Differentiating wild from captive animals: an isotopic approach

Luiza Brasileiro [Corresp., 1, 2], **Rodrigo R Mayrink** [2, 3], **André C Pereira** [2], **Fábio J V Costa** [4], **Gabriela B Nardoto** [Corresp. 2]

[1] Diretoria de Fiscalização Ambiental, Brasilia Ambiental, Brasília, DF, Brazil

[2] Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, Brazil

[3] Setor Técnico-Científico, Policia Federal, Belo Horizonte, MG, Brazil

[4] Instituto Nacional de Criminalística, Policia Federal, Brasília, DF, Brazil

Corresponding Authors: Luiza Brasileiro, Gabriela B Nardoto
Email address: brasileiro.luiza@gmail.com, gbnardoto@unb.br

Background. The illegal wildlife trade is one of the most widespread and lucrative black markets. The irregular trade of animals has several environmental impacts, such as the welfare harms of individuals, extinction of species and populations, introduction of invasive species and zoonotic diseases, disruption of ecosystem services, and on food security. *Ex-situ* conservation might play an essential role in biodiversity conservation. However, that strategy is far from being a consensus since several studies suggest a relationship between authorized and illegal animal markets. The development of new techniques to differentiate whether animals or their products are captive-bred or wild-caught is fundamental, since the traditional control techniques are usually inaccurate and easily defrauded. Stable isotopes analysis has been used to identify animal provenance and some studies have successfully demonstrated its potential to differentiate wild from captive animals. Here we performed a literature review examining an extensive collection of publications to develop an overall picture of the application of stable isotopes to distinguish between wild and captive animals.

Survey methodology. Peer-reviewed publications were searched in the Web of Science database and in the references list from the main studies and reviews on the subject. We selected and analyzed 47 studies that used $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ in tissues from fish, amphibian, reptile, bird, and mammal groups.

Results. Studies are using stable isotopes in wild and captive animals all over the world, but concentrated in Europe (n = 21), and covering all main vertebrate groups, mainly fishes (n = 14). Most publications used $\delta^{13}C$ and $\delta^{15}N$, usually together, followed by $\delta^{2}H$ specially, when involving geographic variation. Every study that proposed to use stable isotopes to differentiate wild and captive animals was totally or partially well succeeded. However, when analyzing all publications together, we found significant differences between wild and captive animals only for $\delta^{18}O$ mean values and for $\delta^{13}C$ and $\delta^{15}N$ standard deviation and range. We also found heterogeneous variation in the distinction between wildlife and captivity by analyzing the different continents, taxonomic groups, and diet.

Conclusions. The use of stable isotopes showed to be effective in distinguishing between wild and captive animals. However, local environmental factors and the specific characteristics and objectives of each research seem to have a more significant influence on this potential than universal factors for all species and at a large scale. Nevertheless, we consider this review has taken a step further in understanding how stables isotopes may be used to distinguish between wild and captive animal and we expect to contribute to expand the use and acceptance of SIA as a reliable tool in combatting wildlife

crimes.

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Luiza Brasileiro[1,2], Rodrigo R. Mayrink[1,3], André C. Pereira[1], Fábio J. V. Costa[4], Gabriela B. Nardoto[1]

[1] Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, Brazil
[2] Diretoria de Fiscalização Ambiental, Brasília Ambiental, Brasília, DF, Brazil
[3] Setor Técnico-Científico, Polícia Federal, Belo Horizonte, MG, Brazil
[4] Instituto Nacional de Criminalística, Polícia Federal, Brasília, DF, Brazil.

Corresponding Author:
Luiza Brasileiro[1] Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, Brazil
Email address: brasileiro.luiza@gmail.com

Gabriela B. Nardoto[1]
Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, Brasília, Distrito Federal 70910-900, Brazil
Email address: gbnardoto@unb.br

Abstract

Background. The illegal wildlife trade is one of the most widespread and lucrative black markets. The irregular trade of animals has several environmental impacts, such as the welfare harms of individuals, extinction of species and populations, introduction of invasive species and zoonotic diseases, disruption of ecosystem services, and on food security. *Ex-situ* conservation might play an essential role in biodiversity conservation. However, that strategy is far from being a consensus since several studies suggest a relationship between authorized and illegal animal markets. The development of new techniques to differentiate whether animals or their products are captive-bred or wild-caught is fundamental, since the traditional control techniques are usually inaccurate and easily defrauded. Stable isotopes analysis has been used to identify animal provenance and some studies have successfully demonstrated its potential to differentiate wild from captive animals. Here we performed a literature review examining an extensive collection of publications to develop an overall picture of the application of stable isotopes to distinguish between wild and captive animals.

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Results. Studies are using stable isotopes in wild and captive animals all over the world, but concentrated in Europe (n = 21), and covering all main vertebrate groups, mainly fishes (n = 14). Most publications used $\delta^{13}C$ and $\delta^{15}N$, usually together, followed by $\delta^{2}H$ specially, when involving geographic variation. Every study that proposed to use stable isotopes to differentiate wild and captive animals was totally or partially well succeeded. However, when analyzing all publications together, we found significant differences between wild and captive animals only for $\delta^{18}O$ mean values and for $\delta^{13}C$ and $\delta^{15}N$ standard deviation and range. We also found heterogeneous variation in the distinction between wildlife and captivity by analyzing the different continents, taxonomic groups, and diet.

Conclusions. The use of stable isotopes showed to be effective in distinguishing between wild and captive animals. However, local environmental factors and the specific characteristics and objectives of each research seem to have a more significant influence on this potential than universal factors for all species and at a large scale. Nevertheless, we consider this review has taken a step further in understanding how stables isotopes may be used to distinguish between wild and captive animal and we expect to contribute to expand the use and acceptance of SIA as a reliable tool in combatting wildlife crimes.

Introduction

Illegal wildlife trade is one of the most widespread and lucrative black markets in the world, costing between US$5 and 23 billion a year and impacting local and global species and ecosystems (GIF, 2017). In 2015, the United Nations General Assembly adopted a resolution for tackling illicit trafficking in wildlife. The Sustainable Development Goals has specific targets to combat poaching and trafficking of protected species (Derek, Amy & Farooq, 2015). The

withdrawal of individuals from their original locality has direct and indirect effects on biological and ecological functions in both population and community levels (Harrison, 2011). The patterns of this activity can change considerably by country or region and involve illicit hunt, capture, poaching, rearing, transportation, and trade of wildlife (or its products) for pets, sport, human consumption, ornamental, medicinal or religious purposes (Reuter & O'Regan, 2017). The animal illegal trade has several environmental impacts, such as the welfare harms of individuals, extinction of species and populations, introduction of invasive species and zoonotic diseases, disruption of ecosystem services, and on food security (Baker et al., 2013; Dirzo et al., 2014; García-Díaz et al., 2015; Smith et al., 2017; Biggs et al., 2021).

The *ex-situ* conservation can have an essential role in biodiversity conservation. However, the Convention on Biological Diversity (CDB) emphasizes that this strategy should be used primarily to complement *in-situ* conservation measures. At the same time, the Convention of International Trade in Endangerment Species of Wild Fauna and Flora (CITES) foresees licensed breeders for some species as a strategy for *ex-situ* conservation. The idea is that authorized breeders can supply the demand for wild animals with captive-bred individuals and thus reduce the pressure on free-living populations (Challender & MacMillan, 2014; Challender, Harrop & MacMillan, 2015). However, that strategy is far from being a consensus (Tensen, 2016; Janssen & Chng, 2018). Although most species raised in captivity also occur in nature, the laws, rules, and management strategies are usually very different according to their origin. Several studies suggest a relationship between authorized and illegal animal markets, where illegally captured animals supply the former, intensifying the irregular trade and the impact on natural populations (Livingstone & Shepherd, 2016; Tensen, 2016; de Lucena Soares et al., 2020).

The development of new techniques to differentiate whether animals or their products are captive-bred or wild-caught is fundamental to enforce wildlife laundering, since the traditional control techniques (such as trader declarations, government-issued licenses, bands, or microchips) are usually inaccurate and easily defrauded. The differentiation between wild and captive animals is also needed for analyzing animal-based human food origin, such as fish and shrimp, and even to characterize the potential invasive populations, such as wild animals that escaped from captive and settled in nature (Hammershøj et al., 2005; García-Díaz et al., 2015).

In the last decades, stable isotopes analysis (SIA) has been applied in several studies involving ecological, forensic, and commercial subjects, such as animal migration, illegal trade, and food certification (Camin et al., 2016; Hobson & Wassenaar, 2019; Meier-Augenstein, 2019; Truonghuynh, Li & Jaganathan, 2020). More recently, SIA has also been used to track individuals' life stories and presumed differences in their diet in studies involving wild (free-living) and captive (farmed) individuals (Stoskopf, Barrick & Showers, 2001; Fernandez-Jover et al., 2020; Jenkins et al., 2020). Moreover, there are some studies that have successfully demonstrated the potential application of SIA to differentiate wild from captive animals (Natusch et al., 2017; Brandis et al., 2018; Andersson et al., 2021) but also applying this tool in real cases of suspected fraud (Dittrich, Struck & Rödel, 2017; Alexander et al., 2019; Jiguet, Kardynal &

Hobson, 2019). Lyons & Natusch (2015) evaluated several methodologies to differentiate between free-living and captive snakes and concluded that SIA may be the best tool in forensic context, since animals access different water and food sources in the wild and in captivity, their tissues are expected to reflect such differences.

Carbon, hydrogen, oxygen, and nitrogen account together for approximately 95% or more of the chemical composition of living organisms. These light elements can be analyzed for their stable isotopic ratios, and results expressed in δ values related to the proportion between the heavier and lighter isotope forms. $\delta^{13}C$ and $\delta^{15}N$ variation in animal tissues reflects mainly their isotopic diet composition. While $\delta^{13}C$ reflects the proportion of C_3/C_4 plants in the diet, the $\delta^{15}N$ reflects the trophic level of the animal within food chains complexity. $\delta^{2}H$ and $\delta^{18}O$ have close relationship with the drinking water and food water, and their variation in animal tissue is more complex, reflecting environmental influences (mainly temperature and altitude) on isotopic fractionation of drinking water, amount of drinking water related to water from food in the diet and metabolic water use (Daniel Bryant & Froelich, 1995). Although few studies use $\delta^{34}S$ compared with the other four elements mentioned before, sulfur isotopic ratio presents a significant potential for application in animal tracking. Sulfur is an essential constituent of amino acids, and its isotopic ratio reflects anthropogenic influences in the environment and marine sources in the diet (Hobson & Clark, 1992; Fry, 2008; Hobson & Wassenaar, 2019).

Specimens raised in captivity are fed under a regular and industrialized diet, with tap water available *ad libitum*, whereas in wild origin specimens the source and frequency of food and water can be highly variable (Natusch et al., 2017). Wild animals are also more susceptible to nutritional and water stress, which can influence their tissue isotopic ratios (Doi, Akamatsu & González, 2017; Magozzi et al., 2019). These differences are reflected in animal tissue stable isotope ratios.

Although SIA applications on differentiating wild and captive animals are of increasing interest, there is no compilation gathering the information available in the literature on the topic. In this study, we examined an extensive collection of publications using SIA in wild and captive animals. The available data in the literature was organized in a database, making the systematized information available for academic and applied purposes. In addition, we performed qualitative and quantitative meta-analyzes to develop an overall picture of the application of stable isotopes to distinguish between wild and captive animals in wildlife crime enforcement.

Survey methodology

Data Source and compilation

Peer-reviewed publications were searched in the Web of Science database (https://clarivate.com/webofsciencegroup/solutions/web-of-science/) from 1945 - 2021 using the terms “isotop*” AND “wild OR free-rang*” AND “captiv* or farm*” as a topic. To exclude domestic animals from the results, we added the search terms “NOT ‘chicken OR hen* OR cattle OR pig’”. The search returned 295 hits, which were initially sorted based on the title, keywords, and abstract. We considered studies of stable isotope involving any non-domestic vertebrate

species. In a second instance, we selected only research related to carbon, nitrogen, hydrogen, oxygen, or sulfur isotopes and that meet one of the following criteria: (1) used stable isotope to differentiate wild from captive animals; (2) conducted the isotopic analyzes in wild and captive animals in the same study; (3) could be directly related to works that meet one of the first two criteria. Paleontological publications were excluded, as well as those studies exclusively using compound-specific isotope analysis (CSIA) techniques. We also excluded studies that did not show the basic isotopic statistical information, such as average, standard deviation or error. Data were extracted only from original research papers rather than those found in reviews or meta-analysis studies to avoid duplicates. After these two steps of filtering, 47 studies remained to be analyzed in this review (Table S1). To ensure that all relevant papers were included, we also checked the references list from the main studies and reviews on the subject.

Data were initially collected from the texts and tables of articles. When they were not or were only partially available, we contacted the authors asking for the missing or the raw data. As a last resort, we estimated isotopic values from the figures, when available, using PlotDigitizer software, version 2.1.1 (PlotDigitizer, 2022).

Data and metadata structure

Variables related to the taxon classification, biology and morphology of animals, samples data (tissue analyzed, geographic location, year and period), rearing system, isotopic records (values of mean, standard deviation, minimum and maximum, range), methodological records from the isotope analyses (quality control estimates of analyses, lipid extraction, and international reference material), and identification of publication were registered (Table 1 and Table S1). Regarding the rearing system, the animals were classified as “wild”, “captive”, “presumed wild”, or “presumed captive”. The last two categories were used when the origin of the samples analyzed was uncertain and inferred in the research, such as in Dittrich, Struck, & Rödel (2017) and Hill et al. (2020). However, for analysis purposes, “presumed wild” and “presumed captive” were treated as “wild” and “captive”, respectively. All stable isotope results are expressed in the conventional delta (δ) notation, in units per mil (‰).

Whenever possible, selected metadata used the same language or criteria as other isotopic databases such as the R package Sider (Healy et al., 2018) and IsoBank (https://isobank.tacc.utexas.edu/). When not provided, the geographical coordinates of the samples were estimated based on the authors’ most detailed geographic information (e.g., city, region, fishing area zones). Sampling latitude and longitude were identified using Google Earth Pro software, version 7.3.48573 and presented in decimal degrees considering geodesic projection (horizontal datum) WGS-84. Graphics design was performed on Venngage v. 2.3, 2001.

We compared mean, standard deviation and range isotopic values of wild and captivity-raised animal using Student’s *t*-test and verified differences in stable isotope ratios between diets using one-way ANOVA, followed by a Tukey HSD posthoc test. We used a significance level of 5% in hypothesis testing. We performed all statistical tests in R, v. 1.4.1106 (R Development Core Team).

To better understand the general findings in the use of stable isotopes to distinguish between wild and captive animals, we performed a deeper qualitative analyze including specifically the studies that had this purpose. We focused on information about local, taxon, and the $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ $\delta^{18}O$ and $\delta^{34}S$ from the target tissue used in every study. This analysis overview was summarized in Table S2.

Quality assurance and quality control

As part of the quality assurance, data were carefully checked in different steps of the database building, trying to keep the information as close as possible to the original one. We made a double-check for values of mean, standard deviation, and range of isotopic ratios extracted by PlotDigitizer. We also double-checked in the original source outliers detected by boxplots for the same variables.

Species names were kept as in the original publications and as "fishes" are a paraphyletic group, we checked the taxonomic class of each species using Eschmeyer's Catalog of Fishes (Van der Laan & Fricke, 2022). Species diets classifications were checked using the R package Sider (Healy et al., 2018) or looked for in peer-reviewed papers.

Results

General trends

In total, 47 publications were analyzed. Most of them used stable isotopes to distinguish wild from captive animals in different contexts such as forensic, ecological, and commercial, or analyzed stable isotopes in wild and captive vertebrates focused on dietary analysis. Some studies measured SIA in wild or captive animals of a same species and that come from the same geographic region were also included in this survey.

We found studies distributed in 37 countries worldwide (Fig. 1A) with the United States and Italy standing out in the number of publications (n = 6, each). Regarding the continents, Europe (n = 21) and Africa (n = 3) had the largest and smallest number of studies, respectively.

Most of the studies focused only on one species or group of species from the same taxonomic class. Amphibia was the least representative group, present in only 4.3% of the studies, while fish accounted for over 45% of the publications (Fig. 1B).

Most studies used stable isotopes of carbon, nitrogen, or both (Fig. 1C), especially those related with dietary research, when the controlled captive environment was used to make inferences about resource use in free-living animals or to distinguish between wild and captive animals in local or regional scale. On the other hand, hydrogen stable isotope ratios in animal tissue were primarily used when the research involved geographic variation.

Fifty-five species from different vertebrate taxonomic groups (mammals, birds, reptiles, amphibians, and fishes) were heterogeneously studied around the world (Fig. 2). While studies on reptiles and amphibians were concentrated in Asia and Oceania, studies with fish occurred more worldwide distributed, but especially in Europe (Fig. 2).

Muscle, and inert organic tissues, such as feathers, hair, skin, scales, and claws, were the most analyzed tissues (see Table S1). Muscle and bone were more commonly used in works

related to animal products or resources, especially fish, while inert tissues were widely used in studies involving living animals.

Considering all publications together, we did not find significant differences between captive and wild animals when the mean isotopic ratios were compared, except for $\delta^{18}O$ (Table 2). However, wild animals showed significantly higher values for $\delta^{13}C$ and $\delta^{15}N$ standard deviation and range. On the other hand, for $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ there was a tendency towards a higher standard deviation for wild animals, but it was not significant (Table 2). Despite the relevant role statistical parameters related to data dispersion (such as standard deviation and range) can play in differentiating between wild and captive animals, only Molkentin et al., (2007) tested for such distinction, and they found significant differences in $\delta^{13}C$ variation of wild and farmed salmons.

The differences between wild and captive animals found for each individual study (see below) in $\delta^{13}C$ and $\delta^{15}N$ mean ratios are heterogeneous, varying with the geographic location and taxonomic group (Fig. 3). While in some continents isotopic mean ratios tended to be higher in captive animals (such as $\delta^{13}C$ in North America and $\delta^{15}N$ in Europe), in others, the values were higher in wild animals (such as $\delta^{13}C$ in Africa and $\delta^{15}N$ in South America).

We found significant variation in the means only for $\delta^{13}C$ in North America ($t_{35.08} = 4.14$, $p < 0.01$) and $\delta^{15}N$ in South America ($t_{26.42} = -2.79$, $p < 0.01$; Fig. 3A and 3B) when comparing wild and captive animals per continent. However, there was a tendency of greater isotopic values variation in samples from wild animals than in captive ones, although significant differences were found only in the standard variation of $\delta^{13}C$ in Europe ($t_{39.62} = -3.40$, $p < 0.01$) and of $\delta^{15}N$ in Asia ($t_{14.33} = -2.27$, p = 0.04), and in the range of $\delta^{15}N$ in Europe ($t_{19.31} = -2.10$, $p = 0.05$).

Different isotopic statistical parameters also drove the distinction of the rearing system for the taxonomic groups (Fig. 3C and 3D). All statistical parameters of $\delta^{15}N$ differed the rearing system in amphibians (mean: $t_{2..05} = -9.76$, $p < 0.01$; standard deviation: $t_{2.24} = -5.09$, $p = 0.03$; range: $t_{1.54} = -8.41$, $p = 0.03$), while only the range distinguished in fish ($t_{44.89} = -2.33$, $p = 0.02$). For other groups there were no differences in isotopic parameters for $\delta^{15}N$. The mean for $\delta^{13}C$ differed between the rearing system in birds ($t_{20.32} = 4.41$, $p < 0.01$) and reptiles ($t_{13.26} = 2.33$, $p = 0.04$), but not for other groups, neither did the other isotopic parameters. Although we found no other significant differences, the isotopic space occupied by individuals considering C and N simultaneously tended to diverge in all groups, either at the mean position or range (Fig.1S).

The $\delta^{13}C$ and $\delta^{15}N$ isotopic means significantly differed according to the animal main diet ($\delta^{3}C$: $F_{2,232} = 13.36$, p < 0.01; $\delta^{15}N$: $F_{2,190} = 35.63$, $p < 0.01$; Fig. 4). Herbivores exhibited the highest $\delta^{13}C$ values, while $\delta^{15}N$ of carnivores were significantly higher than herbivores and omnivores.

Due to the small number of studies, we did not perform more detailed analyses for $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$. However, individual studies indicated the potential of these elements to differentiate between wild and captive animals.

Publications that aimed to distinguish between wild and captive animals

The first studies using stable isotopes to distinguish wild and captive animals dated around two decades ago and aimed to evaluate the potential of SIA as a tool to differentiate wild from recent farm-scaped salmons (*Salar salar,* Dempson & Power, 2004) and minks (*Mustela vison,* Hammershøj, Asferg & Kristensen, 2004). Since then, the number of studies using this tool and its applications has been growing. Currently, its use involves several branches of science, such as commercial, forensic, or ecological purposes.

Of the 47 studies reviewed, 32 used stable isotopes to differentiate between wild and captive animals, including all vertebrate groups (fishes, amphibians, reptiles, birds, and mammals) (see Table S2). Fish was the most studied group, especially in commercial questions, followed by birds and mammals focused on forensic or ecological goals.

The $\delta^{13}C$ and $\delta^{15}N$ were the main isotopes used, usually together, to distinguish between captive and wild animals, especially on a local scale, followed by $\delta^{2}H$, particularly when some geographic variation was involved (Table S2). To identify such differences the studies performed discriminant tests, frequentist statistics (such as *t*-tests and ANOVA), or both. The exception is Rojas et al. (2007), where the authors relate a trend of higher $\delta^{13}C$ in wild salmons, compared to captive ones, but no statistical analysis was performed. Table S2 shows the summary of the research that used stable isotopes to differentiate wild from captive animals, including an overview of the main results found in each study. About 87% of the papers found significant differences between wild and captive animals. When discriminant tests were used, the accuracy ranged from 58% to 100.

A couple of studies showed some overlap between wild and captive samples, depending on the characteristics of captivity analyzed (e.g., more or less intensive) or how long an animal had changed from the breeding system. On the other hand, Van Schingen et al. (2016) simulated and tested differences between three different breeding systems, which they called “wild”, “captive” or “semi-captive”. Despite the overlap, they found between semi-captive and the two other groups, discriminant tests using $\delta^{13}C$ and $\delta^{15}N$ correctly classified 89% of the semi-captive samples. Other studies could isotopically distinguish animals from different breeding systems at even more detailed levels, such as differentiating free-living individuals from those conventionally and organically farmed or even distinguishing different breeders of the same species.

Discussion

Stable isotopes are important biomarkers of animal provenance. Besides the geographic origin, for which they have already been widely used (Kelly, Heaton & Hoogewerff, 2005; Camin et al., 2016; Vander Zanden et al., 2018; Hobson & Wassenaar, 2019), SIA raised as a potential tool to identify different rearing systems in the last few decades. Such information has been applied in different fields of science, such as anthropology (Somerville, Nelson & Knudson, 2010; Sugiyama, Fash & France, 2018), commercial (Molkentin et al., 2015; Vasconi et al., 2019), ecology (Hammershøj et al., 2005; Kays & Feranec, 2011), and forensics (Kelly, Thompson & Newton, 2008; Alexander et al., 2019; Jiguet, Kardynal & Hobson, 2019). The development of tools to identify the actual origin of an animal rearing system, in turn, has crucial applications in

304 animal traffic enforcement through the identification of wildlife laundering, animal-based food
305 certification, and even illegal introductions of species.
306 The 32 analyzed publications that used stable isotopes to specifically look for differences
307 between wild and captive animals were partially or totally succeeded, which highlights the
308 relevance of this tool to identify the origin of animals rearing system. Despite these consistent
309 findings in individual surveys, we did not find a general trend in how this differentiation occurs.
310 This is not entirely surprising, considering the complexity and variability in the analyzed data
311 involving different tissues from different species, originating from different biomes, ecosystems,
312 and captives with very distinct conditions.
313 Therefore, it is essential to consider research-specific characteristics when designing and
314 interpreting surveys using stable isotopes to differentiate between free-living and captive
315 animals. For example, it is expected that captivity animals have access to a more homogeneous
316 diet, and consequently, isotopic ratios variability to be lower compared to wild reared animals.
317 This pattern was observed in most studies. However, if the free-living population in a
318 hypothetical research is limited to a few individuals or it comes from specific regions or
319 ecosystems, and the captive collections are from many farms with diverse characteristics, this
320 trend may be reversed (see Anderson et al., 2021). In addition, it is essential to consider the
321 biological context of the species. Different element isotopes may be helpful in identifying
322 differences between captive and wild animals depending on whether the animal is migratory, if it
323 changes and how it changes its habitat according to life stage, or what its primary diet is.
324 The choice of the tissue used in the research is also fundamental. Tissues with a higher
325 turnover rate, such as blood, are more subject to seasonal or physiological variations, especially
326 in the wild, where animals are more exposed to uncontrolled environmental conditions. In these
327 cases, samples ideally should be collected at similar periods in both wild and captive. A great
328 variability in isotopic values was found in studies made in large period of time, such as Codron
329 et al. (2013) and Farabegoli et al. (2018). On the other hand, the influence of such external
330 factors is minimized in studies with animal products or using inert tissues, such as feathers,
331 claws, and scales. At the same time, if the animal has changed from free-living to captive or vice
332 versa, it is important to consider which environment the isotopic composition of each tissue is
333 expressing, according to the time elapsed since the change. Or, if this period is unknown,
334 simultaneous analysis of more than one tissue can indicate the period when the change occurred.
335 It is also important to mention that samples had lipids extracted in some research, but not
336 in all of them. Some studies did not even mention if this procedure was taken, as did not present
337 the analytical error associated with isotope-ratio mass spectrometry measures. These
338 methodological aspects could also have contributed to the great heterogeneity found in the results
339 of the different publications.
340 Fish was the most studied group in the publications analyzed in this review, especially in
341 Europe, where surveys often addressed issues related to the origin of animal-based food. All fish
342 studies involved species of a single Class (Actinopteri), and most of them involved species used
343 for human consumption (e.g., European seabass, meagre, and different species of salmon).

Mammals, birds, and reptiles research were mainly associated with ecological or forensic purposes, such as identifying the origin of a wolf population (*Canis lupus*) in an area where they were previously extinct (Kays & Feranec, 2011); the potential use of stable isotopes to identify the provenance of invasive alien species (*Trachemis scripta*) (Hill et al., 2020); or to detect crocodile lizard (*Shinisaurus crocodilurus*), short-beak echidnas (*Tachyglossus aculeatus*), and yellow-crested cockatoos (*Cacatua sulphurea*) laundering (van Schingen et al., 2016; Brandis et al., 2018; Andersson et al., 2021)

δ^{13}C and δ^{15}N were the elements most used, followed by δ^{2}H, δ^{18}O, and δ^{34}S. This is not surprising, considering that those two former elements isotopic ratios are effect of diets and that wild *versus* captive isotopic differences are based on the assumption that the animals have different diets in these two systems. In general, our results suggest that this assumption is valid. However, some overlap in isotope ratios can still occur. Since the food received by animals in captivity tends to be a simplification of their diet in nature, the more similar the captivity is to the natural environment, the greater the tendency of overlapping isotopic values of the tissues analyzed. In Liu et al. (2020), for example, δ^{13}C and δ^{15}N could clearly distinguish between wild-caught and pond-farmed carps, but the lake-farmed ones had overlapping isotopic ratios with the two other groups. In this case, the lake is a more extensive captivity compared to the pound, considered as a "semi-captive" environment.

One possible solution to minimize this overlap is to analyze more isotopes simultaneously. Studies in free-living and captive individuals in the same region combining δ^{13}C and δ^{15}N tend to lead to a more accurate differentiation than a single isotope. Pereira et al. (2019) found the worst performance in the discriminant analysis using stable isotopes to distinguish between wild or captive arapaima fish (*Arapaima* spp.) in Brazil with 58% of correct classification. But besides some uncertainty of the origin of the fish purchased at the markets, they used only δ^{13}C to do the classification. Possibly the additional use of δ^{15}N could provide better discrimination.

In addition, if the research involves individuals from different geographic locations, δ^{2}H and δ^{18}O would perform better if used together with carbon and nitrogen. Although less explored, -these water elements isotopes may also vary due to different water availability and consumption, and possible different metabolic responses in captivity. Research involving species with different levels of anthropogenic impact, a mixture of terrestrial and aquatic resources, or various aquatic sources in their diet, as in Liu et al. (2020), could show benefit from δ^{34}S analysis.

The analysis of more than one statistical parameter, such as means and data dispersion variables could also help to identify the breeding system, especially if the intention is to detect the origin of a group instead of a specific individual. The mean isotopic ratios will depend on specific environmental and physiological factors, such as tissue, groups' diet and geographic location. These factors, in turn, may vary according to the taxon, scale, seasonality, and other specific characteristics of each research. Measures of dispersion, such as standard deviation and

range, are also exposed to external influences but are expected to be smaller in captive-bred animals since their conditions tend to be more homogeneous.

Finally, two others possibilities to reduced overlaps which is already being adopted by some researchers are the use of complementary methodology such as elemental data (e.g., Brandis et al., 2018; Gopi et al., 2019) fatty acid profile (Farabegoli et al., 2018; Vasconi et al., 2019) or the use of compound-specific isotope analysis (CSIA) (Aursand, Mabon & Martin, 2000; Molkentin et al., 2015; Wang et al., 2018; Andersson et al., 2021). CSIA is a robust tool for measuring the molecular-level isotopic composition of organic chemical compounds such as hydrocarbons, fatty acids, and amino acids. The principle is similar to bulk tissue isotope analysis. Moreover, since many subparts of the macromolecules have specific biochemical pathways, CSIA has the potential to provide more detailed information on the rearing system based on metabolic route of compounds (e.g., amino acids, fatty acids) (Whiteman et al., 2019). For example, in Molkentin et al. (2015) and Wang et al. (2018), the isotopic analyses of bulk tissue could differentiate conventionally farmed from wild salmons. However, there was some overlap with organically farmed animals. The authors used CSIA to analyze $\delta^{13}C$ of fatty acid and amino acids, respectively, and both obtained a more precise separation of three rearing systems: wild, conventionally, and organically farmed salmon.

Conclusions

Our study reveals that SIA has been proved to be useful to distinguish between wild and captive in different vertebrate groups and methodological designs worldwide. Local environmental factors and the specific characteristics and objectives of each research seem to have a more significant influence on this potential than universal factors for all species and at a large scale (global or continental). These findings indicate the importance of considering and presenting such factors when performing research. Also, it is fundamental the information about some methodological procedures (such as the lipids extraction or analytical error), and the presentation of basic statistical parameters (such as the mean and some data dispersion variable) to evaluate if the results of a specific research could be applied to another one or could be used to confidentially identify irregularities in animas trade.

We consider this review has taken a step further in understanding how stables isotopes may be used to distinguish between wild and captive animals, besides highlighting some essential factors that should be considered in using or analyzing the use of this technique. We expect the present study to contribute to expanding the use and acceptance of SIA as a reliable tool in combatting wildlife crimes, and, as consequence, contribute to the efficiency of ex situ conservation strategies and the protection of natural populations.

Acknowledgements

We thank the Environmental Isotope Studies at the University of Brasilia group for all scientific discussion and contributions.

References

421 Alexander J, Downs CT, Butler M, Woodborne S, Symes CT. 2019. Stable isotope analyses as
422 a forensic tool to monitor illegally traded African grey parrots. *Animal Conservation*
423 22:134–143. DOI: 10.1111/acv.12445.
424 Andersson AA, Gibson L, Baker DM, Cybulski JD, Wang S, Leung B, Chu LM, Dingle C. 2021.
425 Stable isotope analysis as a tool to detect illegal trade in critically endangered
426 cockatoos. *Animal Conservation* 24:1021–1031. DOI: 10.1111/acv.12705.
427 Aursand M, Mabon F, Martin GJ. 2000. Characterization of farmed and wild salmon (*Salmo*
428 *salar*) by a combined use of compositional and isotopic analyses. *Journal of the*
429 *American Oil Chemists' Society* 77:659–666. DOI: 10.1007/s11746-000-0106-5.
430 Baker SE, Cain R, van Kesteren F, Zommers ZA, D'Cruze N, Macdonald DW. 2013. Rough
431 Trade: Animal Welfare in the Global Wildlife Trade. *BioScience* 63:928–938. DOI:
432 10.1525/bio.2013.63.12.6.
433 Biggs D, Caceres-Escobar H, Kock R, Thomson G, Compton J. 2021. Extend existing food
434 safety systems to the global wildlife trade. *The Lancet Planetary Health* 5:e402–e403.
435 DOI: 10.1016/S2542-5196(21)00142-X.
436 Brandis KJ, Meagher PJB, Tong LJ, Shaw M, Mazumder D, Gadd P, Ramp D. 2018. Novel
437 detection of provenance in the illegal wildlife trade using elemental data. *Scientific*
438 *Reports* 8:15380. DOI: 10.1038/s41598-018-33786-0.
439 Camin F, Bontempo L, Perini M, Piasentier E. 2016. Stable Isotope Ratio Analysis for Assessing
440 the Authenticity of Food of Animal Origin: Authenticity of animal origin food….
441 *Comprehensive Reviews in Food Science and Food Safety* 15:868–877. DOI:
442 10.1111/1541-4337.12219.
443 Challender DWS, Harrop SR, MacMillan DC. 2015. Understanding markets to conserve trade-
444 threatened species in CITES. *Biological Conservation* 187:249–259. DOI:
445 10.1016/j.biocon.2015.04.015.

446 Challender DWS, MacMillan DC. 2014. Poaching is more than an Enforcement Problem:
447 Poaching is more than an enforcement problem. *Conservation Letters* 7:484–494. DOI:
448 10.1111/conl.12082.
449 Codron J, Kirkman K, Duffy KJ, Sponheimer M, Lee-Thorp JA, Ganswindt A, Clauss M, Codron
450 D. 2013. Stable isotope turnover and variability in tail hairs of captive and free-ranging
451 African elephants (*Loxodonta africana*) reveal dietary niche differences within
452 populations. *Canadian Journal of Zoology* 91:124–134. DOI: 10.1139/cjz-2012-0155.
453 Daniel Bryant J, Froelich PN. 1995. A model of oxygen isotope fractionation in body water of
454 large mammals. *Geochimica et Cosmochimica Acta* 59:4523–4537. DOI: 10.1016/0016-
455 7037(95)00250-4.
456 Dempson JB, Power M. 2004. Use of stable isotopes to distinguish farmed from wild Atlantic
457 salmon, Salmo salar. *Ecology of Freshwater Fish* 13:176–184. DOI: 10.1111/j.1600-
458 0633.2004.00057.x.
459 Derek O, Amy C, Farooq U. 2015. Universal Sustainable Development Goals; Understanding
460 the transformational challenge for developing countries. In: *Stakeholder Forum*. 1–24.
461 Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. 2014. Defaunation in the
462 Anthropocene. *Science* 345:401–406. DOI: 10.1126/science.1251817.
463 Dittrich C, Struck U, Rödel M-O. 2017. Stable isotope analyses-A method to distinguish
464 intensively farmed from wild frogs. *Ecology and Evolution* 7:2525–2534. DOI:
465 10.1002/ece3.2878.
466 Doi H, Akamatsu F, González AL. 2017. Starvation effects on nitrogen and carbon stable
467 isotopes of animals: an insight from meta-analysis of fasting experiments. *Royal Society*
468 *Open Science* 4:170633. DOI: 10.1098/rsos.170633.
469 Farabegoli F, Pirini M, Rotolo M, Silvi M, Testi S, Ghidini S, Zanardi E, Remondini D, Bonaldo A,
470 Parma L, Badiani A. 2018. Toward the Authentication of European Sea Bass Origin
471 through a Combination of Biometric Measurements and Multiple Analytical Techniques.

Journal of Agricultural and Food Chemistry 66:6822–6831. DOI: 10.1021/acs.jafc.8b00505.

Fernandez-Jover D, Mladineo I, Grubišić L, Lušić J, Sanchez-Jerez P. 2020. Changes in trophic behaviour and trace metal concentrations in wild fish in a tuna-farming environment. The key role of a sound baitfish choice. *Regional Studies in Marine Science* 38:101357. DOI: 10.1016/j.rsma.2020.101357.

Fry B. 2008. *Stable isotope ecology*. New York, NY: Springer.

García-Díaz P, Ross JV, Ayres C, Cassey P. 2015. Understanding the biological invasion risk posed by the global wildlife trade: propagule pressure drives the introduction and establishment of Nearctic turtles. *Global Change Biology* 21:1078–1091. DOI: 10.1111/gcb.12790.

Gopi K, Mazumder D, Sammut J, Saintilan N, Crawford J, Gadd P. 2019. Isotopic and elemental profiling to trace the geographic origins of farmed and wild-caught Asian seabass (Lates calcarifer). *Aquaculture* 502:56–62. DOI: 10.1016/j.aquaculture.2018.12.012.

Hammershøj M, Asferg T, Kristensen NB. 2004. Comparison of methods to separate wild American mink from fur farm escapees. *Mammalian Biology* 69:281–286. DOI: 10.1078/1616-5047-00145.

Hammershøj M, Pertoldi C, Asferg T, Bach Møller T, Bastian Kristensen N. 2005. Danish free-ranging mink populations consist mainly of farm animals: Evidence from microsatellite and stable isotope analyses. *Journal for Nature Conservation* 13:267–274. DOI: 10.1016/j.jnc.2005.03.001.

Harrison RD. 2011. Emptying the Forest: Hunting and the Extirpation of Wildlife from Tropical Nature Reserves. *BioScience* 61:919–924. DOI: 10.1525/bio.2011.61.11.11.

Healy K, Guillerme T, Kelly SBA, Inger R, Bearhop S, Jackson AL. 2018. SIDER: an R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. *Ecography* 41:1393–1400. DOI: 10.1111/ecog.03371.

498 Hill KGW, Nielson KE, Tyler JJ, McInerney FA, Doubleday ZA, Frankham GJ, Johnson RN,
499 Gillanders BM, Delean S, Cassey P. 2020. Pet or pest? Stable isotope methods for
500 determining the provenance of an invasive alien species. *NeoBiota* 59:21–37. DOI:
501 10.3897/neobiota.59.53671.
502 Hobson KA, Clark RG. 1992. Assessing Avian Diets Using Stable Isotopes I: Turnover of ^{13} C in
503 Tissues. *The Condor* 94:181–188. DOI: 10.2307/1368807.
504 Hobson KA, Wassenaar LI (eds.). 2019. *Tracking animal migration with stable isotopes*.
505 London: Academic Press.
506 Janssen J, Chng SCL. 2018. Biological parameters used in setting captive-breeding quotas for
507 Indonesia's breeding facilities: Setting Captive-Breeding Quotas. *Conservation Biology*
508 32:18–25. DOI: 10.1111/cobi.12978.
509 Jenkins E, Gulka J, Yurkowski DJ, Le François NR, Wong E, Davoren GK. 2020. Isotopic
510 Discrimination (δ ^{15} N, δ ^{13} C) in Captive and Wild Common Murres (*Uria aalge*) and
511 Atlantic Puffins (*Fratercula arctica*). *Physiological and Biochemical Zoology* 93:296–
512 309. DOI: 10.1086/709460.
513 Jiguet F, Kardynal KJ, Hobson KA. 2019. Stable isotopes reveal captive vs wild origin of illegally
514 captured songbirds in France. *Forensic Science International* 302:109884. DOI:
515 10.1016/j.forsciint.2019.109884.
516 Kays R, Feranec RS. 2011. Using Stable Carbon Isotopes to Distinguish Wild from Captive
517 Wolves. *Northeastern Naturalist* 18:253–264. DOI: 10.1656/045.018.0301.
518 Kelly S, Heaton K, Hoogewerff J. 2005. Tracing the geographical origin of food: The application
519 of multi-element and multi-isotope analysis. *Trends in Food Science & Technology*
520 16:555–567. DOI: 10.1016/j.tifs.2005.08.008.
521 Kelly A, Thompson R, Newton J. 2008. Stable hydrogen isotope analysis as a method to identify
522 illegally trapped songbirds. *Science & Justice* 48:67–70. DOI:
523 10.1016/j.scijus.2007.09.012.

524 Liu Z, Yuan Y, Zhao Y, Zhang Y, Nie J, Shao S, Rogers KM. 2020. Differentiating wild, lake-
525 farmed and pond-farmed carp using stable isotope and multi-element analysis of fish
526 scales with chemometrics. *Food Chemistry* 328:127115. DOI:
527 10.1016/j.foodchem.2020.127115.
528 Livingstone E, Shepherd CR. 2016. Bear farms in Lao PDR expand illegally and fail to conserve
529 wild bears. *Oryx* 50:176–184. DOI: 10.1017/S0030605314000477.
530 de Lucena Soares HK, dos Santos Soares VM, de Faria Lopes S, de Lucena RFP, Barboza
531 RRD. 2020. Rearing and trade of wild birds in a semiarid region of Brazil. *Environment,*
532 *Development and Sustainability* 22:4323–4339. DOI: 10.1007/s10668-019-00386-5.
533 Lyons J, Natusch D. 2015. Methodologies for differentiating between wild and captive-bred
534 CITES-listed snakes. :6.
535 Magozzi S, Vander Zanden HB, Wunder MB, Bowen GJ. 2019. Mechanistic model predicts
536 tissue–environment relationships and trophic shifts in animal hydrogen and oxygen
537 isotope ratios. *Oecologia* 191:777–789. DOI: 10.1007/s00442-019-04532-8.
538 Meier-Augenstein W. 2019. From stable isotope ecology to forensic isotope ecology —
539 Isotopes' tales. *Forensic Science International* 300:89–98. DOI:
540 10.1016/j.forsciint.2019.04.023.
541 Molkentin J, Lehmann I, Ostermeyer U, Rehbein H. 2015. Traceability of organic fish –
542 Authenticating the production origin of salmonids by chemical and isotopic analyses.
543 *Food Control* 53:55–66. DOI: 10.1016/j.foodcont.2015.01.003.
544 Natusch DJD, Carter JF, Aust PW, Van Tri N, Tinggi U, Mumpuni, Riyanto A, Lyons JA. 2017.
545 Serpent's source: Determining the source and geographic origin of traded python skins
546 using isotopic and elemental markers. *Biological Conservation* 209:406–414. DOI:
547 10.1016/j.biocon.2017.02.042.
548 Reuter P, O'Regan D. 2017. Smuggling wildlife in the Americas: scale, methods, and links to
549 other organised crimes. *Global Crime* 18:77–99. DOI: 10.1080/17440572.2016.1179633.

Rojas JMM, Serra F, Giani I, Moretti VM, Reniero F, Guillou C. 2007. The use of stable isotope ratio analyses to discriminate wild and farmed gilthead sea bream (Sparus aurata). *Rapid Communications in Mass Spectrometry* 21:207–211. DOI: 10.1002/rcm.2836.

van Schingen M, Ziegler T, Boner M, Streit B, Nguyen TQ, Crook V, Ziegler S. 2016. Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (Shinisaurus crocodilurus) from Vietnam. *Global Ecology and Conservation* 6:232–241. DOI: 10.1016/j.gecco.2016.03.004.

Smith KM, Zambrana-Torrelio C, White A, Asmussen M, Machalaba C, Kennedy S, Lopez K, Wolf TM, Daszak P, Travis DA, Karesh WB. 2017. Summarizing US Wildlife Trade with an Eye Toward Assessing the Risk of Infectious Disease Introduction. *EcoHealth* 14:29–39. DOI: 10.1007/s10393-017-1211-7.

Somerville AD, Nelson BA, Knudson KJ. 2010. Isotopic investigation of pre-Hispanic macaw breeding in Northwest Mexico. *Journal of Anthropological Archaeology* 29:125–135. DOI: 10.1016/j.jaa.2009.09.003.

Stoskopf MK, Barrick RE, Showers WJ. 2001. Oxygen isotope variability in bones of wild caught and constant temperature reared sub-adult American alligators. *Journal of Thermal Biology* 26:183–191. DOI: 10.1016/S0306-4565(00)00041-3.

Sugiyama N, Fash WL, France CAM. 2018. Jaguar and puma captivity and trade among the Maya: Stable isotope data from Copan, Honduras. *PLOS ONE* 13:e0202958. DOI: 10.1371/journal.pone.0202958.

Tensen L. 2016. Under what circumstances can wildlife farming benefit species conservation? *Global Ecology and Conservation* 6:286–298. DOI: 10.1016/j.gecco.2016.03.007.

Truonghuynh HT, Li GB, Jaganathan GK. 2020. Isotope Analysis as a Means of Tracing Aquatic Products Authenticity, Source and Geographic Origins. *Italian Journal of Food Science* 32. DOI: 10.14674/IJFS-1778.

575 Vander Zanden HB, Nelson DM, Wunder MB, Conkling TJ, Katzner T. 2018. Application of
576 isoscapes to determine geographic origin of terrestrial wildlife for conservation and
577 management. *Biological Conservation* 228:268–280. DOI:
578 10.1016/j.biocon.2018.10.019.
579 Vasconi M, Lopez A, Galimberti C, Moreno Rojas JM, Muñoz Redondo JM, Bellagamba F,
580 Moretti VM. 2019. Authentication of farmed and wild european eel (Anguilla anguilla) by
581 fatty acid profile and carbon and nitrogen isotopic analyses. *Food Control* 102:112–121.
582 DOI: 10.1016/j.foodcont.2019.03.004.
583 Wang YV, Wan AHL, Lock E-J, Andersen N, Winter-Schuh C, Larsen T. 2018. Know your fish:
584 A novel compound-specific isotope approach for tracing wild and farmed salmon. *Food*
585 *Chemistry* 256:380–389. DOI: 10.1016/j.foodchem.2018.02.095.
586 Whiteman J, Elliott Smith E, Besser A, Newsome S. 2019. A Guide to Using Compound-Specific
587 Stable Isotope Analysis to Study the Fates of Molecules in Organisms and Ecosystems.
588 *Diversity* 11:8. DOI: 10.3390/d11010008.
589

Figure 1

Distribution of studies using stable isotopes of carbon, nitrogen, hydrogen, oxygen, and sulfur in wild and captive animals worldwide (A), by taxonomic group (B), and by elements isotope ratios (C).

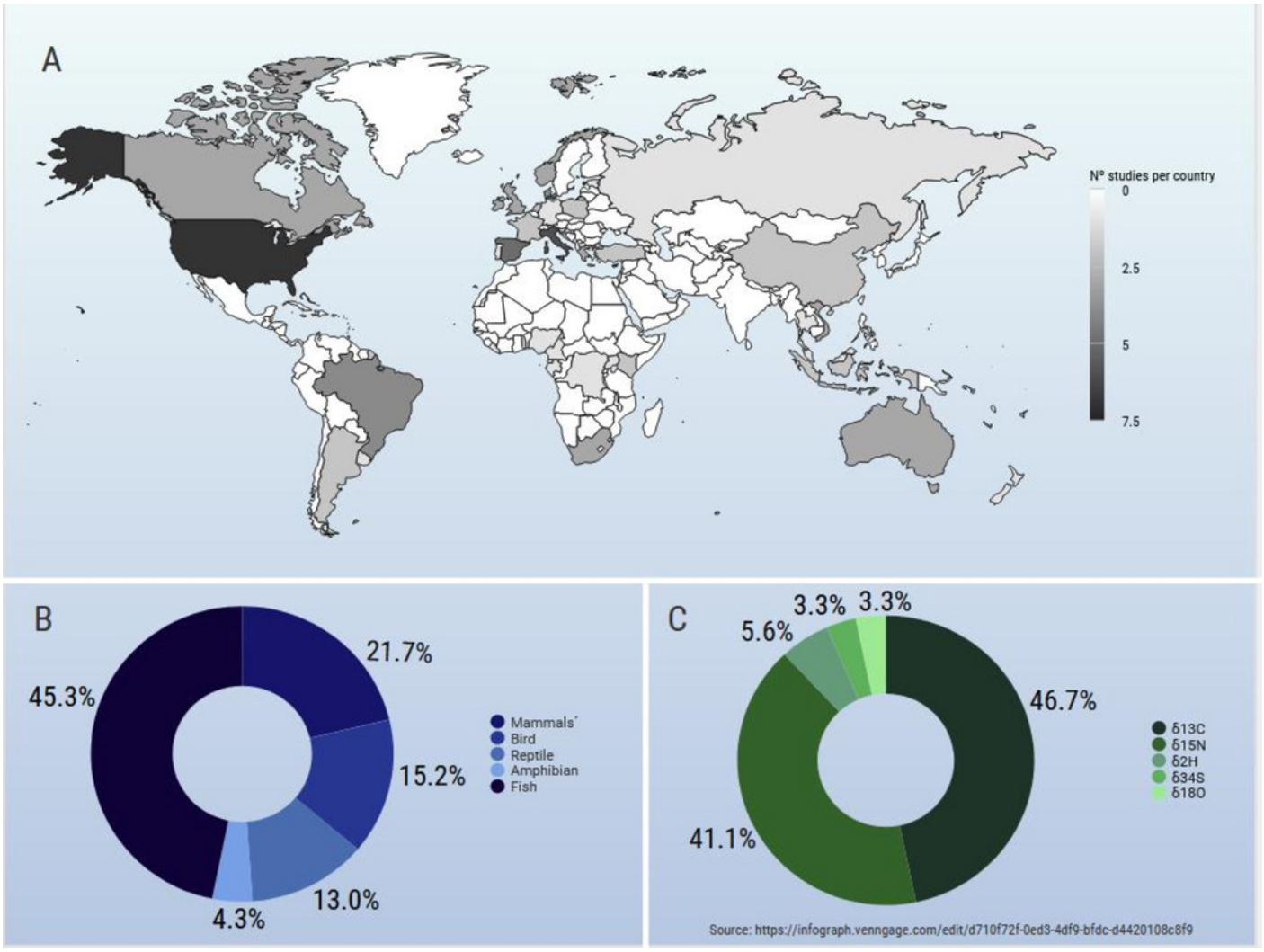

Figure 2

Percentage of studies involving the different taxonomic groups (mammals, birds, reptiles, amphibians, and fishes) per continent (Africa, Asia, Europe, North America, Oceania, and South America) (A) and of studies involving the continents per taxonomic gro

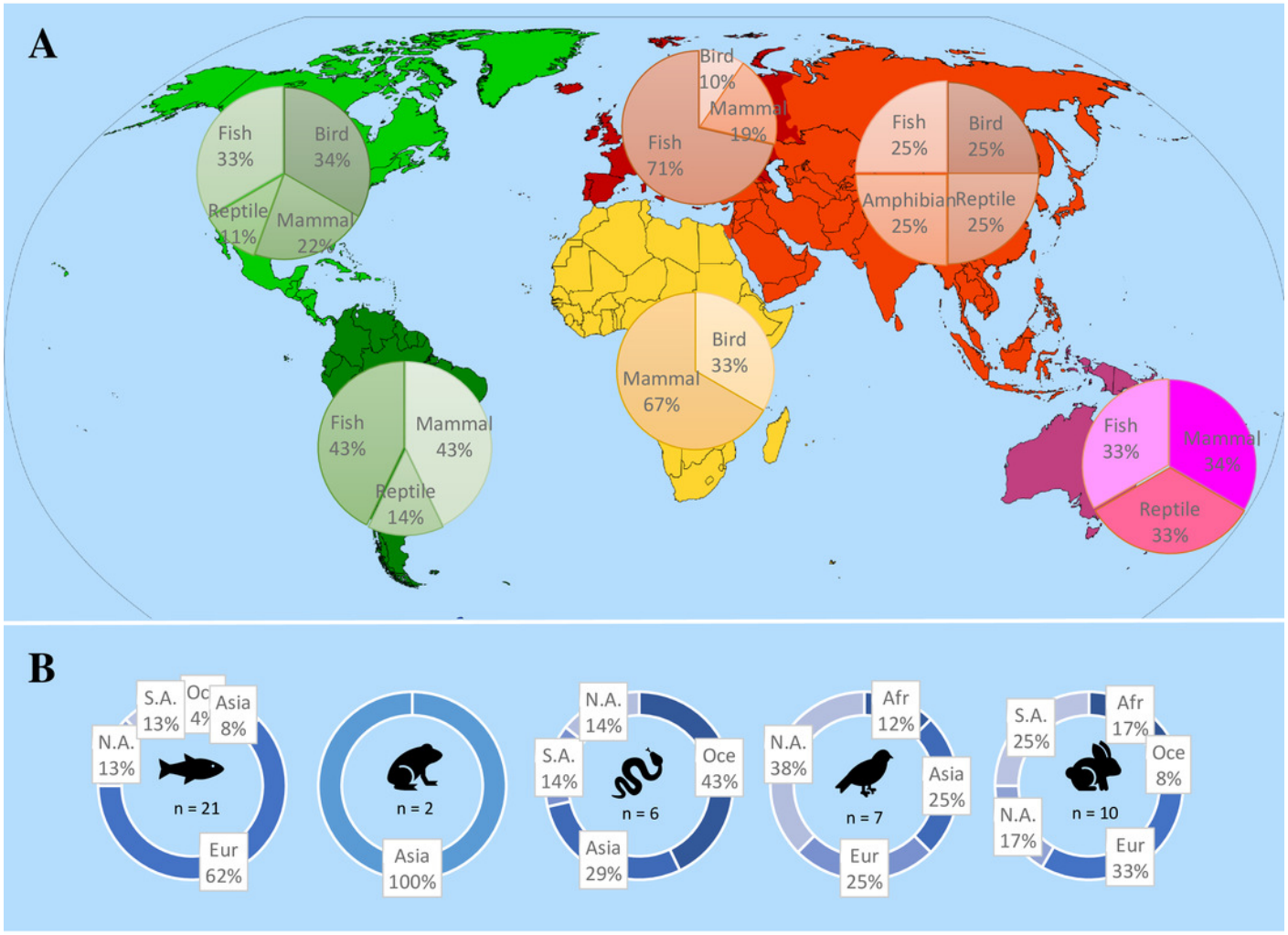

Figure 3

δ^{13}C (A and C) and δ^{15}N (B and D) means isotopic ratios for wild and captive animals by continent (left) and by taxon group (right), considering all review publications simultaneously.

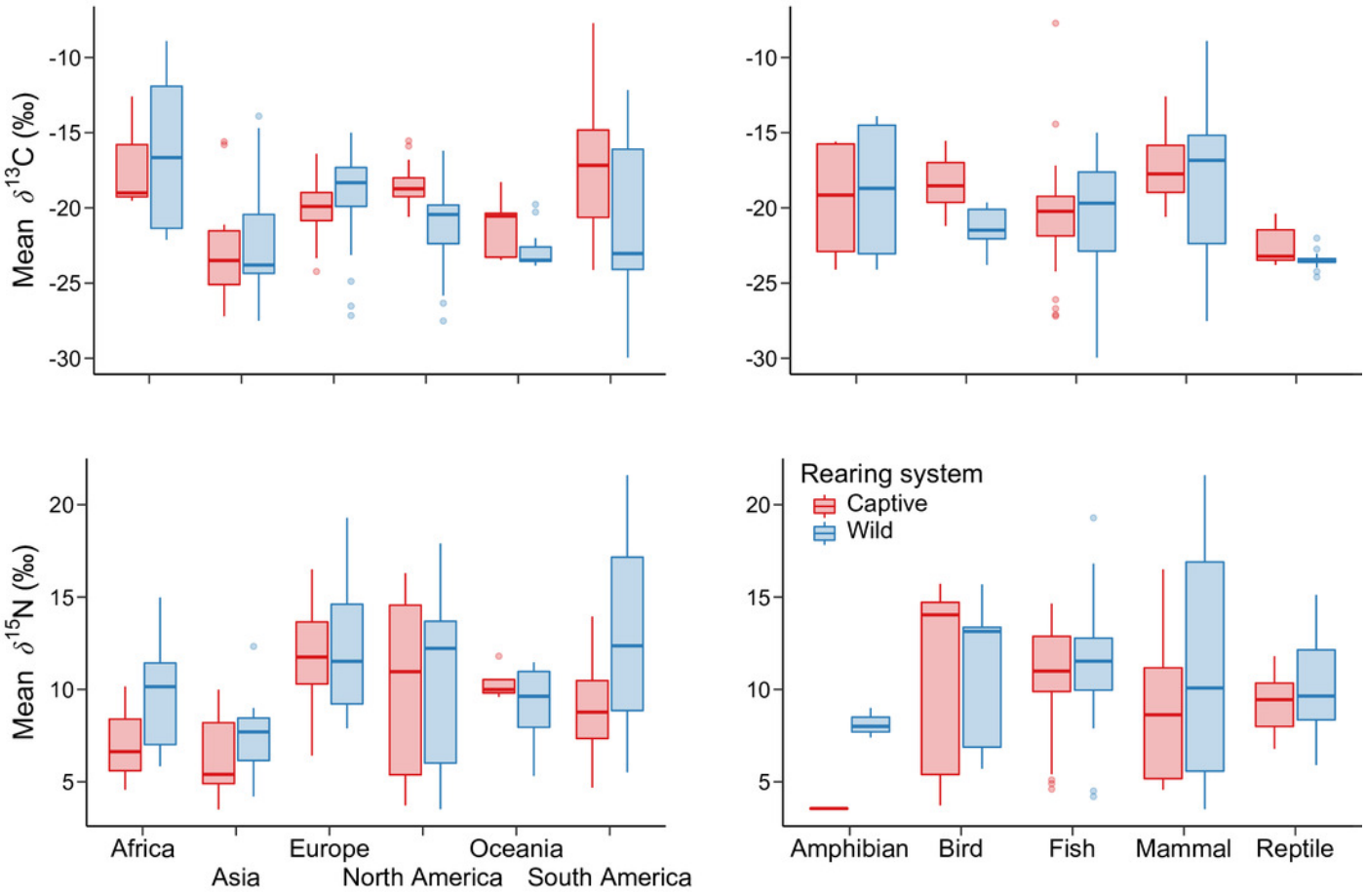

Figure 4

Animals $\delta^{13}C$ (left) and $\delta^{15}N$ (right) ratios mean according to the diet.

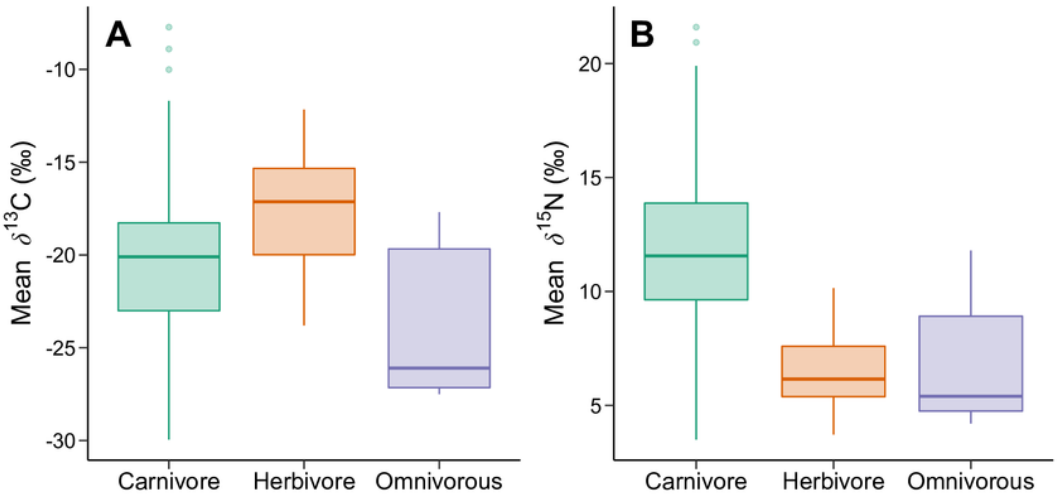
A
B
Mean δ^{13}C (‰)
Mean δ^{15}N (‰)
-10
-15
-20
-25
-30
20
15
10
5
Carnivore
Herbivore
Omnivorous
Carnivore
Herbivore
Omnivorous

Table 1(on next page)

List and description of the variables selected to be included in the database.

Table 1. List and description of the variables selected to be included in the database

VARIABLE	EXPLANATION
Reference	Publication included in the data collection.
Taxon group	Mammal, Bird, Reptile, Amphibian, Fish.
Taxon	Most detailed taxon identified (usually species or genus)
Life-stage	Adult or subadult
Size-range or weight	Body size in centimeters or weight in kilograms
Diet	Herbivore, carnivore or omnivore
Continent	Where data were collected: Africa, Asia, Europe, Oceania, North America, and South America.
Multiple countries?	Yes or no. Were samples collected in more than one country?
Country/Region	Country(ies) or subcontinental region where data were collected.
Region/city	City, estate, or region within a country.
Lat	Latitude (m). UTM system
Long	Longitude (m). UTM system
Month/period	Month or other information available about samples collection period.
Year	Year of samples collection.
Tissue	Animal tissue used in the isotopic analysis. E.g., feather, muscle, blood.
Subtissue	A specific part of a given tissue. E.g., red blood cells, type of feathers.
System	Rearing system: wild, captive, presumed wild or presumed captive
Subgroup	When there are different treatments within a wild or captive condition.
N	The number of sampled animals.
Breeding system change	Time the animal changed from wild to captive or captive to wild (in months).
Mean δ^zx (‰)	isotopic ratio means.
SD δ^zx (‰)	isotopic ratio standard deviation
MIN δ^zx (‰)	isotopic ratio minimum value
MAX δ^zx (‰)	isotopic ratio maximum value
Range δ^zx (‰)	Difference between maximum and minimum isotopic ratios
Lipid extraction	Yes or no. Were lipids extracted during sample preparation?
Analytical error	Error that might be associated with isotope-ratio mass spectrometry
Reference standard	Compounds with well-defined isotopic compositions used to ensure accuracy in mass spectrometric measurements of isotope ratios

Observation	Any additional relevant information
Related publication	DOI or link to the publication

2

Table 2(on next page)

Comparison of the mean isotopic ratios, standard deviation, and range of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ in captive and free-living animals considering all 47 analyzed publica

Significant differences are indicated by different letters.

Table 2. Comparison of the mean isotopic ratios, standard deviation, and range of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ in captive and free-living animals considering all 47 analyzed publications. Significant differences are indicated by different letters.

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{18}O$	$\delta^{34}S$
μ_w	-20.42 ± 4.13[a]	10.92 ± 4.20[a]	-68.80 ± 33.90[a]	23.20 ± 1.83[a]	1.50 ± 8.74[a]
μ_c	-19.68 ± 3.09[a]	10.18 ± 3.45[a]	-61.21 ± 38.89[a]	19.05 ± 1.66[b]	8.16 ± 7.28[a]
SD_w	0.90 ± 0.63[a]	0.86 ± 0.70[a]	10.35 ± 3.70[a]	1.89 ± 0.48[a]	2.17 ± 2.32[a]
SD_c	0.68 ± 0.61[b]	0.56 ± 0.53[b]	6.73 ± 5.71[a]	1.44 ± 0.50[a]	1.24 ± 2.34[a]
$Range_w$	3.31 ± 2.35[a]	3.50 ± 2.53[a]	37.02 ± 24.49[a]	7.3 ± 2.24[a]	6.24 ± 6.48[a]
$Range_c$	2.48 ± 1.98[b]	2.04 ± 1.70[b]	29.29 ± 29.64[a]	6.02 ± 2.46[a]	7.92 ± 9.73[a]