocodiles (*C. moreletii* and *C. acutus*) (mean values ± SD): N, sampled individuals; TL, total length; N/A, not available; * isotopic values not included in the statistical analysis.

*Tabla 1s. Valores de $\delta^{13}C$ y $\delta^{15}N$ (‰) determinados en muestras de escamas y músculo de cocodrilos silvestres y criados (*C. moreletii *y* C. acutus*) (valores medios ± DE): N, individuos estudiados; TL, longitud total; N/A, no disponible; * valores isotópicos no incluidos en el análisis estadístico.*

Location/origin	Species	N and TL (cm)	Tissue type	$\delta^{13}C$ value	$\delta^{15}N$ value
Wild					
Carpintero lagoon, Tampico, Tamaulpas	*C. moreletii*	187	Scute	–23.05 ± 0.17	12.90 ± 0.07
			Muscle	–24.11 ± 0.71	14.13 ± 0.28
		203	Scute	–22.13 ± 0.28	13.44 ± 0.19
			Muscle	–25.01 ± 0.46	14.09 ± 0.63
		218	Scute	–23.97 ± 0.35	14.69 ± 0.13
			Muscle	–24.88 ± 0.27	15.15 ± 0.43
Isla Navidad, Colima	*C. acutus*	206	Scutes	–24.99 ± 0.31	11.14 ± 0.21
		248		–27.18 ± 0.22	10.30 ± 0.27
Boca Negra estuary, Puerto Vallarta, Jalisco	*C. acutus*	95	Scutes	–25.52 ± 0.44	12.97 ± 0.62
		122		–25.98 ± 0.80	12.64 ± 0.45
		103		–26.11 ± 0.34	13.31 ± 0.71
La Manzanilla estuary, La Huerta, Jalisco	*C. acutus*	32*	Scutes	–18.23 ± 0.45	13.34 ± 0.34
	Recently hatched*	34*		–17.17 ± 0.32	13.66 ± 0.14
		30*		–18.55 ± 0.21	13.04 ± 0.27
	Adults	289	Scutes	–23.11 ± 0.40	14.18 ± 0.09
		320	Scutes	–24.67 ± 0.21	12.57 ± 0.42
Melaque, Jalisco	*C. acutus*	182	Scutes	–26.88 ± 0.53	13.58 ± 0.12
Farmed or captive					
Farmed Veracruz	*C. moreletii*	117	Scutes	–20.79 ± 0.31	8.09 ± 0.33
			Muscle	–21.58 ± 0.31	8.15 ± 0.23
		128	Scutes	–22.98 ± 0.29	8.79 ± 0.17
			Muscle	–22.26 ± 0.40	8.45 ± 0.25
Farmed Campeche 1	*C. moreletii*	105	Muscle	–19.72 ± 0.11	6.23 ± 0.08
		111		–20.18 ± 0.27	6.78 ± 0.38
Farmed Campeche 2	*C. moreletii*	113		–18.90 ± 0.17	8.14 ± 0.19
Farmed Guerrero	*C. moreletii*	N/A	Muscle	–23.55 ± 0.28	7.89 ± 0.57
	C. acutus	44	Muscle	–23.19 ± 0.02	7.74 ± 0.09
			Scutes	–20.28 ± 0.02	7.80 ± 0.21
		105	Muscle	–21.98 ± 0.04	7.17 ± 0.44
			Scutes	–21.25 ± 0.44	8.00 ± 0.11
Farmed Chiapas, processed skin product	*C. acutus*	N/A	Cured scutes	–19.44 ± 0.31	8.74 ± 0.39
Farmed Quintana Roo	*C. moreletii*	111	Scutes	–20.69 ± 0.28	7.54 ± 0.12
			Muscle	–19.95 ± 0.37	6.77 ± 0.32
Captive in rural farm	*C. acutus*	144	Scutes	–25.77 ± 0.28	8.97 ± 0.35
			Muscle	–22.09 ± 0.16	8.61 ± 0.11
Captive Jalisco	*C. acutus*	62	Scutes	–24.29 ± 0.22	7.50 ± 0.09