Differentiating wild from captive animals: an isotopic approach

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Background. Wildlife farming can be an important but complex tool for conservation. Wildlife farming should meet a variety of biophysical, market, and regulatory conditions to achieve conservation benefits. Regulatory controls, in turn, are often based on certification and traceability requirements to identify the animals' origin. The traditional techniques for discriminating between wild and captive animals are insufficient to prevent doubts or misdeclaration, especially when labels are not expected or mandatory. There is a pressing need to develop more accurate techniques to discriminate between wild and captive animals and their products. Stable isotope analysis has been used to identify animal provenance, and some studies have successfully demonstrated its potential to differentiate wild from captive animals. In this literature review, we examined an extensive collection of publications to develop an overall picture of the application of stable isotopes to distinguish between wild and captive animals focusing on evaluating the potential and patterns of this tool.

Survey methodology. We searched peer-reviewed publications in the Web of Science database and the references list from the main studies and reviews on the subject. We selected and analyzed 47 studies that used δ^{13}C, δ^{15}N, δ^{2}H, δ^{18}O , and δ^{34}S in tissues from fish, amphibian, reptile, bird, and mammal groups. Then, we built a database from the isotope ratios and metadata extracted from the publications.

Results. Studies have been using stable isotopes in wild and captive animals worldwide, with a particular concentration in Europe, covering all main vertebrate groups. 80.8% of the studies combined stable isotopes of carbon and nitrogen, and 88.2% used at least one of those elements. Fish is the most studied group, while amphibians are the least. Muscle was used in 83.3% of the works with fishes, while inert tissues were used in 87.0% of studies involving the other taxonomic groups. δ^{13}C and δ^{15}N standard deviation and range were significantly higher in the wild than in captive animals, suggesting a more variable diet in the first group. δ^{13}C tended to be higher in wild fishes but in captive mammals, birds, reptiles, and amphibians. δ^{15}N was higher in the wild terrestrial animals when controlling for diet. Only 5.7% of the studies failed to differentiate wild and captive animals using stable isotopes.

Conclusions. This review reveals that SIA can help distinguish between wild and captive in different vertebrate groups, rearing conditions, and methodological designs. Some aspects should be carefully considered to use the methodology properly, such as the wild and captive conditions, the tissue analyzed, and how homogeneous the samples are. Despite the increased use of SIA to distinguish wild from captive animals, some gaps remain since some taxonomic groups (e.g., amphibians), countries (e.g., Africa), and isotopes (e.g., d^2H, d^{18}O, and d^{34}S) have been little studied.

Differentiating wild from captive animals: an isotopic approach

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Abstract

26 **Background.** Wildlife farming can be an important but complex tool for conservation.

27 Wildlife farming should meet a variety of biophysical, market, and regulatory conditions to

28 achieve conservation benefits. Regulatory controls, in turn, are often based on certification and

29 traceability requirements to identify the animals' origin. The traditional techniques for

30 discriminating between wild and captive animals are insufficient to prevent doubts or

31 misdeclaration, especially when labels are not expected or mandatory. There is a pressing need to

32 develop more accurate techniques to discriminate between wild and captive animals and their

33 products. Stable isotope analysis has been used to identify animal provenance, and some studies

34 have successfully demonstrated its potential to differentiate wild from captive animals. In this

35 literature review, we examined an extensive collection of publications to develop an overall

36 picture of the application of stable isotopes to distinguish between wild and captive animals

37 focusing on evaluating the potential and patterns of this tool.

38 **Survey methodology**. We searched peer-reviewed publications in the Web of Science database

39 and the references list from the main studies and reviews on the subject. We selected and

40 analyzed 47 studies that used δ^{13}C, δ^{15}N, δ^2H, δ^{18}O, and δ^{34}S in tissues from fish, amphibian,

41 reptile, bird, and mammal groups. Then, we built a database from the isotope ratios and metadata

42 extracted from the publications.

43 **Results.** Studies have been using stable isotopes in wild and captive animals worldwide, with a

44 particular concentration in Europe, covering all main vertebrate groups. A total of 80.8% of the

45 studies combined stable isotopes of carbon and nitrogen, and 88.2% used at least one of those

46 elements. Fish is the most studied group, while amphibians are the least. Muscle was used in

47 83.3% of the works with fishes, while inert tissues were used in 87.0% of studies involving the

48 other taxonomic groups. δ^{13}C and δ^{15}N standard deviation and range were significantly higher in

49 the wild than in captive animals, suggesting a more variable diet in the first group. δ^{13}C tended to

50 be higher in wild fishes and in captive mammals, birds, reptiles, and amphibians. δ^{15}N was

51 higher in the wild terrestrial animals when controlling for diet. Only 5.7% of the studies failed to

52 differentiate wild and captive animals using stable isotopes.

53 **Conclusions.** This review reveals that stable isotope analysis (SIA) can help distinguish between

54 wild and captive in different vertebrate groups, rearing conditions, and methodological designs.

55 Some aspects should be carefully considered to use the methodology properly, such as the wild

56 and captive conditions, the tissue analyzed, and how homogeneous the samples are. Despite the

57 increased use of SIA to distinguish wild from captive animals, some gaps remain for some

58 taxonomic groups (e.g., amphibians), geographic regions (e.g., Africa), and isotopes (e.g., d^{2}H,

59 d^{18}O, and d^{34}S), which have been little studied.

60

Introduction

61

62 Human activities are the most relevant causes of defaunation, mainly habitat degradation and
63 species overexploitation (Young et al., 2016). The maintenance and management of wild species
64 in captivity can be an important tool for conservation, either by maintaining gene banks or target
65 species that would be unlikely to survive in the natural environment (CDB, 1992; Maxted, 2013)
66 or by decreasing the pressure on wild population by wildlife farming (Bulte & Damania, 2005;
67 Damania & Bulte, 2007; Nogueira & Nogueira-Filho, 2011; Rizzolo, 2020).
68   Wildlife farming is a broad term related to the domestication, production, trade, and
69 consumption of live wildlife and their products, involving a variety of contexts and species born
70 or raised in captivity (Rizzolo, 2020). The legalization of the production and trade of wild
71 animals can be considered a form of supply-side conservation, avoiding wild stock depletion and
72 deforestation, which benefits the recovery and maintenance of the wild population (Bulte &
73 Damania, 2005; Damania & Bulte, 2007; Nogueira & Nogueira-Filho, 2011; Phelps, Carrasco &
74 Webb, 2014).
75   However, wildlife farming is not a simple and uncontentious conservation strategy
76 (Tensen, 2016; Janssen & Chng, 2018; Challender et al., 2019). The increase in the wildlife
77 market brings concerns about production control, management, and trade (Lyons & Natusch,
78 2011; García-Díaz et al., 2015; Janssen & Chng, 2018). There are a variety of biophysical,
79 market, and regulatory conditions that wildlife farming should meet to achieve conservation
80 benefits (Phelps, Carrasco & Webb, 2014; Tensen, 2016; Janssen & Chng, 2018; Challender et
81 al., 2019). In turn, regulatory controls are often based on certification and traceability
82 requirements to identify the animals' origin preventing incorrect or fraudulent statements, alien
83 species invasion, and minimizing damage to wild populations and human health (EC, 2022;
84 Smith et al., 2017; Staerk *et al.,* 2018).
85   One of the main problems in regulating wildlife farming is identifying the traded animals'
86 real origin (wild or captive) (Tensen, 2016; EC, 2022). The traditional techniques for
87 discriminating between wild and captive animals, such as trader declarations, government-issued
88 licenses, bands, or microchips, may not be sufficient to prevent doubts or misdeclaration (Lyons
89 & Natusch, 2011; Livingstone & Shepherd, 2016; Tensen, 2016). The efficiency of such
90 methods is even more limited when labels are not expected or mandatory, such as in the trade of
91 animal parts or aquaculture products in most countries, and to identify unmarked escaped or
92 released animals (e.g., Oceana, 2015). Thus, there is a pressing need to develop more accurate
93 techniques to discriminate between wild and captive animals and their products.
94   Stable isotopes are endogenous markers capable of identifying the origin of various
95 samples, such as food, water, or organic materials. The technique has become consolidated as
96 providing biomarkers of animals' geographic origin (Hobson & Wassenaar, 2019). It has also
97 helped track individuals' ecological traits, such as habitat use and diet (Shipley & Matich, 2020),
98 making stable isotopes a potential tool for identifying the origin of animals also regarding the
99 rearing system: wild or captive (Camin et al., 2016; Truonghuynh, Li & Jaganathan, 2020).
100   Stable isotope analysis (SIA) is based on the variation in the ratio between an element's
101 light and heavy forms (expressed by the letter δ) in response to environmental patterns. As

102 animals access and metabolize environmental resources, their tissues incorporate and express the
103 natural variability in the stable isotopic ratios. Typically, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ are categorized as
104 local spatial assays, and their variation in animal tissues reflects their diet composition, such as
105 the primary energy source (e.g., C_3 or C_4 plants; marine or terrestrial resources) and trophic
106 position, while δ^2H and $\delta^{18}O$ reflect geographic origin and movement in response to hydrological
107 process (Hobson & Clark, 1992; Fry, 2008; Hobson & Wassenaar, 2019).
108   However, stable isotope ratios in animal tissues may be influenced by complex
109 physiological processes leading to changes in the isotopic signal compared to the diet (diet-tissue
110 fractionation) and between different tissues (tissue-tissue fractionation). The isotopic
111 fractionation and the speed of incorporation in animals' organic structures, in turn, depends on
112 several environmental, nutritional, and physiological factors, such as tissue turnover rate
113 (Tieszen et al., 1983; Hobson & Clark, 1992; Vander Zanden et al., 2015), diet composition
114 (DeNiro & Epstein, 1977; Magozzi et al., 2019; Whiteman et al., 2021), reproductive and
115 nutritional states (Doi, Akamatsu & González, 2017; Shipley & Matich, 2020). Comparing wild
116 and captive animals using stable isotopes should consider additional points to avoid confounding
117 effects, such as the characteristics of captivity (e.g., intensive or extensive farming), and whether
118 the animal changed the captive/wild state and the timescales involved.
119   There are a variety of wildlife farming modalities according to different criteria, such as
120 the management intensity (herding, ranching, and farming) (Hudson et al. 1989) or according to
121 the system used to produce specimens (born in captivity, bred in captivity or ranched) (CITES
122 classification; Lyons, Natusch and Jenkins, 2017). These modalities can subject captive animals
123 to significantly different conditions, influencing their isotopic signature. However, the potential
124 of SIA to differentiate wild from captive animals increases under the general assumption that
125 individuals are likely to access different resource items from distinct geographic origins in these
126 two environments (e.g., Dempson and Power, 2004; Kays and Feranec, 2011; Chaguri et al.,
127 2017; Natusch et al., 2017). Additionally, captive specimens are less subjected to some of the
128 main factors influencing isotopic fractionation and tissue turnover, such as natural habitat
129 gradients, seasonality, complex food chain, diet quality variation, and nutritional stress (Shipley
130 & Matich, 2020).
131   Several studies have successfully demonstrated the potential application of SIA to
132 differentiate wild from captive animals (Dittrich, Struck & Rödel, 2017; Natusch et al., 2017;
133 Brandis et al., 2018; Alexander et al., 2019; Andersson et al., 2021; Hopkins et al., 2022).
134 However, there is no scientific compilation of the topic. This study examined an extensive
135 collection of publications using SIA in wild and captive animals. The available data in the
136 literature was organized in a database, making the systematized information available for
137 academic and applied purposes. We performed qualitative and quantitative analyses to develop
138 an overall picture of the application of stable isotopes to distinguish between wild and captive
139 animals focusing on: (1) evaluating the potential of this tool to distinguish individuals from wild
140 and captive; (2) searching for discernible patterns of how such differences occur.
141

Survey methodology

Data Source and compilation

We searched peer-reviewed publications in the Web of Science database (https://clarivate.com/webofsciencegroup/solutions/web-of-science/) from 1945 to 2021 using the terms "isotop*" AND "wild OR free-rang*" AND "captiv* or farm*" as a topic. We added the search terms "NOT 'chicken OR hen* OR cattle OR pig'" to exclude domestic animals from the results. The search returned 295 hits, initially sorted based on the title, keywords, and abstract. First, we considered studies of stable isotopes involving any nondomestic vertebrate species. In a second instance, we selected only research related to carbon, nitrogen, hydrogen, oxygen, or sulfur isotopes that met one of the following criteria: (1) used stable isotope to differentiate wild from captive animals; (2) conducted the isotopic analysis in wild and captive individuals of the same species in the same study. Paleontological publications and those studies exclusively using compound-specific isotope analysis (CSIA) techniques were excluded. We also excluded studies that did not show 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 filtering steps, 47 studies remained to be analyzed in this review (Table S1). We also checked the references list from the main studies and reviews to ensure that all relevant papers were included.

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 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

We selected variables related to the taxon classification, biology and morphology of animals, samples data (tissue, geographic location, year, and period), rearing system, isotopic records (values of mean, standard deviation, minimum and maximum, range), methodological records from the isotope analyses (analytical error, lipid extraction, and international reference material), and identification of publication were registered (Table 1 and Table S1). Regarding the rearing system, we classified the animals as "wild" or "captive." On some occasions, the origin of the samples was uncertain; thus, we followed the authors' conclusions about their wild or captive origin. All stable isotope results are expressed in the conventional delta (δ) notation, in units per mil (‰).

We used the systematized metadata of our database to present an overall picture of the studies analyzing stable isotopes in wild and captive animals. Additionally, we used the isotopic ratios' mean, standard deviation, and range of wild and captive animals from the database to evaluate general differences and similarities. For this, we performed Student's t-test for differences in $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$ considering the whole database in total. We also tested for differences between wild and captive animals for $\delta^{13}C$ and $\delta^{15}N$ per continent, taxon group, and/or dietary category. We also performed Student's t-test or one-way ANOVA to

182   compare $\delta^{13}C$ and $\delta^{15}N$ in wild and captive animals considering the terrestrial taxon together
183   (amphibian, reptile, bird, and mammal) and fish per continent. Due to the lack of suitable studies,
184   we did not perform more detailed analyses for δ^2H, $\delta^{18}O$, and $\delta^{34}S$. In addition, despite the
185   possible effects of different types of captivity on the isotopic signature of animals, we could not
186   access details of captivity conditions in most studies, making it impossible to categorize them as
187   suggested by Hudson et al. (1989) or CITES. Thus, in the database, they were called "captivity"
188   only.
189   To gain a deeper understanding, we also assessed each study individually. For studies
190   that analyzed isotopic differences between the two environments, we relied on the results
191   reported by the authors. For studies that measured but did not compare isotopic ratios in the wild
192   and captivity, we tested for such differences (*t*-test, ANOVA, Wilcox-test, Kruskal-Wallis, and
193   linear mixed model) when the original data were available. In this approach, we considered the
194   different categories of wild or captive when explicitly presented by the authors. Inferential tests
195   were preceded by analyses of normality (Shapiro-Wilk test) and equality of variances. We
196   performed statistical tests in the R platform, version 4.1.0 (R Development Core Team), with a
197   significance level of 5% in all hypothesis testing.
198
199   **Quality assurance and control**
200   As part of the quality assurance, we carefully checked the data in different steps of the database
201   building, trying to keep the information as close as possible to the original one. For example, we
202   double-checked for mean, standard deviation, and range of isotopic ratios extracted by
203   PlotDigitizer. We also double-checked the original source outliers detected by boxplots for the
204   same variables.
205   When not provided, the geographic coordinates of the samples were estimated based on
206   the authors' most detailed geographic information (e.g., city, region, fishing area zones). Species
207   names were kept as in the original publications, and as "fishes" are a paraphyletic group, we
208   checked the taxonomic class of each species using Eschmeyer's Catalog of Fishes (Van der Laan
209   & Fricke, 2022). Species diet classifications were checked using the R package Sider (Healy et
210   al., 2018) or searched for in peer-reviewed papers.
211

Results

213   **An overall picture of the use of stable isotopes to differentiate wild and captive animals**
214   The first studies using stable isotopes to distinguish wild and captive animals dated around two
215   decades ago and aimed to evaluate the potential of SIA as a tool to differentiate wild from recent
216   farm-escaped salmon (*Salar salar,* Dempson & Power, 2004) and mink (*Mustela vison,*
217   Hammershøj, Asferg & Kristensen, 2004). Since then, the number of studies using this tool and
218   its applications has been growing.
219   From the 47 selected publications, 33 used stable isotopes to distinguish wild from
220   captive animals (Table S2), and 14 analyzed stable isotopes in wild and captive vertebrates for
221   different purposes (Table S3). We found studies distributed in 37 countries worldwide (Fig. 1A),

222 with the United States and Italy having more publications (n = 6 each). Regarding the continents,
223 Europe (n = 21) and Africa (n = 3) had the largest and smallest number of studies, respectively.
224   Fifty-five species from different vertebrate taxonomic groups (mammals, birds, reptiles,
225 amphibians, and fishes) were studied (Fig. S1). The most studied groups varied on different
226 continents: while studies on reptiles and amphibians were concentrated in Asia and Oceania,
227 studies with fish were distributed worldwide (Fig. S1). All studies focused on one species (n =
228 36) or a group of species from the same taxonomic class (n = 11). Amphibia was the least
229 representative group, recording in only one study, while fish accounted for over 46% of the
230 publications (Fig. 1B).
231   Carbon and nitrogen stable isotopes in combination accounted for 80.8% of the studies,
232 and 88.2% used at least one of those elements (Fig. 1C). Hydrogen, oxygen, and sulfur stable
233 isotopes together account for less than 15% of the publications (Fig. 1C). Muscle and inert
234 organic structures were the most analyzed tissues presenting in 46.81% and 42.55% of the
235 studies, respectively. Muscle was used in 83.33% of the works with fishes, while inert tissues
236 were used in 86.96% of studies involving the other taxonomic groups.
237   The experimental design, captivity condition, and description of the publications varied
238 widely (Table S4). While some studies measured stable isotopes for only two categories (wild
239 and captive) of the same species at the same moment and in the same geographic regions, others
240 had a complex design, considering several types of wild or captive, various species, countries,
241 period, and samples origin.
242
243 **Patterns and potential of SIA in distinguishing between wild and captive animals**
244 *Analyses of the systematized database*
245 The mean for $\delta^{18}O$ and the standard deviation and range for $\delta^{13}C$ and $\delta^{15}N$ were significantly
246 higher in the wild than in captive animals (Table 2). $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ standard deviation
247 tended to be higher in wild animals, but it was not significant (Table 2). The isotopic differences
248 in the rearing system varied with the geographic location, taxonomic group, and diet (Fig. S2).
249      $\delta^{13}C$ of the terrestrial taxon (amphibian, reptile, bird, and mammal) were significantly
250 higher in captive animals ($t_{121} = 2.40$, $p = 0.02$). At the same time, the $\delta^{15}N$ was higher in the
251 wild animals but only when controlling for diet type ($F_{1,1} = 363.4$, $p = 0.03$). Captive fishes
252 exhibited significantly lower $\delta^{13}C$ in Europe ($t_{37} = -2.07$; $p = 0.05$). The isotopic space occupied
253 by individuals considering C and N simultaneously tended to diverge in all taxonomic groups,
254 either at the mean position or range (Fig. S3).
255
256 *Analysis of the publications individually*
257 Overall, 83.9% of the studies found significant differences or no overlapping among categories
258 of wild and captive animals analyzed, while 16.1% distinguished among some modalities where
259 authors used different wild or captive conditions. In addition, no publication failed to
260 differentiate between all categories of wild and captive (Table S2). Accurately identifying the
261 group to which individuals belonged (wild or captive) ranged from 58% to 100% when using

262 discriminant tests. To identify differences between wild and captive individuals, the studies
263 performed graphical analyses of overlapping, discriminant tests, frequentist statistics (such as *t*-
264 tests and ANOVA), or the combination of the last two statistical methods.
265   From the studies using δ^{13}C and δ^{15}N, 90.3% and 89.3% reported significant differences
266 between wild and captive animals, respectively. The δ^{13}C was usually higher in the wild than in
267 captive fishes. The opposite was found for the other taxonomic groups (Fig. 2). On the other
268 hand, δ^{15}N was consistently higher in wild birds and did not show clear trends in the other
269 taxonomic groups (amphibians could not be evaluated since there was only one study) (Fig. 2).
270 The studies used δ^{2}H and δ^{18}O when geographic variation was also involved (δ^{2}H in birds and
271 reptiles and the δ^{18}O in fishes and amphibians). The δ^{34}S was used in two studies with birds or
272 fishes, involving expected differences in the proportion of marine/terrestrial diet (Table S2).
273   Regarding the publications that measured but did not compare stable isotopes in wild and
274 captive animals, we accessed the original data for 10 of 14 studies. We could distinguish
275 between all (64.3%) or some (21.4%) categories in which authors used different wild or captive
276 conditions. We found no differences between treatments in 14.3% of the publications (Table S5).
277
278 **Discussion**
279 **An overall picture of the use of stable isotopes to differentiate wild and captive animals**
280 The development and understanding of methods to accurately identify the origin of an animal are
281 crucial to ensure that wildlife farming fulfills its role as a conservation strategy (Phelps, Carrasco
282 & Webb, 2014; Tensen, 2016; Lyons, Natusch, and Jenkins, 2017; Natusch, 2018). Stable
283 isotope analyses are an important biomarker of animal provenance. Here we summarize the main
284 aspects of the use of stable isotopes as a tool to differentiate wild and captive animals.
285   Fish was the most studied group involving taxa of a single class (Actinopteri) and the
286 species mainly used for human consumption (e.g., European seabass, meagre, and different
287 species of salmon). Only two fish species were considered globally threatened by IUCN and
288 included in the CITES appendix (Tables S3). These findings suggest that beyond environmental
289 impacts, identifying seafood origin involves concerns related to food safety, leading to labeling
290 regulation about the rearing system in European Union and the United States (EC, 2001, AMS,
291 2009). However, few countries have clear requirements for the origin of the breeding system yet
292 (El Sheikha & Xu, 2017).
293   Mammals, birds, and reptiles studies were mainly associated with ecological or forensic
294 purposes, such as identifying the origin of a wolf population (*Canis lupus*) in an area where they
295 were previously extinct (Kays & Feranec, 2011), the use of stable isotopes to identify the
296 provenance of invasive alien species (*Trachemis scripta*) (Hill et al., 2020), or to detect crocodile
297 lizard (*Shinisaurus crocodilurus*), short-beak echidnas (*Tachyglossus aculeatus*), and yellow-
298 crested cockatoos (*Cacatua sulphurea*) laundering (van Schingen et al., 2016; Brandis et al.,
299 2018; Andersson et al., 2021). Most studies in mammals, birds, or reptiles involved living
300 species listed in the CITES appendices and relied on inert keratinous tissues, demonstrating SIA
301 as a noninvasive biomarker of the rearing system.

302   Around 90% of the studies relied on $\delta^{13}C$ and $\delta^{15}N$ in animal tissues, which was not a
303   surprise, considering the assumption that wild and captive animals have different diets, which is
304   reflected in their isotopic ratios (e.g., Dempson and Power, 2004; Natusch et al., 2017; Hill et al.,
305   2020). The δ^2H and $\delta^{18}O$ have been largely used to infer animals' geographic origin and
306   movement due to their pattern of variation in response to hydrological processes and the linkage
307   with those in animal tissues (Hobson & Wassenaar, 2019). However, the tissue-environment
308   relationship of δ^2H and $\delta^{18}O$ may also be affected by local factors, such as food-web
309   relationships (Vander Zanden et al., 2016), physiology, and the proportion of water in the
310   animals' diet (Magozzi et al., 2019). The lack of dietary and trophic studies using δ^2H and $\delta^{18}O$
311   is also reflected in the wild-versus-captive studies. No research used $\delta^{34}S$ exclusively to
312   distinguish wild from captive animals.
313
314   **Patterns and potential of SIA in distinguishing between wild and captive animals**
315   *Analyses of the systematized database*
316   We observed significant differences in the $\delta^{18}O$ between wild and captive animals. These
317   findings suggest that δ^2H and $\delta^{18}O$ may be underused in differentiating wild and captive animals
318   since these elements represented less than 10% of the isotopes analyzed. The $\delta^{13}C$ and $\delta^{15}N$
319   standard deviations were significantly higher in the wild compared to captive, supporting the
320   assumption that wild conditions tend to be more variable than captive ones, regardless of the
321   specific circumstances. Such dispersion variables can be particularly helpful in identifying the
322   rearing system of a group instead of one particular individual. Few studies mention data
323   dispersion variables to infer animals' origin (Molketin et al, 2007, Busetto et al, 2008, Van
324   Schingen et al, 2016, Dittrich et al, 2016). Our results suggest those variables could be relevant
325   in differentiating wild and captive animals.
326   Although most studies could distinguish isotopically wild from captive animals (see
327   below), there were few differences when considering the entire database simultaneously. Some
328   patterns emerged as analyses were performed on more homogeneous groups (e.g., only terrestrial
329   taxon or fish in Europe), suggesting that the isotopic differences between wild and captive
330   animals are not unidirectional. Rather, such differences appear to vary by location and taxonomic
331   group. Thus, using SIA to identify the rearing system origin of animals may perform better the
332   more homogeneous the samples.
333
334   *Analysis of the publications individually*
335   More than 80% of the publications that looked for isotopic differences between wild and captive
336   animals were successful. In some research, the animals' origin was inferred by the authors or
337   informed by traders or labels, which may have influenced the results. The studies with the lowest
338   performance involved fish (Pereira et al., 2019; Molkentin et al., 2007, 2015; Wang et al., 2018;
339   Vasconi et al., 2019; Liu et al., 2020). The complex experimental design, the largest number of
340   variables involved (including different fish farming models) (Table S3), and the uncertainty of
341   the samples' origin were probably the main reasons this group performed worse than terrestrial

342   taxon. Conversely, other studies could isotopically distinguish animals from different rearing
343   systems at even more detailed levels, such as distinguishing different breeders of the same
344   species (Castelli & Reed, 2017).
345   Despite the specifics of each study, $\delta^{13}C$ was consistently lower in the wild than in
346   captive terrestrial animals (Fig. 2), indicating consumption of higher levels of C4 plant-based
347   food for captive individuals compared to wild populations of the same taxon. Such a pattern
348   indicates the composition of industrial food provided to these captive animals, based mainly on
349   less expensive items, such as corn (Kays & Feranec, 2011). In contrast, wild fishes exhibited
350   higher $\delta^{13}C$ than captive ones in most studies. Multiple factors can explain these findings, such
351   as captive fishes tend to have higher lipid concentrations than wild ones, leading to lower $\delta^{13}C$
352   (DeNiro & Epstein, 1977; Focken & Becker, 1998; Serrano, Blanes & Orero, 2007; Fasolato et
353   al., 2010), most fish studies included carnivore marine or migratory species in Europe, where the
354   composition of conventional aquafeeds is based on terrestrial plant ingredients such as cereals,
355   soy, legumes, and plant-derived oils, which has lower $\delta^{13}C$ than fish-based diet typical of
356   carnivore wild marine fishes (Schoeninger & DeNiro, 1984; Farabegoli et al., 2018; Wang et al.,
357   2018).
358   Finally, we found differences between wild and captive animals in more than 85% of the
359   studies that did not compare individuals in these two environments. These results suggest the
360   potential of isotopes to differentiate wild and captive animals even when the research was not
361   designed to look for such differences. They are also particularly relevant, considering the
362   publication bias of positive results (Mlinarić, Horvat & Šupak Smolčić, 2017), which could
363   overestimate the capacity of SIA in identifying animals' origin. The only two studies that found
364   no differences relied exclusively on $\delta^{13}C$ and had high variability and unbalanced samples (Cree
365   et al., 1999; Hammershøj et al., 2004). Using more than one isotopic element and more sensitive
366   or complex statistical tests (e.g., multivariate analysis) could help find masked differences
367   between wild and captive groups.
368
369   *Improving isotopic research to distinguish between wild and captive animals*
370   Recognizing the differences in the experimental design and objectives of each study, some
371   approaches can be considered to enhance and improve the use of stable isotopes to distinguish
372   between wild and captive animals. First and crucial, the study should define known captive or
373   wild origin samples for comparison whenever possible, whether directly sampled or from the
374   literature.
375   Second, the choice of the tissue used in the research is fundamental. Metabolically
376   active tissues reflect distinct temporal integration time according to their turnover rate, varying
377   from a few days (e.g., blood plasma), months (e.g., muscle), or lifetime (E.g., bone collagen)
378   (Tieszen et al., 1983; Vander Zanden et al., 2015; Carter, Bauchinger & McWilliams, 2019).
379   Conversely, the isotopic ratio of keratinous tissues, such as feathers, claws, and scales, will
380   reflect where they were synthesized since they are metabolically inert after their formation
381   (Mizutani et al., 1990; Hobson & Clark, 1992). Therefore, under changes between rearing

382   systems, the study should identify the exact period of change to avoid any confounding effect of
383   feeding changes and consider the tissue that captures the time elapsed since the rearing change.
384   Third, to improve the evaluation and understanding of the data, special attention must be
385   given to presenting the methodology and results. Measures of central tendency, such as each
386   treatment's mean and standard deviation, should always be reported. The wild and captive
387   conditions should also be carefully considered and described to ensure correct interpretation of
388   the results.
389   Despite increased publications using stable isotopes as tracers of wildlife origin in recent
390   decades, there are still some important gaps that future studies could address. Some taxon groups
391   are highly underrepresented, especially amphibians, with only one study. The influence of
392   different categories of captivity on isotope ratios also needs to be better explored and understood.
393   Most studies are in Europe and North America, while some high diversity and threatened regions
394   in South America and Africa have been little studied. Regarding the isotopes, $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$
395   were only occasionally analyzed, and the potential of $\delta^{18}O$ and $\delta^{2}H$ to distinguish wild and
396   captive animals based on differences in physiological conditions in these environments remains
397   unexplored.
398

Conclusions
400   Our study reveals that SIA can help distinguish between wild and captive in different vertebrate
401   groups, rearing conditions, and methodological designs. Despite the variety of publications
402   reviewed, we could observe some distinct patterns in how these differences occur, such as the
403   higher diet variability in wild animals and the preferential use of plant-based food in captivity.
404   Nevertheless, some aspects should be carefully considered for the proper use of the
405   methodology, such as knowing the wild and captive conditions of the animals studied and having
406   samples of known origin to use as a basis for comparison. Additionally, the methodology seems
407   to perform better the more homogeneous samples are since the direction of differences between
408   wild and captive animals can vary greatly according to local and taxonomic specificities.
409   However, many gaps remain to be filled, especially in the unbalanced taxon, region, and
410   isotope studied. We expect the present study to expand the use and acceptance of SIA as a
411   reliable tool in identifying animals' rearing system origin and, consequently, contributing to the
412   efficiency of wildlife farming as a conservation strategy and protecting natural populations.
413

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418

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594

Figure 1

Distribution of studies using stable isotopes.

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).

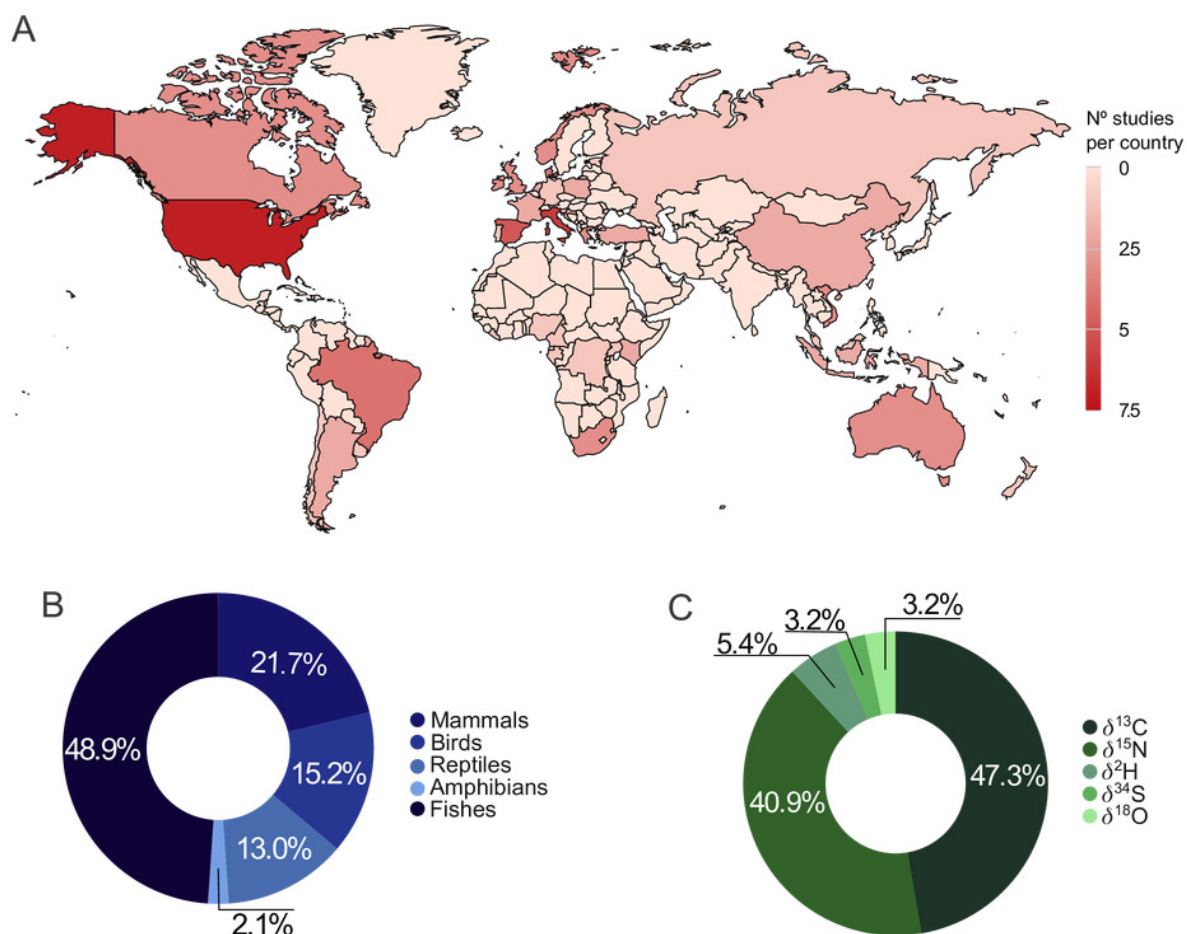
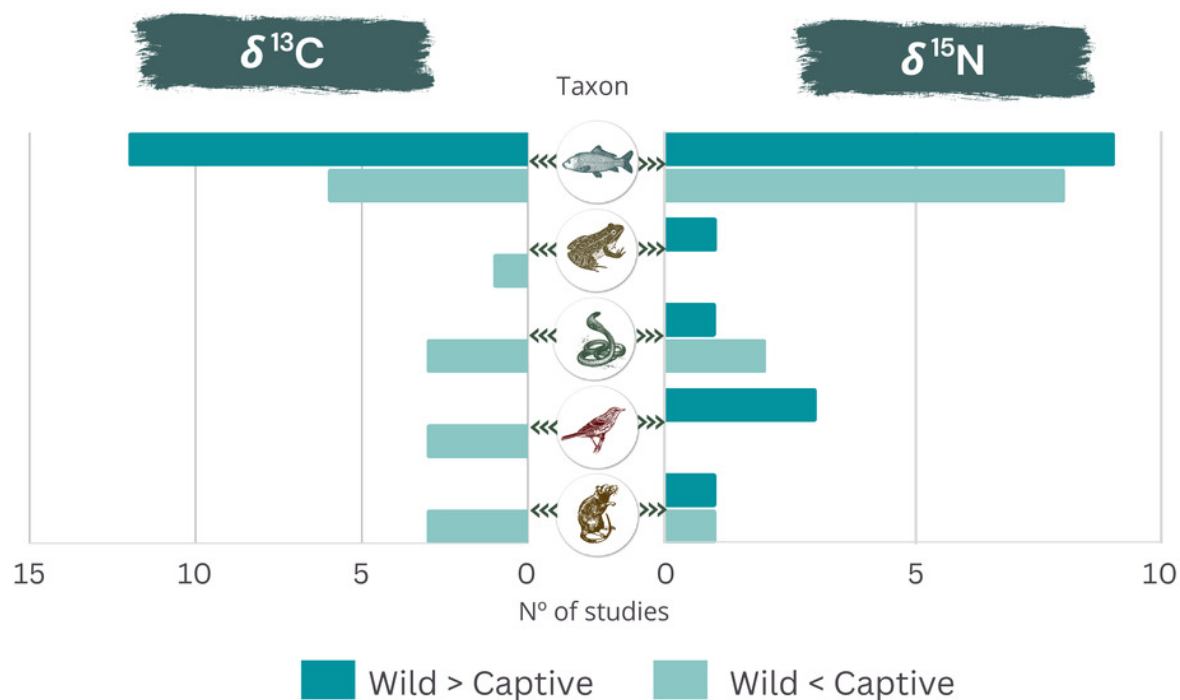



Figure 2

Quantitative comparison between wild and captive animals by taxonomic group.

Quantitative comparison (higher or lower) of δ^{13}C (left) and δ^{15}N (right) between wild and captive animals by taxonomic group.

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

1   **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).
Sub-tissue	A specific part of a given tissue (e.g., red blood cells, type of feathers).
Rearing System	wild or 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 $\delta^z x$ (‰)	isotopic ratio means.
SD $\delta^z x$ (‰)	isotopic ratio standard deviation
MIN $\delta^z x$ (‰)	isotopic ratio minimum value
MAX $\delta^z x$ (‰)	isotopic ratio maximum value
Range $\delta^z x$ (‰)	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 |

Comparison of the mean isotopic ratios, standard deviation, and range in captive and wild animals considering all analyzed publications.

Comparison of the mean isotopic ratios, standard deviation, and range of δ^{13}C, δ^{15}N, δ^{2}H, δ^{18}O, and δ^{34}S in captive and wild animals considering all 47 analyzed publications. Significant differences are indicated by different letters ($p < 0.05$).

1 **Table 2**. Comparison of the mean isotopic ratios, standard deviation, and range of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$,

2 $\delta^{18}O$, and $\delta^{34}S$ in captive and wild animals considering all 47 analyzed publications. Significant

3 differences are indicated by different letters (p < 0.05).

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{18}O$	$\delta^{34}S$
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