UNIVERSIDADE DE BRASÍLIA
Faculdade UnB de Planaltina - FUP
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Luiza Brasileiro Reis Pereira

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Tese apresentada ao Programa de Pós-Graduação em Ciências Ambientais da Universidade de Brasília, para a obtenção do Título de Doutor em Ciências Ambientais.

Orientadora: Gabriela Bielefeld Nardoto

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Vida livre ou cativeiro? Ferramentas isotópicas para identificar a origem de pássaros silvestres

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“A utopia está lá no horizonte. Me aproximo dois passos, ela se afasta dois passos. Caminho dez passos e o horizonte corre dez passos. Por mais que eu caminhe, jamais alcançarei. Para que serve a utopia? Serve para isso: para que eu não deixe de caminhar.”

(Eduardo Galeano)

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RESUMO

Nos últimos séculos tem sido desencadeado um período de intensa perda de espécies, comparado às cinco ondas de extinção em massa já existentes na Terra. A sexta onda de extinção, ao contrário das anteriores, é causada basicamente por ações antrópicas levando a uma perda de fauna global e afetando os diferentes grupos taxonômicos. Dentre as principais ações antrópicas que têm efeito direto sobre a fauna está a sua sobre-exploração cujo efeito, apesar de já conhecido, nunca foi efetivamente combatido. A caça, coleta, captura e o comércio irregular de animais silvestres embora ocorra em todo mundo, apresenta particularidades locais, e, no Brasil, parece estar voltado especialmente para o abastecimento de um mercado consumidor de animais de estimação, especialmente pássaros canoros. Apesar de já ser possível identificar padrões quanto ao tráfico de animais silvestres no país, algumas questões ainda precisam ser melhor debatidas, como a possível relação entre a criação autorizada e a ilegal de espécimes nativos. Estudos têm apontado que a existência de um mercado legal de animais silvestres apenas pode funcionar para fins conservacionistas se, dentre outros critérios, não houver fraude na origem das espécies comercializadas. Nesse sentido, é fundamental o desenvolvimento de novas técnicas que permitam distinguir animais legais (nascidos em cativeiro) dos "esquentados" (capturados na natureza e colocados em cativeiro com aparência de regulares). O principal objetivo dessa Tese é o aprimoramento de uma ferramenta para a distinção entre pássaros silvestres oriundos de vida livre e cativeiro por meio dos isótopos estáveis. No primeiro capítulo, foi realizada uma revisão na literatura das publicações já existentes utilizando isótopos estáveis em vertebrados de vida livre e cativeiro, demonstrando a relevância da ferramenta, especialmente C e N, em distinguir esses dois grupos em diferentes táxons. O segundo capítulo traz análises de diferenças isotópicas em penas de indivíduos da família Thraupidae de vida livre e cativeiro em todo o Brasil, além de modelos de classificação com base nos valores de $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ e $\delta^{18}O$ para identificar a origem de amostras resultantes de apreensões de aves de origem desconhecida. Os valores de $\delta^{13}C$, $\delta^{2}H$ e $\delta^{15}N$, diferiram significativamente entre os indivíduos de vida livre e cativeiro, sendo $\delta^{13}C$ e $\delta^{2}H$ os principais preditores utilizados pelo modelo de classificação, com acurácia média de 0.92. Por fim, no terceiro capítulo foram elaboradas novas *isoscapes* de $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ e $\delta^{18}O$ para penas de indivíduos de vida livre e de cativeiro da família Thraupidae, com poder preditivo comparáveis a outros modelos elaborados para o Brasil. O desenvolvimento das ferramentas aqui propostas poderá servir como base para outros estudos tanto ecológicos como forenses e auxiliar órgãos governamentais no combate ao tráfico de animais silvestres.

Palavras-chave: tráfico de animais, isótopos estáveis, rastreamento forense, perícia criminal, ilegalidades contra a fauna, vida livre, cativeiro, aves

ABSTRACT

In recent centuries, the planet has witnessed an intense period of species loss comparable to the five previous mass extinction events. Unlike the earlier waves, the sixth mass extinction is predominantly driven by anthropogenic actions, leading to global fauna decline and affecting different taxonomic groups. Species overexploitation is among the main anthropogenic activities directly impacting fauna, and being well-known, it has never been effectively addressed. Although widespread worldwide, hunting, collection, poaching, and illegal wildlife trade (IWT) have distinct local characteristics. In Brazil, these activities seem particularly aimed at supplying a pet trade market, especially for songbirds. Although it is already possible to identify patterns regarding wildlife trafficking in the country, certain issues still require further discussion, such as the potential link between authorized breeding and the illegal trade of native species. Studies suggest that the existence of a legal wildlife market can only serve conservation purposes if there is no "laundering" of trafficked species. The development of new techniques to distinguish legally bred (captive-born) animals from "laundered" ones (wild-caught and presented as captive-bred) is crucial. The main objective of this dissertation is to improve a new tool for distinguishing wild birds from those originating in captivity through stable isotope analysis. In the first chapter, I conducted a literature review of the existing studies employing stable isotopes in wild and captive vertebrates, highlighting the relevance of this tool in differentiating these two rearing systems across various taxa, particularly using C and N. The second chapter presents analyses of isotopic differences in feathers from wild and captive birds of the family Thraupidae across Brazil, including classification models based on δ13C, δ15N, δ2H, and δ18O values to identify the origin of samples seized from birds of unknown provenance. The δ13C, δ15N, and δ2H values showed significant differences between wild and captive individuals. Among these, $\delta^{13}C$ and $\delta^{2}H$ stood out as the primary predictors in the classification model, which achieved an average accuracy of 0.92. Finally, the third chapter provides newly developed isoscapes for $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$ in feathers from wild and captive individuals of the Thraupidae family with predictive power comparable to other models created for Brazil. I expect that the tools developed in this study will serve as a foundation for other ecological and forensic investigations, supporting governmental agencies in combating wildlife trafficking.

Keywords: wildlife trafficking, stable isotopes, forensic tracking, criminal investigation, wildlife crimes, wild-caught, captive, birds.

SUMÁRIO

1. INTRODUÇÃO GERAL

Nos últimos séculos tem sido desencadeado um período de intensa perda de biodiversidade comparável às cinco ondas de extinção em massa já ocorridas na história da Terra (Barnosky et al. 2011). A sexta onda de extinção, no entanto, tem suas causas atribuídas especialmente a ações humanas, impactando os diversos *taxa* em todo o mundo. De tão intenso, o termo "*defaunation*" tem sido utilizado em analogia ao termo *"deforestation"* para se referir à perda de fauna não apenas em relação a extinção de espécies, mas também de populações ou abundância de indivíduos (Dirzo et al. 2014; Young et al. 2016; Gardner et al. 2019). Além das consequências negativas intrínsecas à perda de biodiversidade, Dirzo *et al*. (2014) elencam diversos serviços ecossistêmicos prejudicados com a perda de fauna e afirmam que, embora as principais causas já sejam bem conhecidas, nenhuma tem sido efetivamente mitigada em escala global.

Dentre as principais ações antrópicas que têm impactos negativos diretos sobre a fauna está a sua sobre-exploração, incluindo a caça, coleta, captura e o tráfico. Além da defaunação, o comércio ilegal de animais silvestres (IWT, do inglês *Illegal wildlife trade*) leva a outros inúmeros impactos socioambientais indiretos, como o comprometimento do bem estar animal (Regueira e Bernard 2012; Baker et al. 2013), a introdução de espécies exóticas (García-Díaz et al. 2015), disseminação de doenças epizootias e zoonóticas (Smith et al. 2017; Keskin et al. 2023) e o comprometimento da segurança e estabilidade nacional, à medida que pode envolver uma cadeia complexa, incluindo caçadores furtivos, atores armados não estatais, grupos criminosos internacionais e corrupção institucional (Lawsson and Vines, 2014; Zain, 2020; UNODC, 2024). O IWT é uma realidade em todo o mundo, porém os padrões podem mudar consideravelmente de acordo com o país ou região (Reuter e O'Regan 2017) e, portanto, a sua caracterização local é fundamental para o direcionamento de ações mais efetivas na prevenção ou combate.

O Brasil é um dos países de maior biodiversidade do planeta e encontra-se na região mais sensível à perda de fauna (Dirzo et al. 2014), o que junto com aspectos socioeconômicos e culturais o tornam caraterístico de países-fonte para o tráfico (Destro, De Marco, & Terribile, 2020, Wyatt, 2018, Young, 2016). Levantamentos realizados, tanto em escala regional quanto nacional, apontam que o tráfico ocorre em todo o país, e com padrões semelhantes quanto aos principais *taxa* impactados e o foco do mercado consumidor. Com exceção de alguns locais na região Norte, aves é o grupo mais impactado, representando 80% dos animais apreendidos por órgão ambientais no país (Destro et al. 2012; Dias Júnior, Cunha, e Dias 2014; Gutjahr et al. 2016; Oliveira, de Freitas Torres, e da Nóbrega Alves 2020). Dentre as aves, pássaros canoros

é o grupo mais afetado, especialmente algumas espécies da família Thraupidae (Tabela 1). Esse perfil sugere que o tráfico no Brasil ocorre especialmente para atender a uma demanda interna por animais de estimação particularmente focada em pássaros canoros.

Tabela 1. Cinco espécies mais apreendidas em diferentes levantamentos realizados no Brasil. Destro *et al.* (2012) realizaram levantamento dos animais recebidos nos Centros de Triagem de Animais Silvestres (CETAS) do IBAMA; Costa *et al.* (2019) realizaram levantamento em publicações referentes ao tráfico de animais; Freeland Brasil, 2023 realizaram levantamento em notícias publicadas na mídia. Das espécies elencadas, *C. brissonii* pertence à família Cardinalidae, e as demais à família Thraupidae (Clements et al., 2023).

Referência	Destro et al. 2012	Costa et al., 2018*	Freeland, 2023
Período	2005 - 2009	1998 - 2017	2018-2022
1º	*Sicalis flaveola*	*Sicalis flaveola*	*Sporophila caerulescens*
2º	*Saltator similis*	*Cyanoloxia brissonii*	*Sicalis flaveola*
3º	*Sporophila caerulescens*	*Sporophila caerulescens*	*Saltator similis*
4º	*Cyanoloxia brissonii*	*Sporophila angolensis*	*Cyanoloxia brissonii*
5º	*Sporophila angolensis*	*Saltator similis*	*Sporophila nigricollis*

* Avaliaram apenas aves

O abastecimento do mercado interno em um país de dimensões continentais pode ter impactos significativos sobre as populações locais (Morton et al. 2021). Em um levantamento sobre o tráfico realizado diretamente em oito feiras livres na Região Metropolitana de Recife - PE, Regueira e Bernard (2012) observaram uma média de 97 pássaros comercializados irregularmente por feira em cada visita. Considerando um cenário em que os animais levariam não mais que uma semana para serem vendidos, os autores estimaram a comercialização anual de mais de 40.000 aves apenas nas feiras estudadas. Adicionalmente, dados da FreeLand Brasil, mostram que entre 2018 e 2022 mais de 180 mil espécimes silvestres, um milhão de quilos de pescado e 38 mil quilos de caça foram notificados pela mídia no Brasil (Freeland, 2023).

Considerando as más condições em que os animais são mantidos; que muitos vêm a óbito antes de serem comercializados (Regueira e Bernard 2012; Ratchford, Allgood, e Todd 2013), bem como as subnotificações, as estimativas de animais comercializados ilegalmente podem chegar a números muito mais elevados. O impacto da atividade tem se mostrado tão intenso, que em vários locais já tem sido reportado o declínio ou mesmo extinção de espécie anteriormente consideradas comuns (Silva, 2012.; Fernandes-Ferreira et al. 2012; Oliveira, de Freitas Torres, e da Nóbrega Alves 2020).

Devido à natureza difusa, críptica e dinâmica da atividade, identificar o local da captura de animais silvestres não é tarefa simples (Destro et al. 2012; Wyatt et al. 2018; Destro, De Marco, e Terribile 2020). Nesse cenário, a criação autorizada de animais silvestres aparece como uma possível ferramenta de prevenir o comércio ilegal de animais silvestres, seja por meio da conservação *ex-situ* (CDB, 1992; Maxted, 2013), ou diminuindo a pressão sobre as populações naturais por meio da oferta de indivíduos oriundos de criação autorizada de animais silvestres em cativeiro, aqui designado como *wildlife farming* (Bulte e Damania 2005; Damania e Bulte 2007; Nogueira e Nogueira-Filho 2011; Rizzolo 2021).

A Convenção sobre o Comércio Internacional das Espécies Silvestres Ameaçadas de Extinção (CITES) é o principal marco legal que regulamenta o comércio internacional de vida silvestre, protegendo cerca de 5.950 espécies de animais e 32.800 espécies de plantas de todo o mundo. Nas quatro décadas seguintes, após a sua entrada em vigor em 1975, o volume notificado de indivíduos silvestres comercializadas internacionalmente quadruplicou, e houve considerável deslocamento da predominância de animais oriundos da natureza para os oriundos de cativeiro no mesmo período (Harfoot et al. 2018). No entanto, grande parte do comércio de animais silvestres ocorre nacional ou regionalmente, não estando sob a proteção da CITES (Lawson e Vines 2014; Morton et al. 2021; Hughes et al. 2023). Além disso, estudos têm sugerido uma relação entre o comércio legal e ilegal de fauna silvestres onde animais capturados ilegalmente são "esquentados" e comercializados como legais (Livingstone e Shepherd 2016; Challender et al. 2019; de Lucena Soares et al. 2020). Assim, se não for bem planejado, regulamentado e controlado, o *wildlife farming* pode ter o efeito contrário ao desejado, intensificando o IWT e o impacto sobre as populações silvestres (Phelps, Carrasco, e Webb 2014; Livingstone e Shepherd 2016; Tensen 2016; Challender et al. 2019; Morton et al. 2021).

No Brasil, a criação de animais silvestres é autorizada, gerida e controlada pelos órgãos ambientais federais e estaduais, sendo que, em regra, apenas animais silvestres nascidos em cativeiro são legalizados. Atualmente, são quase um milhão de criadores e mais de 3 milhões de pássaros silvestres nativos registrados apenas no sistema de criação amadorista (SisPass), e a maioria das espécies criadas por criadores licenciados também está entre as mais frequentemente apreendidas pelos órgãos ambientais (Tabela 2) (Destro et al. 2012; Charity e Ferreira 2020).

Tabela 2. Lista das cinco espécies mais abundantes entre criadores amadorísticos de Passeriforme no Brasil. Os levantamentos foram realizados no Sistema de Controle e Monitoramento da Atividade de Criação Amadora de Pássaros (SisPass) por Destro *et al.* (2012)

e em consulta ao IBAMA por meio da Lei de Acesso à Informação (Lei nº 12.527/2011) (Documento SEI nº 9193817).

Referência	Destro et al. 2012	IBAMA, 2021*
Período	2005 - 2009	2018 - 2019
1º	*Saltator similis*	*Sporophila angolensis*
2º	*Sporophila angolensis*	*Sporophila maximiliani*
3º	*Sporophila caerulescens*	*Sicalis flaveola*
4º	*Sicalis flaveola*	*Saltator similis*
5º	*Cyanoloxia brissonii*	*Sporophila caerulescens*

*Dados referentes apenas ao Distrito Federal

Identificar se um animal comercializado, assim como seus produtos, é oriundo de cativeiro ou de populações de vida livre é crucial para avaliar a legalidade do comércio e criação de animais silvestres. No entanto, esta tarefa pode se apresentar como um grande desafio, uma vez que as técnicas de controle tradicionais, como licenças, declarações e anilhas, são geralmente imprecisas ou susceptíveis de fraude.

Nesse contexto, surge a necessidade de ferramentas mais robustas e confiáveis para a identificação da origem de animais silvestres, especialmente aquelas que não dependam exclusivamente de registros administrativos ou sistemas de controle externos. Marcadores endógenos que permitam a inferência sobre a sua origem, têm surgido como alternativas importantes na identificação de fraudes na criação e comércio de animais silvestres. Tais ferramentas, apresentam uma nítida vantagem frente às tradicionais, devido ao seu poder de acurácia e dificuldade de manipulação e adulteração.

O uso dos isótopos estáveis tem se mostrado uma importante ferramenta de uso forense devido ao seu potencial em determinar a origem de uma ampla variedade de organismos ou tecidos (Fry 2008; Hobson & Wassenaar 2019; Meier-Augenstein 2019). Como as moléculas orgânicas apresentam razões isotópicas de carbono ($\delta^{13}C$), hidrogênio ($\delta^{2}H$), oxigênio ($\delta^{18}O$) e nitrogênio ($\delta^{15}N$) que refletem o local de origem onde foram incorporados, seja pelo alimento ou pela água, os tecidos dos organismos vivos, ao assimilar tais moléculas, também irão refletir as razões isotópicas condizentes com a sua origem de formação.

Um fator importante a se considerar em estudos isotópicos envolvendo tecidos orgânicos, é a sua taxa de incorporação isotópica, que, por sua vez, pode ser influenciada por fatores como o *táxon*, ontogenia, fisiologia, massa corporal e, especialmente, o tipo do tecido (M. J. Vander Zanden et al. 2015). Enquanto alguns tecidos, como o sangue e órgãos internos,

possuem uma rápida taxa de renovação (ou *turnover*), outros formados por queratina, são metabolicamente inertes após a síntese e, portanto, mantêm um registro isotópico que reflete o local em que o tecido foi sintetizado, como é o caso das garras e penas das aves. Em consequência dessas diferenças na taxa de incorporação isotópica, diferentes tecidos irão fornecer informações sobre momentos distintos da vida de um indivíduo. A escolha do tecido a ser analisado passa, portanto, tanto pelo conhecimento prévio da sua taxa de crescimento e renovação, quanto pela escolha do momento e local da vida do animal em que se está interessado.

Os diferentes tipos de isótopos analisados também podem trazer informações distintas e complementares. As razões isotópicas de hidrogênio ($\delta^{2}H$) e oxigênio ($\delta^{18}O$) dos tecidos animais terrestres são influenciadas principalmente pelos processos hidrológicos em função de fatores geográficos, como a continentalidade (Hobson & Wassenaar, 2019). Já as razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) são moldados principalmente pela dieta, o primeiro em função do tipo de vegetação (plantas C3 ou C4) na base da cadeia alimentar e o segundo por processos do solo e interações tróficas (Fry, 2008). No entanto, dependendo da escala e do objetivo, $\delta^{2}H$ e $\delta^{18}O$ também apresentam potencial aplicação em estudos sobre teias alimentares (H. B. Vander Zanden et al. 2016; Magozzi et al. 2019), assim como $\delta^{13}C$ e $\delta^{15}N$, também têm sido de grande relevância para identificação de origem geográfica (Haveles, Fox, e Fox-Dobbs 2019; Koehler, Kardynal, e Hobson 2019).

Assim, por meio dos isótopos estáveis é possível inferir simultaneamente informações sobre a dieta, a origem geográfica e mudanças de ambiente de animais silvestres. Tais informações são bastante úteis para identificar a origem cativa ou de vida livre de animais silvestres criados ou comercializados, já que as mesmas espécies devem acessar recursos distintos e de origens geográficas diferentes nos dois ambientes (Dempson e Power 2004; Kays e Feranec 2011; Chaguri et al. 2017; Natusch et al. 2017). Em contextos forenses, a metodologia de isótopos estáveis oferece um meio confiável e acessível de diferenciar entre animais silvestres e cativos em todas as formas (vivos, em suas partes ou derivados) (Lyons & Natusch, 2015; Hobson & Wassenaar, 2019). O uso da ferramenta, no entanto, pode ser limitado por diversos fatores como a carência de bancos de bancos de dados de referência, falta de padronização de procedimentos analíticos e tempo de renovação do tecido analisado após a mudança de ambiente.

Informações sobre a origem, selvagem ou cativa, de animais silvestres têm importantes aplicações em diferentes ramos da ciência, como antropologia (Somerville, Nelson, e Knudson 2010), ecologia (Kays e Feranec 2011) e forense (Alexander et al. 2019; Andersson et al. 2021;

Jiguet, Kardynal, e Hobson 2019) tendo, neste último caso, importante potencial de aplicação no combate ao tráfico de animais especialmente em sua forma mais sutil: quando há aparência de legalidade.

Adicionalmente, por meio dos isótopos estáveis, é possível aferir também o provável local de origem de animais comercializados, desde que existam modelos de distribuição geográfica das razões isotópicas (*isoscapes*) acuradas o bastante para os organismos avaliados (ver Sena-Souza, Costa e Nardoto, 2019 para uma revisão). As isoscapes são representações gráficas de modelos espacialmente explícitos que descrevem a distribuição das razões isotópicas em paisagens geográficas. Estes modelos podem ser desenvolvidos para vários isótopos de materiais bióticos e abióticos, desde que haja padrões previsíveis de variação espacial.

O principal objetivo dessa Tese é aprimorar o uso de isótopos estáveis para diferenciar animais silvestres de vida livre e cativeiro, auxiliando na identificação de indivíduos esquentados e esclarecendo aspectos sobre o tráfico de animais no Brasil. A análise simultânea dos quatro principais isótopos componentes dos seres vivos (C, N, H e O), em um grupo diverso de aves que possui grande representatividade ao mesmo tempo entre as espécies mais traficadas e na biodiversidade de avifauna em todo o Brasil, faz esse o primeiro estudo a avaliar a eficácia das análises isotópicas em diferenciar animais de vida livre e cativeiro em um contexto tão diverso e complexo.

1. Estruturação da Tese

A tese está dividida em três capítulos complementares entre si, que, em conjunto, se propõem a analisar e discutir o uso de isótopos estáveis de carbono, nitrogênio, oxigênio e hidrogênio para diferenciar animais silvestres de vida livre daqueles de cativeiro, através do desenvolvimento de modelos isotópicos mais acurados visando a identificação e o combate ao esquentamento e ao tráfico de animais silvestres no Brasil e no mundo (Figura 1).

O primeiro capítulo teve como objetivo uma revisão da literatura sobre a utilização de isótopos estáveis em estudos envolvendo animais silvestres de vida livre e cativeiro no âmbito global e foi publicado em 2023 na revista *PeerJ* (Brasileiro *et al.*, 2023). Este estudo reforçou o potencial das análises isotópicas em diferenciar animais cativos e de vida livre em diferentes grupos de vertebrados, condições de criação e concepções metodológicas, já que mais de 80% dos artigos analisados encontraram diferenças entre as duas categorias. Além de fornecer uma base ao leitor quanto às aplicações já em curso da ferramenta, essa revisão teve como produto

um banco de dados sistematizados das razões isotópicas e metadados associados de estudos que analisaram isótopos estáveis de tecidos de animais silvestres nesses dois ambientes. A ideia é que os valores já disponíveis na literatura possam servir como base de consulta e comparação para outros estudos ou questões aplicadas.

No segundo capítulo foi analisado o uso de $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ e $\delta^{18}O$ para diferenciar pássaros da família Thraupidae de vida livre e cativeiro no Brasil. Além de abranger a maior parte das espécies mais traficadas e mais criadas em cativeiro no Brasil, esta família detém sozinha a maior diversidade de pássaros do mundo, e é a segunda maior em abundância no Brasil, com espécies ocupando grande diversidade de habitats em todo o país, como florestas, áreas abertas, regiões degradadas e áreas urbanas (Pacheco et al. 2021). Todos os isótopos diferiram significativamente entre animais de vida livre e cativeiro, e o melhor modelo testado teve acurácia média de 92% na classificação dos grupos.

O terceiro capítulo teve como objetivo desenvolver isoscapes de indivíduos da família Thraupidae para o território brasileiro. Foram elaboradas isoscapes de $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ e $\delta^{18}O$ para animais de cativeiro e $\delta^{13}C$, $\delta^{15}N$ e $\delta^{18}O$ para animais de vida livre, pois já existe isoscape de $\delta^{2}H$ para traupídeos de vida livre (Alquezar *et al.*, 2022). O poder preditivo das isoscapes, em geral, foi comparável com outros modelos elaborados para o Brasil.

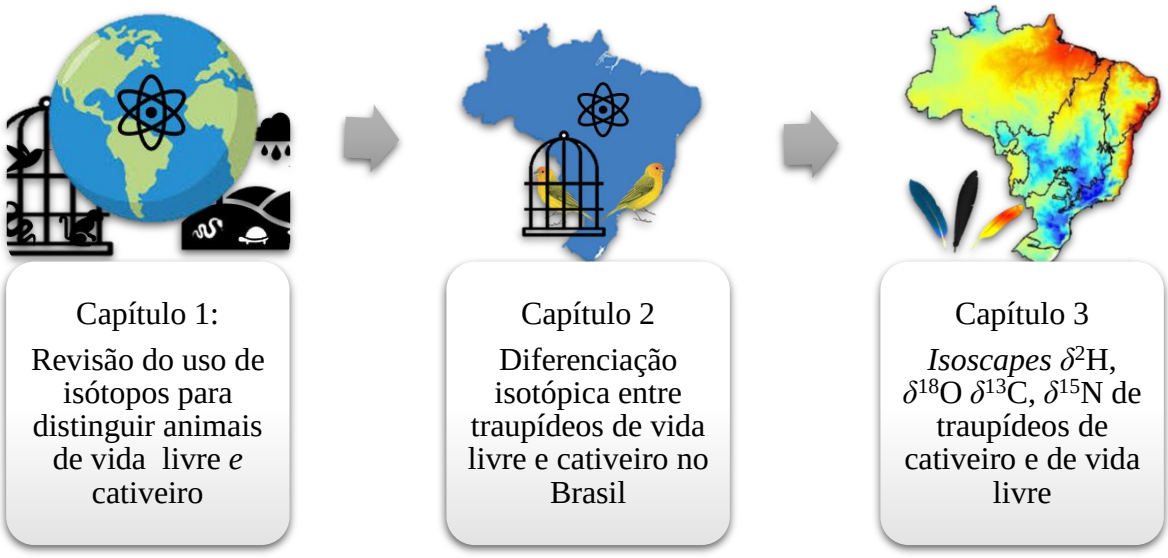

Figura 1. Estrutura da Tese

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CAPÍTULO 1 - DIFFERENTIATING WILD FROM CAPTIVE ANIMALS: AN ISOTOPIC APPROACH

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Differentiating wild from captive animals: an isotopic approach

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Abstract

Background. Wildlife farming can be an important but complex tool for conservation. To achieve conservation benefits, wildlife farming should meet a variety of criteria, including traceability conditions to identify the animals' origin. The traditional techniques for discriminating between wild and captive animals may be 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 patterns and potential 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 $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ in tissues from fish, amphibian, reptile, bird, and mammal groups. 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. $\delta^{13}C$ and $\delta^{15}N$ standard deviation and range were significantly higher in the wild than in captive animals, suggesting a more variable diet in the first group. $\delta^{13}C$ tended to be higher in wild fishes and in captive mammals, birds, reptiles, and amphibians. $\delta^{15}N$ 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 stable isotope analysis 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 for some taxonomic groups (e.g., amphibians), geographic region (e.g., Africa), and isotopes (e.g., $d^{2}H$, $d^{18}O$, and $d^{34}S$) which have been little studied.

Introduction

Human activities are the most relevant causes of defaunation, mainly habitat degradation and species overexploitation (Young *et al.*, 2016). The maintenance and management of wild species in captivity can be an important tool for conservation, either by maintaining gene banks or target species that would be unlikely to survive in the natural environment (CDB, 1992; Maxted, 2013) or by decreasing the pressure on wild population by wildlife farming (Bulte & Damania, 2005; Damania & Bulte, 2007; Nogueira & Nogueira-Filho, 2011; Rizzolo, 2020).

Wildlife farming is a broad term related to the domestication, production, trade, and consumption of live wildlife and their products, involving a variety of contexts and species born or raised in captivity (Rizzolo, 2020). The legalization of the production and trade of wild animals can be considered a form of supply-side conservation, avoiding wild stock depletion and deforestation, which benefits the recovery and maintenance of the wild population (Bulte & Damania, 2005; Damania & Bulte, 2007; Nogueira & Nogueira-Filho, 2011; Phelps, Carrasco, & Webb, 2014).

However, wildlife farming is not a simple and uncontentious conservation strategy (Tensen, 2016; Janssen & Chng, 2018; Challender *et al.*, 2019). The increase in the wildlife market brings concerns about production control, management, and trade (Lyons & Natusch, 2011; García-Díaz *et al.*, 2015; Janssen & Chng, 2018). There are a variety of biophysical, market, and regulatory conditions that wildlife farming should meet to achieve conservation benefits (Phelps, Carrasco, & Webb, 2014; Tensen, 2016; Janssen & Chng, 2018; Challender *et al.*, 2019). In turn, regulatory controls are often based on certification and traceability requirements to identify the animals' origin preventing incorrect or fraudulent statements, alien species invasion, and minimizing damage to wild populations and human health (EC, 2022; Smith et al., 2017; Staerk *et al.*, 2018).

One of the main problems in regulating wildlife farming is identifying the traded animals' real origin (wild or captive) (Tensen, 2016; EC, 2022). The traditional techniques for discriminating between wild and captive animals, such as trader declarations, government-issued licenses, bands, or microchips, may not be enough to prevent doubts or misdeclaration (Lyons & Natusch, 2011; Livingstone & Shepherd, 2016; Tensen, 2016). The efficiency of such methods is even more limited when labels are not expected or mandatory, such as in the trade of animal parts or aquaculture products in most countries, and to identify unmarked escaped or released animals (E.g., Oceana, 2015). Thus, there is a pressing need to develop more accurate techniques to discriminate between wild and captive animals and their products.

Stable isotopes are endogenous markers capable of identifying the origin of various samples, such as food, water, or organic materials. The technique has become consolidated as

biomarkers of animals' geographical origin (Hobson & Wassenaar, 2019). It has also helped track individuals' ecological traits, such as habitat use and diet (Shipley & Matich, 2020), making stable isotopes a potential tool for identifying the origin of animals also regarding the rearing system: wild or captivity (Camin *et al.*, 2016; Truonghuynh, Li, & Jaganathan, 2020).

Stable isotope analysis (SIA) is based on the variation in the ratio between an element's light and heavy forms (expressed by the letter δ) in response to environmental patterns. As animals access and metabolize environmental resources, their tissues incorporate and express the natural variability in the stable isotopic ratios. Typically, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ are categorized as local spatial assays, and their variation in animal tissues reflects their diet composition, such as the primary energy source (e.g., C_3 or C_4 plants; marine or terrestrial resources) and trophic position, while $\delta^{2}H$ and $\delta^{18}O$ reflect geographic origin and movement in response to hydrological process (Hobson & Clark, 1992; Fry, 2008; Hobson & Wassenaar, 2019).

However, stable isotope ratios in animal tissues may be influenced by complex physiological processes leading to changes in the isotopic signal compared to the diet (diet-tissue fractionation) and between different tissues (tissue-tissue fractionation). The isotopic fractionation and the speed of incorporation in animals' organic structures, in turn, depends on several environmental, nutritional, and physiological factors, such as tissue turnover rate (Tieszen *et al.*, 1983; Hobson & Clark, 1992; Vander Zanden *et al.*, 2015), diet composition (DeNiro & Epstein, 1977; Magozzi *et al.*, 2019; Whiteman *et al.*, 2021), reproductive and nutritional states (Doi, Akamatsu, & González, 2017; Shipley & Matich, 2020). Comparing wild and captive animals using stable isotopes should consider additional points to avoid confounding effects, such as the characteristics of captivity (E.g., intensive or extensive farming), and whether the animal changed captive/wild state and the timescales involved.

There are a variety of wildlife farming modalities according to different criteria, such as the management intensity (herding, ranching, and farming) (Hudson et al. 1989) or according to the system used to produce specimens (born in captivity, bred in captivity or ranched) (CITES classification; Lyons, Natusch and Jenkins, 2017). These modalities can subject captive animals to significantly different conditions, influencing their isotopic signature. However, the potential of SIA to differentiate wild from captive animals rises under the general assumption that individuals are likely to access different resources items from distinct geographical origins in these two environments (e.g., Dempson and Power, 2004; Kays and Feranec, 2011; Chaguri et al., 2017; Natusch et al., 2017). Additionally, captive specimens are less subjected to some of the main factors influencing isotopic fractionation and tissue turnover, such as natural habitat

gradients, seasonality, complex food chain, diet quality variation, and nutritional stress (Shipley & Matich, 2020).

Several studies have successfully demonstrated the potential application of SIA to differentiate wild from captive animals (Dittrich, Struck, & Rödel, 2017; Natusch *et al.*, 2017; Brandis *et al.*, 2018; Alexander *et al.*, 2019; Andersson *et al.*, 2021; Hopkins *et al.*, 2022). However, there is no scientific compilation of the topic. This study 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. We performed qualitative and quantitative analyses to develop an overall picture of the application of stable isotopes to distinguish between wild and captive animals focusing on: (1) evaluating the potential of this tool to distinguish individuals from wild and captivity; (2) searching for discernible patterns of how such differences occur.

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 non-domestic vertebrate species. In a second instance, we selected only research related to carbon, nitrogen, hydrogen, oxygen, or sulfur isotopes that meet 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 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 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, taxa group, and/or dietary category. We also performed Student's t-test or one-way ANOVA to compare $\delta^{13}C$ and $\delta^{15}N$ in wild and captive animals considering the terrestrial taxa together (amphibian, reptile, bird, and mammal) and fish per continent. Due to the lack of suitable studies, we did not perform more detailed analyses for $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$. In addition, despite the possible effects of different types of captivity on the isotopic signature of animals, we could not access details of captivity conditions in most studies, making it impossible to categorize them as suggested by Hudson et al. (1989) or CITES. Thus, in the database, they were called "captivity" only.

To gain a deeper understanding, we also assessed each study individually. For studies that analyzed isotopic differences between the two environments, we relied on the results reported by the authors. For studies that measured but did not compare isotopic ratios in the wild and captivity, we tested for such differences (*t*-test, ANOVA, Wilcox-test, Kruskal-Wallis, or linear mixed model) when the original data were available. In this approach, we considered the different categories of wild or captivity when explicitly presented by the authors. Inferential tests were preceded by analyses of normality (Shapiro-Wilk test) and equality of variances. We performed statistical tests in the R platform, version 4.1.0 (R Development Core Team), with a significance level of 5% in all hypothesis testing.

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 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

Quality assurance and control

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

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). 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 diet classifications were checked using the R package Sider (Healy *et al.*, 2018) or looked for in peer-reviewed papers.

Results

An overall picture of the use of stable isotopes to differentiate 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.

From the 47 selected publications, 33 used stable isotopes to distinguish wild from captive animals (Table S2), and 14 analyzed stable isotopes in wild and captive vertebrates with different purposes (Table S3). We found studies distributed in 37 countries worldwide (Fig. 1A), with the United States and Italy having more publications (n = 6 each). Regarding the continents, Europe (n = 21) and Africa (n = 3) had the largest and smallest number of studies, respectively.

Fifty-five species from different vertebrate taxonomic groups (mammals, birds, reptiles, amphibians, and fishes) were studied (Fig. S1). The most studied groups varied on different continents: while studies on reptiles and amphibians were concentrated in Asia and Oceania, studies with fish were distributed worldwide (Fig. S1). All studies focused on one species (n = 36) or a group of species from the same taxonomic class (n = 11). Amphibia was the least representative group, recording in only one study, while fish accounted for over 46% of the publications (Fig. 1B).

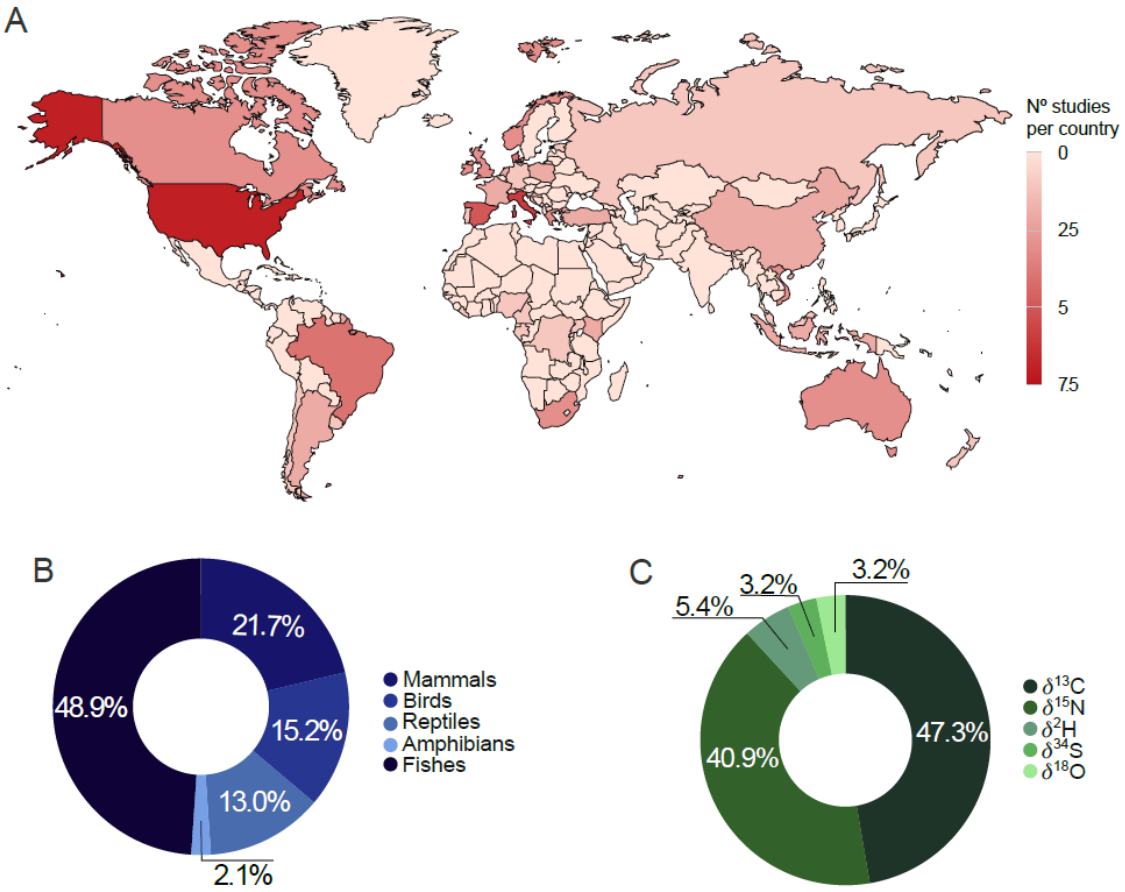

Figure 1. Distribution of studies using stable isotopes of carbon, nitrogen, hydrogen, oxygen, and sulfur in wild and captive animals worldwide (A), by taxonomic group (B), and by elements isotope ratios (C).

Carbon and nitrogen stable isotopes in combination accounted for 80.8% of the studies, and 88.2% used at least one of those elements (Fig. 1C). Hydrogen, oxygen, and sulfur stable isotopes together account for less than 15% of the publications (Fig. 1C). Muscle and inert organic structures were the most analyzed tissues presenting in 46.81% and 42.55% of the studies, respectively. Muscle was used in 83.33% of the works with fishes, while inert tissues were used in 86.96% of studies involving the other taxonomic groups.

The experimental design, captivity condition, and description of the publications varied widely (Table S4). While some studies measured stable isotopes for only two categories (wild and captivity) of the same species at the same moment and in the same geographic regions, others had a complex design, considering several types of wild or captivity, various species, countries, period, and samples origin.

Patterns and potential of SIA in distinguishing between wild and captive animals

Analyses of the systematized database

The mean for $\delta^{18}O$ and the standard deviation and range for $\delta^{13}C$ and $\delta^{15}N$ were significantly higher in the wild than in captive animals (Table 2). $\delta^{2}H$, $\delta^{18}O$, and $\delta^{34}S$ standard deviation tended to be higher in wild animals, but it was not significant (Table 2). The isotopic differences in the rearing system varied with the geographic location, taxonomic group, and diet (Fig. S2).

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 wild animals considering data from the 47 analyzed publications and systematized in the database (Table 1S). Significant 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]

$\delta^{13}C$ of the terrestrial taxon (amphibian, reptile, bird, and mammal) were significantly higher in captive animals ($t_{121} = 2.40$, $p = 0.02$). At the same time, the $\delta^{15}N$ was higher in the wild animals, but only when controlling for diet type ($F_{1,1} = 363.4$, $p = 0.03$). Captive fishes exhibited significantly lower $\delta^{13}C$ in Europe ($t_{37} = -2.07$; $p = 0.05$). The isotopic space occupied by individuals considering C and N simultaneously tended to diverge in all taxonomic groups, either at the mean position or range (Fig. S3).

Analysis of the publications individually

Overall, 83.9% of the studies found significant differences or no overlaps among categories of wild and captive animals analyzed, while 16.1% distinguished among some modalities where authors used different wild or captivity conditions. In addition, no publication failed to differentiate between all categories of wild and captivity (Table S2). Accurately identifying the group to which individuals belonged (wild or captive) ranged from 58% to 100% when using discriminant tests. To identify differences between wild and captive individuals, the studies performed graphical analyzes of overlaps, discriminant tests, frequentist statistics (such as *t*-tests and ANOVA), or the combination of the last two statistical methods.

From the studies using $\delta^{13}C$ and $\delta^{15}N$, 90.3% and 89.3% reported significant differences between wild and captive animals, respectively. The $\delta^{13}C$ was usually higher in the

wild than in captive fishes. The opposite was found for the other taxonomic groups (Fig. 2). On the other hand, $\delta^{15}N$ was consistently higher in wild birds and did not show clear trends in the other taxonomic groups (amphibians could not be evaluated since there was only one study) (Fig. 2). The studies used $\delta^{2}H$ and $\delta^{18}O$ when geographic variation was also involved ($\delta^{2}H$ in birds and reptiles and the $\delta^{18}O$ in fishes and amphibians). The $\delta^{34}S$ was used in two studies with birds or fishes, involving expected differences in the proportion of marine/terrestrial diet (Table S2).

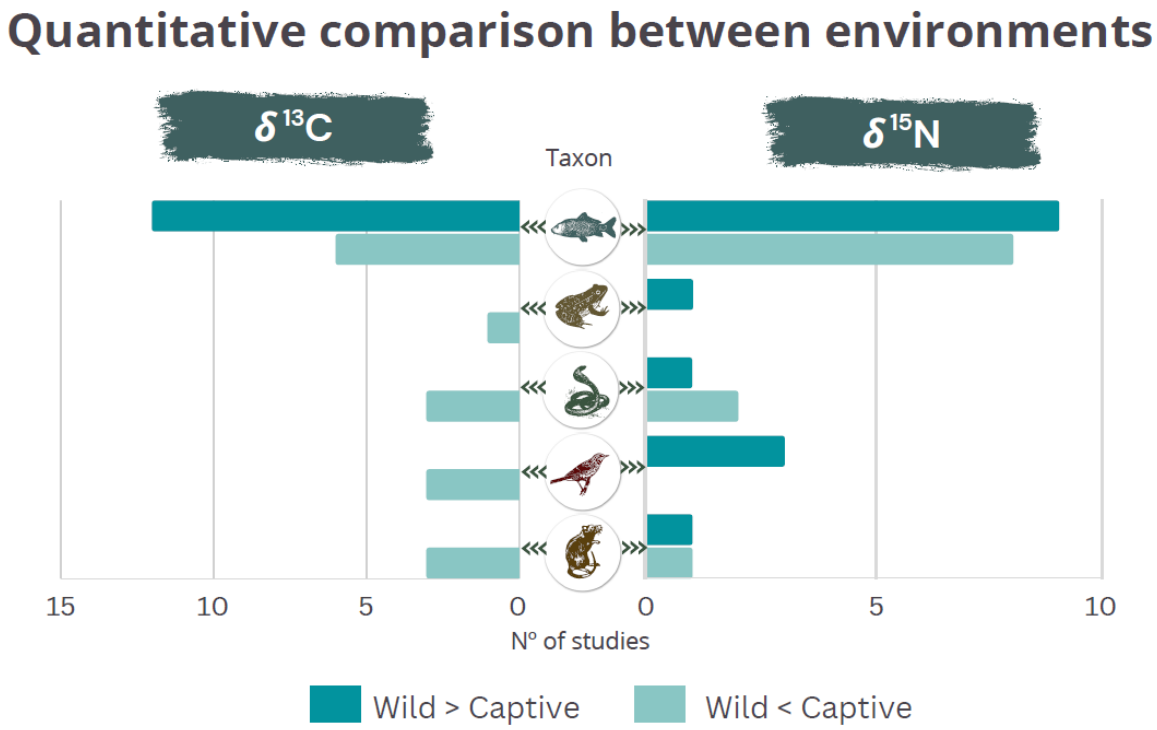

Figure 2. Qualitative comparison (higher or lower) of $\delta^{13}C$ (left) and $\delta^{15}N$ (right) between wild and captive animals by taxonomic group.

Regarding the publications that measured but did not compare stable isotopes in wild and captive animals, we accessed the original data for 10 of 14 studies. We could distinguish between all (64.3%) or some (21.4%) categories where authors used different wild or captivity conditions. We found no differences between treatments in 14.3% of the publications (Table S5).

Discussion

An overall picture of the use of stable isotopes to differentiate wild and captive animals

The development and understanding of methods to accurately identify the origin of an animal are crucial to ensure that wildlife farming fulfills its role as a conservation strategy (Phelps, Carrasco & Webb, 2014; Tensen, 2016; Lyons, Natusch, and Jenkins, 2017; Natusch, 2018).

Stable isotope analyses are an important biomarker of animal provenance. Here we summarize the main aspects of the use of stable isotopes as a tool to differentiate wild and captive animals.

Fish was the most studied group involving taxa of a single Class (Actinopteri) and the species mainly used for human consumption (e.g., European seabass, meagre, and different species of salmon). Only two fish species were considered globally threatened by IUCN and included in the CITES appendix (Tables S3). These findings suggest that beyond environmental impacts, identifying seafood origin involves concerns related to food safety, leading to labeling regulation about the rearing system in European Union and the United States (EC, 2001, AMS, 2009). However, few countries have clear requirements for the origin of the breeding system yet (El Sheikha & Xu, 2017).

Mammals, birds, and reptiles studies 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 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). Most studies in mammals, birds, or reptiles involved living species listed in the CITES appendices and relied on inert keratinous tissues, pointing out SIA as a non-invasive biomarker of the rearing system.

Around 90% of the studies relied on $\delta^{13}C$ and $\delta^{15}N$ in animal tissues, which was not a surprise, considering the assumption that wild and captive animals have different diets, which is reflected in their isotopic ratios (E.g., Dempson and Power, 2004; Natusch et al., 2017; Hill et al., 2020). The $\delta^{2}H$ and $\delta^{18}O$ have been largely used to infer animals' geographic origin and movement due to their pattern of variation in response to hydrological processes and the linkage with those in animal tissues (Hobson & Wassenaar, 2019). However, the tissue-environment relationship of $\delta^{2}H$ and $\delta^{18}O$ may also be affected by local factors, such as food-web relationships (Vander Zanden *et al.*, 2016), physiology, and the proportion of water in the animals' diet (Magozzi et al., 2019). The lack of dietary and trophic studies using $\delta^{2}H$ and $\delta^{18}O$ is also reflected in the wild-*versus*-captive studies. No research used $\delta^{34}S$ exclusively to distinguish wild from captive animals.

Patterns and potential of SIA in distinguishing between wild and captive animals

Analyses of the systematized database

We observed significant differences in the $\delta^{18}O$ between wild and captive animals. These findings suggest that $\delta^{2}H$ and $\delta^{18}O$ may have played an underexploited role in differentiating

wild and captive animals since these elements represented less than 10% of the isotopes analyzed. The $\delta^{13}C$ and $\delta^{15}N$ standard deviations were significantly higher in the wild compared to captivity, supporting the assumption that wild conditions tend to be more variable than captive ones, regardless of the specific circumstances. Such dispersion variables can be particularly helpful in identifying the rearing system of a group instead of one particular individual. Few studies mention data dispersion variables to infer animals' origin (Molketin et al, 2007, Busetto et al, 2008, Van Schingen et al, 2016, Dittrich et al, 2016). Our results suggest those variables could be relevant in differentiating wild and captive animals.

Although most studies could distinguish isotopically wild from captive animals (see below), there were few differences when considering the entire database simultaneously. Some patterns emerged as analyses were performed on more homogeneous groups (e.g., only terrestrial taxon or fish in Europe), suggesting that the isotopic differences between wild and captive animals are not unidirectional. Rather, such differences appear to vary by location and taxonomic group. Thus, using SIA to identify the rearing system origin of animals may perform better the more homogeneous the samples.

Analysis of the publications individually

More than 80% of the publications that looked for isotopic differences between wild and captive animals were successful. In some research, the animals' origin was inferred by the authors, or informed by traders or labels, which may have influenced the results. The studies with the lowest performance involved fish (Pereira et al., 2019; Molkentin et al., 2007, 2015; Wang et al., 2018; Vasconi et al., 2019; Liu et al., 2020). The complex experimental design, the largest number of variables involved (including different fish farming models) (Table S3), and the uncertainty of the samples' origin were probably the main reasons this group performed worse than terrestrial taxon. Conversely, other studies could isotopically distinguish animals from different rearing systems at even more detailed levels, such as distinguishing different breeders of the same species (Castelli & Reed, 2017).

Despite the specifics of each study, $\delta^{13}C$ was consistently lower in the wild than in captive terrestrial animals (Fig. 2), indicating consumption of higher levels of C4 plant-based food for captive individuals compared to wild populations of the same taxon. Such a pattern indicates the composition of industrial food provided to these captive animals, based mainly on less expensive items, such as corn (Kays & Feranec, 2011). Differently, wild fishes exhibited higher $\delta^{13}C$ than captive ones in most studies. Multiple factors can explain these findings, such as captive fishes tend to have higher lipid concentrations than wild ones, leading to lower $\delta^{13}C$ (DeNiro & Epstein, 1977; Focken & Becker, 1998; Serrano, Blanes, & Orero, 2007; Fasolato

et al., 2010), most fish studies included carnivore marine or migratory species in Europe, where the composition of conventional aquafeeds is based on terrestrial plant ingredients such as cereals, soy, legumes, and plant-derived oils, which has lower $\delta^{13}C$ than fish-based diet typical of carnivore wild marine fishes (Schoeninger & DeNiro, 1984; Farabegoli *et al.*, 2018; Wang *et al.*, 2018).

Finally, we found differences between wild and captive animals in more than 85% of the studies that did not compare individuals in these two environments. These results suggest the potential of isotopes to differentiate wild and captive animals even when the research was not designed to look for such differences. They are also particularly relevant, considering the publication bias of positive results (Mlinarić, Horvat, & Šupak Smolčić, 2017), which could overestimate the capacity of SIA in identifying animals' origin. The only two studies that found no differences relied exclusively on $\delta^{13}C$ and had high variability and unbalanced samples (Cree et al, 1999; Hammershøj et al., 2004). Using more than one isotopic element and more sensitive or complex statistical tests (e.g., multivariate analysis) could help find masked differences between wild and captive groups.

Improving isotopic research to distinguish between wild and captive animals

Recognizing the differences in the experimental design and objectives of each research, some approaches can be considered to enhance and improve the use of stable isotopes to distinguish between wild and captive animals. First and crucial, the study should define known captive or wild origin samples for comparison whenever possible, whether directly sampled or from the literature.

Second, the choice of the tissue used in the research is fundamental. Metabolically active tissues reflect distinct temporal integration time according to their turnover rate, varying from a few days (E.g., blood plasma), months (E.g., muscle), or lifetime (E.g., bone collagen) (Tieszen et al., 1983; Vander Zanden et al., 2015; Carter, Bauchinger & McWilliams, 2019). Conversely, the isotopic ratio of keratinous tissues, such as feathers, claws, and scales, will reflect where they were synthesized since they are metabolically inert after their formation (Mizutani *et al.*, 1990; Hobson & Clark, 1992). Therefore, under changes between rearing systems, the study should identify the exact period of change to avoid any confounding effect of the feeding change and consider the tissue that captures the time elapsed since the rearing change.

Third, to improve the evaluation and understanding of the data, special attention must be dedicated to the presentation of the methodology and results. Measures of central tendency, such as each treatment's mean and standard deviation, should always be reported. The wild and

captive conditions should also be carefully considered and described to make possible the correct interpretation of the results.

Despite increased publications using stable isotopes as a tracer of wildlife origin in the last decades, there are still some important gaps that further studies could focus on fulfilling. Some taxon groups are highly underrepresented, especially amphibians, with only one study. The influence of different categories of captivity on isotope ratios also needs to be better explored and understood. Most studies are in Europe and North America, while some high diversity and threatened regions in South America and Africa have been little studied. Regarding the isotopes, $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$ were only occasionally analyzed, and the potential of $\delta^{18}O$ and $\delta^{2}H$ to distinguish wild and captive animals based on differences in physiological conditions in these environments remains unexplored.

Conclusions

Our study reveals that SIA can help distinguish between wild and captive in different vertebrate groups, rearing conditions, and methodological designs. Despite the variety of publications reviewed, we could observe some patterns in how these differences occur, such as the higher diet variability in wild animals and the preferential use of plant-based food in captivity.

Nevertheless, some aspects should be carefully considered for the proper use of the methodology, such as knowing the wild and captive conditions of the animals studied and having samples of known origin to use as a basis for comparison. Additionally, the methodology seems to perform better the more homogeneous samples are since the direction of differences between wild and captive animals can vary greatly according to local and taxonomic specificities.

However, many gaps remain to be filled, especially in the unbalanced taxon, region, and isotope studied. We expect the present study to expand the use and acceptance of SIA as a reliable tool in identifying the animals' rearing system origin and, consequently, contributing to the efficiency of wildlife farming as a conservation strategy and the protection of natural populations.

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SUPPORTING INFORMATION

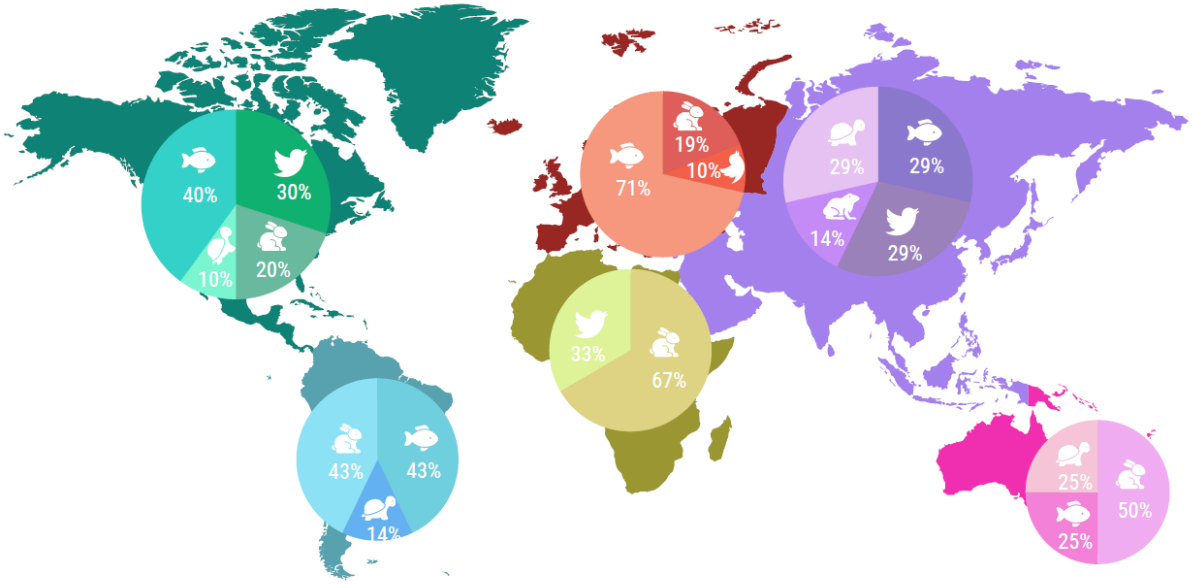

Figure 1S. Percentage of studies involving the different taxonomic groups (mammals, birds, reptiles, amphibians, and fishes) per continent (Africa, Asia, Europe, North America, Oceania, and South America).

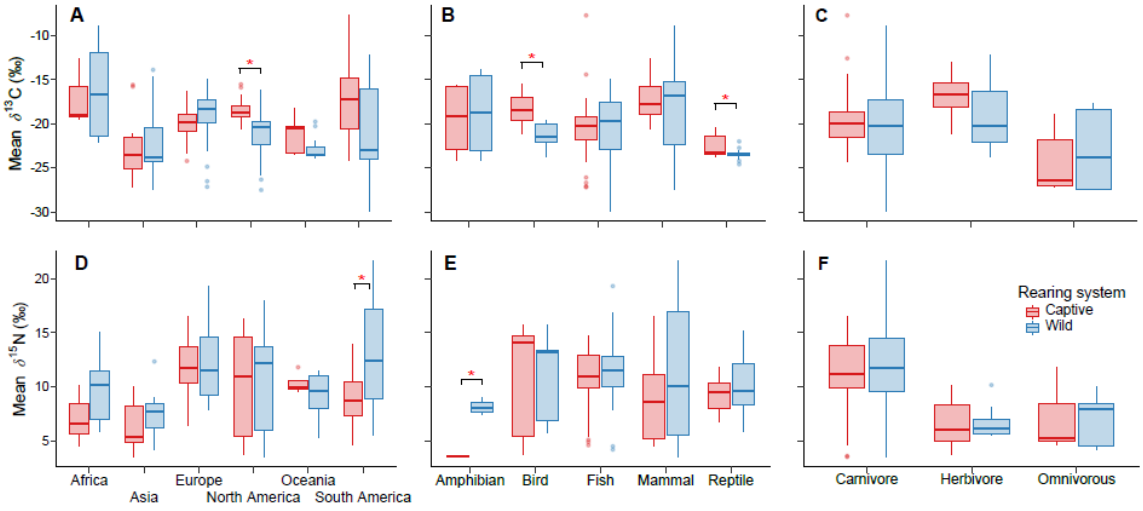

Figure 2S. $\delta^{13}C$ (A, B, C) and $\delta^{15}N$ (D, E, F) means isotopic ratios for wild and captive animals by continent (A, B), by taxonomic group (B, E), and by diet (C, F) considering data from all review publications. The box represents the middle 50% of the data, and whiskers extend to the highest and lowest values within 1.5 times the interquartile range. Asterisks indicate significant differences ($p < 0.05$).

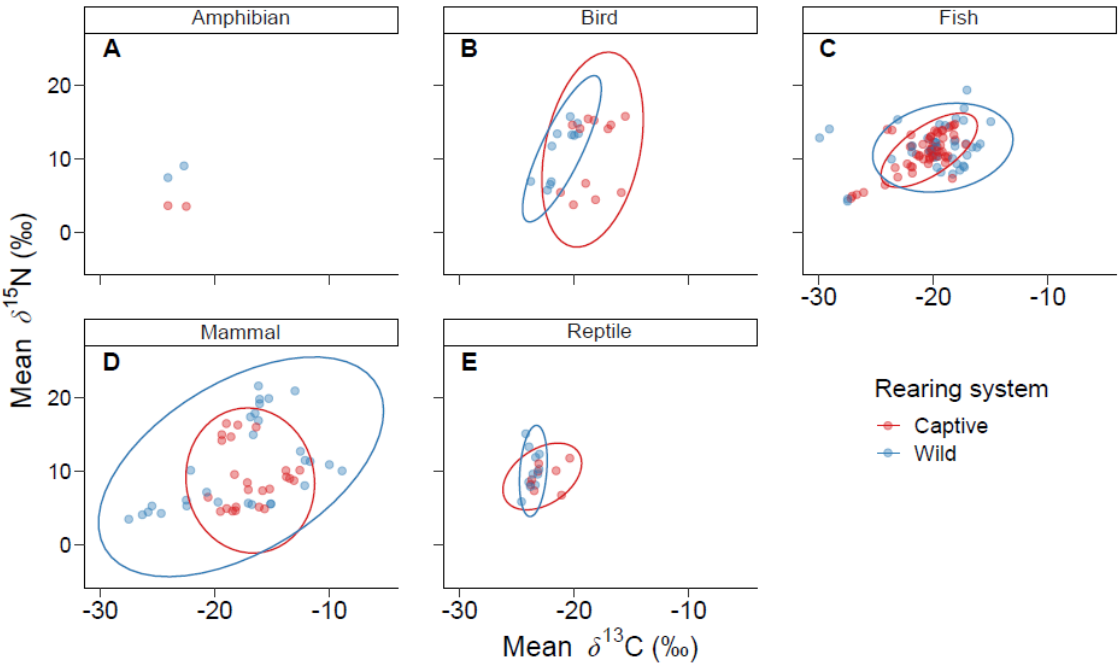

Figure 3S. Biplot of δ^{13}C and δ^{15}N means of captive and wild animals of each taxon group considering all reviewed publications simultaneously.

Table S2. Summary of studies using stable isotopes to differentiate between wild and captive animals organized by taxon group (fish, amphibian, reptile, bird, and mammal). In "Summary Results" the equal sign (=) indicates the absence of significant differences in inferential tests.

	TAXON (SPECIES)	ISOTOPES ANALYZED	TISSUE	LOCAL (COUNTRY)	SUMMARY RESULT	REFERENCE
FISH	*Salmo salar*	$\delta^{13}C$, $\delta^{15}N$	Muscle	Canada	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Dempson & Power, 2004)
	Sparus aurata	$\delta^{13}C$, $\delta^{15}N$	Muscle	Italy; France	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ (Based on no overlaps between groups)	(Rojas *et al.*, 2007)
	Dicentrarchus labrax	$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$	Muscle oil	England; Scotland; Greece	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ $\delta^{18}O_{wild} = \delta^{18}O_{Ncaptive}$	(Bell *et al.*, 2007)*
	Salmo salar	$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$	Muscle	Ireland and Norway	$\delta^{13}C_{wild}$ range < $\delta^{13}C_{cap\text{-}conventional}$ range $\delta^{15}N_{wild}$ variation > $\delta^{13}C_{captive}$ variation $\delta^{15}N_{wild} < \delta^{15}N_{cap\text{-}organic}$ $\delta^{18}O_{wild} = \delta^{18}O_{cap\text{-}conventional} = \delta^{18}O_{cap\text{-}organic}$	(Molkentin *et al.*, 2007)
	Sparus aurata	$\delta^{13}C$, $\delta^{15}N$	Muscle (red and white), liver and gills	Spain	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$	(Serrano, Blanes, & Orero, 2007)
	Psetta maxima	$\delta^{13}C$, $\delta^{15}N$	Muscle	Denmark, Spain, Netherlands	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{13}C_{wild\text{-}Netherlands} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild\text{-}Netherlands} > \delta^{15}N_{wild\text{-}Denmark} > \delta^{15}N_{captive}$	(Busetto *et al.*, 2008)
	Oncorhynchus tshawytscha; O. kisutch; Salmo salar	$\delta^{13}C$, $\delta^{15}N$	Muscle	Pacific and Atlantic Ocean	86 – 100% hits using different multivariate analyses: LDA, QDA, NN, PNN, and NNB	(Anderson, Hobbie, & Smith, 2010)
	Dicentrarchus labrax	$\delta^{13}C$, $\delta^{15}N$	Muscle	FAO zone 37.1 and 27	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Fasolato *et al.*, 2010)
	Pseudoplatystoma fasciatum	$\delta^{13}C$, $\delta^{15}N$	Muscle	Brazil	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ (rainy season)	(Sant'Ana, Ducatti, & Ramires, 2010)
	Salmo salar; Oncorhynchus mykiss	$\delta^{13}C$, $\delta^{15}N$	Muscle	Chile	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ 93.9% hits in Discriminant Analysis	(Schröder & Garcia de Leaniz, 2011)

FISH	*Salmo trutta*	$\delta^{13}C$, $\delta^{34}S$	Scale	Poland	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{34}S_{captive} > \delta^{34}S_{wild}$ (Based on no overlaps between groups)	(Trembaczowski, 2011)
	Oncorhynchus nerka; O. kisutch; Salmo salar; S. trutta	$\delta^{13}C$, $\delta^{15}N$	Muscle	United States, Ireland, Scotland, Norway, Germany	Bulk: $\delta^{13}C_{wild}$, $\delta^{13}C_{organic} > \delta^{13}C_{conventional}$ $\delta^{15}N_{wild}$, $\delta^{15}N_{organic} > \delta^{15}N_{conventional}$ Lipids: $\delta^{13}C_{organic} > \delta^{13}C_{wild}$, $\delta^{13}C_{conventional}$ (Based on no overlaps between groups)	(Molkentin *et al.*, 2015)
	Argyrosomus regius	$\delta^{13}C$, $\delta^{15}N$	Muscle	Portugal	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Chaguri *et al.*, 2017)
	Dicentrarchus labrax	$\delta^{13}C$, $\delta^{15}N$	Muscle	Europe	91% hits in the Discriminant Analysis	(Farabegoli *et al.*, 2018)
	Oncorhynchus gorbuscha, *Oncorhynchus nerka* *Salmo salar*	$\delta^{13}C$, $\delta^{15}N$	Muscle	United States, Norway, Ireland	$\delta^{13}C_{bulk}$: differences between all groups (except wild *vs.* Irish organic *S. salar*; wild *O. gorbuscha vs.* wild *O. nerka* salmon) $\delta^{15}N_{bulk}$: differences between wild and conventionally farmed SCIA allowed more accurate results	(Wang *et al.*, 2018)
	Lates calcarifer	$\delta^{13}C$ e $\delta^{15}N$	Muscle	Australia; Malaysia	$\delta^{13}C_{wild} > \delta^{13}C_{captive}$ (except for Northern Territory – AU: no difference was found) $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Gopi *et al.*, 2019)
	Arapaima spp.	$\delta^{13}C$	Otolith	Brazil	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ (Madeira, Solimões and Lower Amazon) $\delta^{13}C_{wild} > \delta^{13}C_{captive}$ (Central Amazon basin) 58% hits in the Discriminant Analysis	(Pereira *et al.*, 2019)
	Anguilla anguilla	$\delta^{13}C$, $\delta^{15}N$	Muscle	Italy, Denmark, Netherlands	Italy, Denmark, and Netherlands: $\delta^{13}C_{wild\text{-}sea} < \delta^{13}C_{cap\text{-}int.\text{-}males} < \delta^{13}C_{wild\text{-}lagoon} = \delta^{13}C_{cap\text{-}ext} = \delta^{13}C_{cap\text{-}int\text{-}females}$ $\delta^{15}N_{wild\text{-}sea} = \delta^{15}N_{cap\text{-}ext.} = \delta^{15}N_{cap\text{-}int\text{-}female} > \delta^{15}N_{wild\text{-}lagoon} = \delta^{15}N_{cap\text{-}int\text{-}males}$ Italy:	(Vasconi *et al.*, 2019)

					$\delta^{13}C_{wild\text{-}sea} < \delta^{13}C_{wild\text{-}lagoon} = \delta^{13}C_{cap\text{-}ext.}$ $\delta^{15}N_{wild\text{-}sea} = \delta^{15}N_{cap\text{-}ext.} > \delta^{15}N_{wild\text{-}lagoon}$	
	Oncorhynchus mykiss	$\delta^{13}C$, $\delta^{15}N$	Muscle	Argentina	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{15}N_{captive\text{-}farmC} > \delta^{15}N_{wild} = \delta^{15}N_{captive\text{-}farmB} > \delta^{15}N_{captive\text{-}farmA}$	(Nabaes Jodar, Cussac, & Becker, 2020)
	C. carpio; C. idella; H. molitrix; M. piceus	$\delta^{13}C$, $\delta^{15}N$	Muscle, scale	China	$\delta^{13}C_{wild} < \delta^{13}C_{lake\text{-}farmed} = \delta^{13}C_{pond\text{-}farmed}$ $\delta^{15}N_{wild} < \delta^{15}N_{pond\ farmed}$ Discriminant model using isotopic and elemental data: 95-100% hits	(Liu *et al.*, 2020)
AMPHIBIAN	*Hoplobatrachus rugulosus, Fejervarya cancrivora; Limnonectes macrodon*	$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$	Muscle, bone	Vietnam; Indonesia	Muscle: differences in $\delta^{13}C$, $\delta^{15}N$; $SD\delta^{15}N_{wild} > SD\delta^{15}N_{farmed}$ Bone: differences in $\delta^{13}C$ and $\delta^{18}O$ (Vietnam x Indonesia)	(Dittrich, Struck, & Rödel, 2017)
	Shinisaurus crocodilurus	$\delta^{13}C$, $\delta^{15}N$	Skin	Vietnam	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$; $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ Assignment test$_{wild\ x\ captive}$: 100% hits	(van Schingen *et al.*, 2016)
REPTILE	*Python reticulatus*; *Python bivittatus*	$\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$	Skin	Vietnam; Indonesia	*P. bivittattus:* $\delta^{13}C_{wild} < \delta^{13}C_{captive}$; $\delta^{15}N_{wild} > \delta^{15}N_{captive;}$ $\delta^{2}H_{wild} = \delta^{2}H_{Captive}$ 100% hits in the Discriminant Analysis *P. reticulatus*: $\delta^{13}C_{wild\text{-}Vietnam} < \delta^{13}C_{wild\text{-}Indonesia} = \delta^{13}C_{captive\text{-}Vietnam}$ $\delta^{2}H_{wild\text{-}Vietnam} < \delta^{2}H_{wild\text{-}Indonesia} = \delta^{2}H_{captive\text{-}Vietnam}$ $\delta^{15}N_{wild\text{-}Vietnam} = \delta^{15}N_{wild\text{-}Indonesia} = \delta^{15}N_{captive\text{-}Vietnam}$	(Natusch *et al.*, 2017)
	Trachemys scripta elegans	$\delta^{13}C$ e $\delta^{15}N$	Carapace	Australia	$\delta^{15}N_{wild} < \delta^{15}N_{captive}$ Assignment test: minimum accuracy of 96%	(Hill *et al.*, 2020)
	Carduelis carduelis	$\delta^{2}H$	Feather	England	$\delta^{2}H_{C.c.\ major} < \delta^{2}H_{C.c.\ brittanica} = \delta^{2}H_{Captive}$	(Kelly, Thompson, & Newton, 2008)
BIRD	*Colinus virginianus*	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$	Feather	United States	100% hits in the Discriminant Analysis (wild *vs.* captive); 99% hits in the Discriminant Analysis (different farms) Isotopes used: $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$,	(Castelli & Reed, 2017)

MAMMAL	*Psittacus erithacus*	$\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$	Feather	South Africa	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ $\delta^{2}H_{wild} < \delta^{2}H_{captive}$	(Alexander *et al.*, 2019)
	Emberiza hortulana	$\delta^{2}H$	Feather	France	$\delta^{2}H_{wild} > \delta^{2}H_{captive}$	(Jiguet, Kardynal, & Hobson, 2019)
	Cacatua sulphurea, Cacatua sp.	$\delta^{13}C$, $\delta^{15}N$	Feather	China	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$ $LDA_{wild\ vs.\ captive}$: Accuracy = 0.91	(Andersson *et al.*, 2021)
	Mustela vison	$\delta^{13}C$	Teeth; claw	Denmark	$\delta^{13}C_{wild} < \delta^{13}C_{captive}$ 95.9% correct classification to the supposed origin group	(Hammershøj *et al.*, 2005)
	Canis lupus	$\delta^{13}C$, $\delta^{15}N$	Hair; bone	USA	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} = \delta^{15}N_{captive}$ (Based on no overlaps between groups)	(Kays & Feranec, 2011)
	Tachyglossus aculeatus	$\delta^{13}C$, $\delta^{15}N$	Quills	Australia	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ 91.31% correct classification	(Brandis *et al.*, 2018)
	Panthera leo	$\delta^{13}C$ e $\delta^{15}N$	Hair	Australia	$\delta^{15}N_{wild} > \delta^{15}N_{captive}$ Predictive $model_{wild\ vs.\ captive}$: Accuracy = 0.7	(Hutchinson & Roberts, 2020)

*Not included in the database (.xlsx file) because isotopic data were not available

Table S3. Summary of studies measuring stable isotopes in wild and captive animals organized by taxon group (fish, amphibian, reptile, bird, and mammal). In the "Summary proposal," DTDF means diet-tissue discrimination factors. In "Summary results," the equal sign (=) indicates the absence of significant differences in inferential tests.

	TAXON (SPECIE)	ISOTOPES ANALYZED	TISSUE	LOCAL (COUNTRY)	SUMMARY PROPOSAL:	SUMMARY RESULT	REFERENCE
FISH	*Thunnus thynnus*	$\delta^{13}C$, $\delta^{15}N$	Muscle and liver	Italy	To assess the changes occurring during farming, investigate the sources of nutrition for *T. thynmus.*	$\delta^{13}C_{wild} = \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$	(Vizzini, Tramati, & Mazzola, 2010)*
	Salmo trutta;	$\delta^{13}C$, $\delta^{34}S$	Scale	Poland	To analyze the relationship between sulfur in sulfate dissolved in water and in fish scales	$\delta^{13}C_{wild\text{-}river} < \delta^{13}C_{captive;}$ $\delta^{34}S_{wild\text{-}river} < \delta^{34}S_{captive}$	(Trembaczowski & Niezgoda, 2011)
	Belone belone; Boops boops	$\delta^{13}C$, $\delta^{15}N$	Muscle	Croatia	To assess the presence, concentrations, origin, and fate of targeted metals and the effects farming has on wild fish.	$\delta^{13}C_{wild} = \delta^{13}C_{captive}$ *B. belone:* $\delta^{15}N_{wild} = \delta^{15}N_{captive}$ *B. boops:* $\delta^{15}N_{wild} < \delta^{15}N_{captive}$	(Fernandez-Jover *et al.*, 2020)*
	Sphenodon punctatus	$\delta^{13}C$	Blood (RBC)	New Zeland	To make inferences about marine content in the diet of *S. punctatus* in response to seasonality and life story.	$\delta^{13}C_{wild} = \delta^{13}C_{captive}$	(Cree *et al.*, 1999)
REPTILE	*Alligator mississippiensis*	$\delta^{18}O$	Bone	USA	To analyze the inter and intra-bone variability of $\delta^{18}O$ according to temperature regularity.	$\delta^{18}O_{wild} < \delta^{18}O_{captive}$	(Stoskopf, Barrick, & Showers, 2001)
	Bothrops atrox	$\delta^{13}C$, $\delta^{15}N$	Blood and scale	Brazil	To analyze the influence of different landscapes on the diet of *B. atrox*	Blood: $\delta^{13}C_{wild} = \delta^{13}C_{captive}$ $\delta^{15}N_{wild} = \delta^{15}N_{captive}$ Scale: $\delta^{13}C_{wild\_forest} < \delta^{13}C_{captive}$ $\delta^{15}N_{wild\_forest} > \delta^{15}N_{captive}$	(Martinez, 2016)
BIRD	*Cerorhinca monocerata*	$\delta^{13}C$, $\delta^{15}N$	Blood and feather	USA	To examine the effects of growth and nutritional status on stable isotope signatures in *C. monocerata* tissues.	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$	(Sears, Hatch, & O'Brien, 2009)*

	Fratercula arctica; Uria aalge	$\delta^{13}C$, $\delta^{15}N$	Blood (RBC and plasma)	Canada	To estimate the DTDFs for captive *F. artica* and *U. aalge* and to reconstruct the diet of wild breeding individuals of the same species	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ (RBC and plasma, both species)	(Jenkins *et al.*, 2020)
	Mustela vison	$\delta^{13}C$	Claw and teeth	Denmark	To conduct a diet-change experiment to verify if the SIA could identify farm-scaped minks (*M. vison*).	$\delta^{13}C_{wild} = \delta^{13}C_{captive}$ (Claw and teeth)	(Hammershøj, Asferg, & Kristensen, 2004)
	Hydrochoerus hydrochaeris	$\delta^{13}C$, $\delta^{15}N$	Blood, claw, hair, and muscle	Brazil	To analyze the diet composition of *H. hydrochaeris* and the reliability of using stable isotopes as a proxy	Blood, claw, and hair: $\delta^{13}C_{wild} = \delta^{13}C_{captive}$ $\delta^{15}N_{wild} < \delta^{15}N_{captive}$ Muscle: $\delta^{13}C_{wild} > \delta^{13}C_{captive}$ $\delta^{15}N_{wild} = \delta^{15}N_{captive}$	(Navarro, 2009)
MAMMAL	*Phoca vitulina*	$\delta^{13}C$, $\delta^{15}N$	Blood (serum)	USA	To determine the trophic level and DTDFs of different tissues and groups of harbor seals	$\delta^{13}C_{wild} > \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Germain *et al.*, 2012)
	Loxodonta africana	$\delta^{13}C$, $\delta^{15}N$	Hair	South Africa	To compare the patterns of seasonal dietary variability across individuals of *L. Africana.*	$\delta^{13}C_{wild} < \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Codron *et al.*, 2013)
	Otaria flavescens	$\delta^{13}C$, $\delta^{15}N$	Blood (RBC and serum)	Uruguay; Spain	To estimate DTDF for females and pups *O. flavescens* in the wild and captive.	$\delta^{13}C_{wild} > \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Drago *et al.*, 2015)*
	Otaria flavescens	$\delta^{13}C$, $\delta^{15}N$	Vibrissae	Argentina; Spain	To analyze the fluctuation in stable isotope values along the vibrissae from wild adult breeding *O. flavescens*	$\delta^{13}C_{wild} > \delta^{13}C_{captive;}$ $\delta^{15}N_{wild} > \delta^{15}N_{captive}$	(Cardona *et al.*, 2017)

*We could not access or infer the original database. The inferences were based on the mean and standard deviation.

Table S4. Global status of the species used in "wild *vs.* captive" studies on the IUCN red list, CITES appendices, and summary of the captive information available in each paper. LC = "Least Concern"; NT = "Near Threatened"; VU = "Vulnerable"; EN = "Endangered"; CR = "Critically Endangered"; RE = "Regionally Extinct", NE = "Not Evaluated" and DD = "Data Deficient".

REFERENCE	TAXON	IUCN STATUS	CITES	CAPTIVE INFORMATION
(Dempson & Power, 2004)	*Salmo salar*	LC	No	Samples from a strain of *S. salar* farmed exclusively in sea-cage culture. The commercial food supplied was also analyzed.
(Rojas et al., 2007)	*Sparus aurata*	LC	No	Samples of *S. aurata* from local producers in four Mediterranean countries. Captive conditions are unknown. However, interviews with fish farmers indicated that farmers in the same country tend to purchase feeds from a unique national provider.
(Bell et al., 2007)	*Dicentrarchus labrax*	LC	No	Samples of *D. labrax* from a research laboratory in Scotland and a farm company in Greece. No further information about the captive was provided. However, the authors mention that sea bass used to be fed a commercial Atlantic cod diet, with lower lipid content than bass diets produced in southern Europe.
(Molkentin et al., 2007)	*Salmo salar*	LC	No	Samples of conventionally and organically reared *S. salar* were purchased. The captive conditions are unknown. However, there are some standards for organic aquaculture, such as all feeding stuff shall be of a certified organic origin; fish meal or oil shall come from the same geographical region and shall be obtained from by-products of wild-caught fish for human consumption; the use of synthetic feed additives is not allowed.
(Serrano, Blanes, & Orero, 2007)	*Sparus aurata*	LC	No	Samples of sea-cage-farmed *S. aurata* were purchased from local commercial markets. No further information about captive was provided, but the authors used morphology and lipid content to confirm the fish origin (wild or captivity)
(Busetto et al., 2008)	*Psetta maxima*	LC	No	Samples of *P. maxima* were collected at the wholesale fish market and by local retailers. No further information about the captive was provided.
(Anderson, Hobbie, & Smith, 2010)	*Oncorhynchus tshawytscha* *Oncorhynchus kisutch;* *Salmo salar*	NE NE LC	No	Samples of *O. tshawytscha, O. kisutch,* and *S. salar* from five aquaculture facilities. No further information about the captive was provided.
(Fasolato et al., 2010)	*Dicentrarchus labrax*	LC	No	Samples of *D. labrax* from three intensive farms in Italy and Greece. No further information about the captive was provided. However, the authors mention that the European sea bass can be farmed extensively in brackish lagoons and intensively in floating cages or in-shore ponds, employing high nutritional feed.

(Sant'Ana, Ducatti, & Ramires, 2010)	*Pseudoplatystoma fasciatum*	NE	No	Samples from *P. fasciatum* were obtained from two local fish farms in the dry and rainy seasons, where they were fed a commercial diet and small fish.
Schröder and Leaniz, 2011	*Salmo salar* *Oncorhynchus mykiss*	LC NE	No	Samples of *S. salar* and *O. mykiss* were collected at two local fish farms. No further information about the captive was provided.
(Trembaczowski & Niezgoda, 2011)	*Salmo trutta* *Oncorhynchus mykiss*	LC	No	Samples of *O. mykiss* were caught in commercial 'put&take' fishery supplied directly from commercial pond farms. Fishes used to stay no longer than a month in such ponds. Samples of *S. trutta* from a hatchery from where almost all trouts and graylings were used to stock rivers in the study region.
(Molkentin et al., 2015)	*Salmo salar;* *Salmo trutta*	LC LC	No	Samples of organically and conventionally farmed salmons were purchased from retail stores and wholesale (*S. salar*) or directly from fish farms (*S. trutta*). The commercial food supplied was also analyzed. No further information about the captive was provided. However, guidelines for organic farming require at least 40% of animal content for carnivorous species. In conventional farms, more than 60% of vegetable ingredients are allowed.
(Chaguri et al., 2017)	*Argyrosomus regius*	LC	No	Samples from *A. regius* cultivated in earth ponds from a local aquaculture facility. No further information about the captive was provided.
(Farabegoli et al., 2018)	*Dicentrarchus labrax*	LC	No	Samples of *D. labrax* from intensive (up to 30 kg m^{-3}), semi-intensive (up to 1 kg m^{-3}), and extensive (up to 0.0025 kg m-3) rearing farms. The intensive farms were equipped with either floating or submersible cages, the semi-intensive with earthen tanks, and the extensive farm was based in valliculture.
(Wang et al., 2018)	*Salmo salar*	LC	No	Samples of *S. salar* came from a known organic producer or were purchased at the supermarket labeled as organically or conventionally reared. The authors assume that farmed salmon obtained from supermarkets were raised according to the EU regulation: organic salmon are fed at least 40% marine originated diet, and conventional are fed less than 22% of marine origin since 2015.
(Gopi et al., 2019)	*Lates calcarifer*	LC	No	Samples of *L. calcarifer* came from the wholesale market through collaboration with industry and research partners. The samples were randomly collected from different ponds at each farm. No further information about the captive was provided. However, the study mentions that *L. calcarifer* has been farmed in brackish water, freshwater, and marine conditions. Pond or net-cage culture is the preferred method of cultivation.
(Pereira et al., 2019)	*Arapaima spp.*	DD	II	Samples from *Arapaima spp.* farms or markets. No further information about the captive was provided.

(Vasconi et al., 2019)	*Anguilla anguilla*	CR	II	Samples from *A. anguilla* farm or purchased on a retail market labeled as from Netherlands, Denmark, or Italy. The authors point out two main rearing systems of eels in Europe: extensive rearing in ponds or vallicoltura (practiced by Italian farmers) or intensive rearing in which eels are kept at their optimum temperature and fed with extruded dry feed several times a day (practiced by Dutch and Danish farmers).
(Nabaes Jodar, Cussac, & Becker, 2020)	*Oncorhynchus mykiss*	NE	No	Samples from three *O. mykiss* farms in the same region of wild ones (Alicurá reservoir). No further information about the captive was provided.
(Liu et al., 2020)	*Cyprinus carpio* *Ctenopharyngodon idella;* *Hypophthalmichthys molitrix;* *Mylopharyngodon piceus*	VU LC NT LC	No	Samples of lake-farmed and pond-farmed carp. Lake-farmed carp were collected from 4 large fish enclosures (each>10 km2) and fed under protocols that comply with Organic Aquaculture Certification Standards. Pond-farmed carp were collected from 20 ponds. Water was piped directly from a regional lake into these large artificial ponds (depth>3 m) and exchanged every 15 days. The density of carp in pond-farmed systems was higher than lake-farmed carp by at least 20 fish per m^2, and the fishes were intensively fed (> 5 kg per m^2 per day) using plant and animal-based proteins.
(Dittrich, Struck, & Rödel, 2017)	*Hoplobatrachus rugulosus;* *Fejervarya cancrivora;* *Limnonectes macrodon*	LC LC LC	No	Samples from three species of deep-frozen frog legs from Vietnam and Indonesia were bought in supermarkets in Germany. Captive conditions are unknown. However, according to the package labels and the authors' conclusions, Vietnamese samples are from frog farms, and Indonesian samples are from wild or free-ranging farming.
(van Schingen et al., 2016)	*Shinisaurus crocodilurus*	EN	I	Samples from *S. crocodilrus* born in captivity. Adults are kept in groups of three to four individuals in outdoor enclosures of about 2–7 m^2, while juveniles are kept in small groups or pairs within plastic boxes inside the station during the first months. Animals are fed once or twice a week, mainly with beetle larvae and sometimes earthworms and crickets, while juveniles are fed more frequently.
(Natusch et al., 2017)	*Python reticulatus;* *Python bivittatus*	LC VU	II	Samples from *P. reticulatus* reared on a diet of wild-caught rats (*Rattus argentiventer*) at a commercial breeding facility. Samples from *P. bivittatus* born on a python farm and raised in a dietary experiment for 13 months. The experiment included wild rats or sausages made from reconstituted waste protein, the predominant items on captive *P. bivittatus* diet in Viet Nam.
(Hill et al., 2020)	*Trachemys scripta*	LC	No	Samples from *T. scripta* seized by state wildlife compliance agencies. Specimens were classified as "wild" or "captive" based on the assumed environmental history. Captive conditions are unknown.

(A. Kelly, Thompson, & Newton, 2008)	*Carduelis carduelis*	LC	III	Samples from captive-bred *S. canaria*. No further information about the captive was provided.
(Castelli & Reed, 2017)	*Colinus virginianus*	NT	I	Samples from hunting farms of *C. virginianus*. The commercial food supplied was also analyzed. No further information about captive was provided; however, the standard management of *C. virginianus* includes getting the eggs from dealers, moving young chicks into flight pens with a density of $2ft^2$, and high-protein food.[1]
(Alexander et al., 2019)	*Psittacus erithacus*	EN	I	Samples from *P. erithacus* kept in captivity for at least one year. No further information about the captive was provided.
(Jiguet, Kardynal, & Hobson, 2019)	*Emberiza hortulana*	LC	III	Samples from *E. hortulana* seized by the police. Captive conditions are unknown; however, the authors selected individuals with plumage dysfunctions characteristics of long-term captivity since there is no captive breeding of the species.
(Andersson et al., 2021)	*Cacatua sulphurea*	CR	I	Samples from five species of the genus *Cacatua* kept in captivity for at least one year and completed at least one molt during that time. Birds were held by research centers, private owners, pet shops, and zoos. The commercial food supplied was also analyzed.
(Mette Hammershøj et al., 2005)	*Mustela vison*	LC	No	Samples from free-ranging *M. vison* to identify individuals' origin. Captive conditions are unknown. However, the authors assume that the food used in most farm mink in Denmark has a high marine fish content and is acquired from a few feed mills.
(Kays & Feranec, 2011)	*Canis lupus*	LC	I; II	Samples from captive *C. latrans* fed scrap beef and kibbled dog food. No further information about the captive was provided.
(Brandis et al., 2018)	*Tachyglossus aculeatus*	LC	No	Samples from *T. aculeatus* kept at the Zoo for between 22 months and 20 years since they are challenging to breed in captivity. All captive echidnas were fed the same diet.
(Hutchinson & Roberts, 2020)	*Panthera leo*	VU	I; II	Samples from South African *P. leo* provided by taxidermists. Captive conditions are unknown.

1. https://extension.psu.edu/bobwhite-quail-production

Table S5. Comparison of the mean isotopic ratios in captive and wild animals of the publication that measured but did not tests for differences. We could access or infer the original data from 10 of the 14 publications.

REFERENCE	SPECIE	TISSUE	ISOTOPE	TEST	RESULT
Trembaczowski & Niezgoda, 2011	*Salmo trutta*	Scale	$\delta^{13}C$	Wilcox test	$W = 468$; $p < 0.001$
			$\delta^{34}S$	Wilcox test	$W = 380$; $p < 0.001$
Cree et al., 1999	*Sphenodon punctatus*	Red blood cells	$\delta^{13}C$	Wilcox test	$W = 517$; $p = 0.191$
Stoskopf, Barrick & Showers, 2001	*Alligator mississippiensis*	Bone	$\delta^{18}O$	Wilcox test	$W = 5767$; $p < 0.001$
Martinez, 2016	*Bothrops atrox*	Scale	$\delta^{13}C$	Kruskal-Wallis	$H_3 = 7.304$; $p = 0.062$
			$\delta^{15}N$	One way ANOVA	$F_{3,36} = 19.5$; $p < 0.001$
		Blood	$\delta^{13}C$	One way ANOVA	$F_{3,28} = 0.412$; $p = 0.745$
			$\delta^{15}N$	Kruskal-Wallis	$H_3 = 3.150$; $p = 0.368$
Jenkins et al., 2020	*Fratercula arctica*	Plasma	$\delta^{13}C$	Wilcox test	$W = 281$; $p < 0.001$
			$\delta^{15}N$	Wilcox test	$W = 304$; $p < 0.001$
		Red blood cells	$\delta^{13}C$	Student's t-test	$t_{33} = 34.271$; $p < 0.001$
			$\delta^{15}N$	Student's t-test	$t_{33} = 57.588$; $p < 0.001$
	Uria aalge	Plasma	$\delta^{13}C$	Wilcox test	$W = 328$; $p < 0.001$
			$\delta^{15}N$	Wilcox test	$W = 380$; $p < 0.001$
		Red blood cells	$\delta^{13}C$	Student's t-test	$t_{37} = 36.614$; $p < 0.001$
			$\delta^{15}N$	Welch t-test	$t_{26.04} = 35.57$; $p < 0.001$
Hammershøj, Asferg & Kristensen, 2004	*Mustela vison*	Claw	$\delta^{13}C$	Welch t-test	$t_{7.028} = 1.040$; $p = 0.332$
		Teeth	$\delta^{13}C$	Wilcox test	$W = 24$; $p = 0.730$
Navarro, 2009	*Hydrochoerus hydrochaeris*	Blood	$\delta^{13}C$	Wilcox test	$W = 255$; $p = 0.855$

		Claw	$\delta^{15}N$	Wilcox test	$W = 342$; $p < 0.030$
			$\delta^{13}C$	Student's t-test	$t_{48} = 0.381$; $p = 0.704$
		Hair	$\delta^{15}N$	Wilcox test	$W = 433$; $p < 0.010$
			$\delta^{13}C$	Student's t-test	$t_{48} = 0.501$; $p = 0.618$
		Muscle	$\delta^{15}N$	Wilcox test	$W = 420$; $p = 0.017$
			$\delta^{13}C$	Wilcox test	$W = 54.5$; $p < 0.001$
			$\delta^{15}N$	Wilcox test	$W = 300$; $p = 0.227$
Germain et al., 2012	*Phoca vitulina*	Serum	$\delta^{13}C$	Student's t-test	$t_{109} = -7.891$; $p < 0.001$
			$\delta^{15}N$	Wilcox test	$W = 228.5$; $p < 0.001$
Codron et al., 2013	*Loxodonta africana*	Hair	$\delta^{13}C$	Linear mixed model	AIC = 1675.0; $\chi < 0.001$
			$\delta^{15}N$	Linear mixed model	AIC = 1815.8; $\chi < 0.001$
Cardona et al., 2017	*Otaria flavescens*	Vibrissae	$\delta^{13}C$	Linear mixed model	AIC = 641.61; $\chi < 0.001$
			$\delta^{15}N$	Linear mixed model	AIC = 876.58; $\chi < 0.001$

CAPÍTULO 2 – STABLE ISOTOPES ANALYSIS AS A TOOL TO PREVENT ILLICIT WILDLIFE TRADE OF SONGBIRDS IN BRAZIL

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Stable isotopes analysis as a tool to prevent illicit wildlife trade of songbirds in Brazil

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Short title: Stable Isotopes to prevent illegal trade in songbirds

Abstract

The illicit wildlife trade is one of the main causes of biodiversity loss, causing several environmental impacts. In Brazil, the illegal wildlife trade is focused on songbirds, especially from the Thraupidae family, a rich and abundant family that includes some highly threatened species. The regulation of the trade of wild animals was conceived as a conservation strategy. However, if not carefully managed, this activity can have the opposite effect, intensifying the impact on natural populations through wildlife laundering. Identifying whether a traded animal is captive or wild is a challenging task but crucial to assessing the legality of the trade. Stable isotope analysis is a powerful tool for indicating animal origin and diet. This technique has proven helpful in differentiating between wild and captive individuals across various studies involving different vertebrate groups. Here, we investigated the use of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$ to differentiate wild and captive songbirds in Brazil. We analyzed 657 feathers of 58 non-migratory species from the Thraupidae family distributed throughout the country ($n_{wild} = 341$; $n_{captive} = 250$; $n_{unknown} = 66$). We tested the differences of each isotope in the two rearing systems and compared seven classification models, using the most accurate to classify our unknown samples. All isotopes differed between the wild and captive birds. The Random Forest model performed best, using $\delta^{13}C$ and $\delta^{2}H$ as the main predictors to distinguish between the two environments. 37.88% of the unknown samples were classified as wild birds, including at least seven individuals with official rings, which indicated they were supposedly born in captivity. We have demonstrated the ability of stable isotopes to distinguish between wild and captive songbirds in Brazil, especially $\delta^{13}C$ and $\delta^{2}H$. Our results suggest that some songbirds have been laundered, highlighting the potential of stable isotope analysis in detecting this illegal practice in Brazil.

Resumo

O comércio ilegal de animais selvagens é uma das principais causas da perda de biodiversidade, causando vários impactos ambientais. No Brasil, o comércio ilegal de animais selvagens é focado em passeriformes, especialmente da família Thraupidae, família que apresenta alta riqueza, diversidade e abundância, e inclui algumas espécies altamente ameaçadas. A regulamentação do comércio de animais silvestres foi concebida como uma estratégia de conservação, mas se não for gerida com cuidado, essa atividade pode ter o efeito oposto, intensificando o impacto nas populações naturais por meio da lavagem de animais. Identificar se um animal comercializado é de origem cativa ou selvagem é uma tarefa desafiadora, mas crucial para avaliar a legalidade do comércio. A análise de isótopos estáveis é uma ferramenta poderosa para indicar simultaneamente a origem e a dieta de animais. Essa técnica tem se mostrado útil para diferenciar indivíduos de vida livre e cativeiro em diversos estudos envolvendo diferentes grupos de vertebrados. Neste estudo, investigamos o uso de $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ e $\delta^{18}O$ para diferenciar aves canoras de vida livre e cativeiro no Brasil. Analisamos 657 penas de 58 espécies não migratórias da família Thraupidae distribuídas por todo o país (n_{wild} = 341; $n_{captive}$ = 250; $n_{unknown}$ = 66). Testamos as diferenças de cada isótopo nos dois sistemas de criação e comparamos sete modelos de classificação, utilizando o mais acurado para classificar nossas amostras de origem desconhecida. Todos os isótopos diferiram entre os pássaros de vida livre e de cativeiro. O modelo utilizando Random Forest teve o melhor desempenho, sendo $\delta^{13}C$ e $\delta^{2}H$ os principais preditores utilizados para classificar os indivíduos de acordo com o sistema de criação. 37,88% das amostras desconhecidas foram classificadas como pássaros de vida livre, incluindo indivíduos com anilhas oficiais, supostamente nascidos em cativeiro. Nós demonstramos a capacidade dos isótopos estáveis para distinguir entre passeriformes de vida livre e cativeiro no Brasil, especialmente $\delta^{13}C$ e $\delta^{2}H$. Nossos resultados sugerem que alguns indivíduos foram esquentados, destacando o potencial da análise de isótopos estáveis na detecção dessa prática ilegal no Brasil.

Introduction

The illicit wildlife trade (IWT) is one of the main causes of biodiversity loss causing several environmental impacts, such as introduction of invasive species (García-

Díaz *et al.*, 2015), spread of zoonotic diseases (Smith *et al.*, 2017; Keskin *et al.*, 2023), disruption of ecosystem services (Dirzo *et al.*, 2014; Gardner *et al.*, 2019), and defaunation (Dirzo *et al.*, 2014; Young *et al.*, 2016). Additionally, the IWT may involve a complex chain, including poachers, armed non-state actors, international crime groups, and institutional corruption, threatening national stability and provoking substantial socioeconomic losses (Lawson and Vines, 2014; Zain, 2020).

The impact of IWT is particularly concerning in source areas for the trafficking, which typically includes highly biodiverse and socially unequal regions, such as Brazil (Young *et al.*, 2016; Wyatt *et al.*, 2018; Destro, De Marco, & Terribile, 2020). In Brazil, the illegal wildlife trade is focused on birds, accounting for around 80% of the animals seized in the country and affecting about 1.5 million birds annually (Destro *et al.*, 2012; Costa *et al.*, 2018; Develey, 2021). Songbirds are the most affected group, causing the decline or even extinction of species where they were previously abundant (Fernandes-Ferreira *et al.*, 2012; Oliveira, de Freitas Torres, & da Nóbrega Alves, 2020). Many of Brazil's most trafficked songbirds belong to the Thraupidae family, which includes threatened species such as *Gubernatrix cristata* and *Sporophila maximiliani*. The Thraupidae is the second richest bird family in the world, with 384 species (Clements *et al.*, 2023), and in Brazil with 156 species, inhabiting a wide range of terrestrial environments, including forests, open areas, degraded lands, and urban zones across the country (Pacheco *et al.*, 2021).

The Convention of International Trade in Endangerment Species of Wild Fauna and Flora (CITES) foresees licensed breeders for some species as a conservation strategy, supplying the demand for wild animals with captive-bred individuals, reducing illegal capture and poaching (Bulte & Damania, 2005; Damania & Bulte, 2007; Rizzolo, 2020). However, much wildlife trade occurs domestically, not under CITES protection (Lawson & Vines, 2014; Morton *et al.*, 2021; Hughes *et al.*, 2023). In addition, some studies have suggested a relationship between authorized and illegal animal markets, where illegally captured animals supply the former, leading to animal laundering and intensifying the irregular trade (Livingstone & Shepherd, 2016; Challender *et al.*, 2019; de Lucena Soares *et al.*, 2020).

In Brazil, nearly one million breeders and over 3 million birds are registered only within the amateur breeding system, and most species raised by licensed breeders are also among those most frequently seized by environmental agencies (Charity & Ferreira, 2020; Destro et al., 2012). If not well planned, regulated, and controlled, the wildlife

market intended as a conservation strategy can have the opposite effect, intensifying the IWT and the impact on wild populations (Phelps, Carrasco, & Webb, 2014; Livingstone & Shepherd, 2016; Tensen, 2016; Challender *et al.*, 2019; Morton *et al.*, 2021). Identifying whether a commercialized animal (or its derivative) has a captive or wild origin is crucial for assessing the legality of trade. However, this is challenging and continues to pose a significant obstacle in monitoring wildlife trade since the traditional control techniques are usually inaccurate or susceptible to fraud.

Stable isotope analysis is an essential tool for differentiating wild and captive animals in different vertebrate groups (Brasileiro *et al.*, 2023). Variation of $\delta^{13}C$ and $\delta^{15}N$ in animal tissues reflects mainly their isotopic diet composition, such as the primary energy source (C3 or C4 plants) and trophic position within food chain complexity, respectively. The $\delta^{2}H$ and $\delta^{18}O$ reflect the source of the water consumed, geographic origin, and movement in response to the hydrological process (Hobson & Wassenaar, 2019; Magozzi et al., 2019). Since wild and captive animals are likely to access different resource items from distinct geographic origins, they should incorporate and exhibit such differences in their organic tissues (Dempson & Power, 2004; Kays & Feranec, 2011; Chaguri *et al.*, 2017; Natusch *et al.*, 2017). While wild animals typically exhibit greater variability in their isotopic signatures due to diverse diets influenced by natural ecosystems, captive animals usually have more homogeneous diets that reflect their controlled feeding conditions (Kays & Feranec, 2011; Dittrich, Struck, & Rödel, 2017; Natusch *et al.*, 2017; Jiguet, Kardynal, & Hobson, 2019).

Here, we investigated the use of $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ analyses to differentiate wild and captive birds within the diverse Thraupidae family in Brazil. We expect the isotopic values to differ between environments due to variations in water and food sources. We also examined the effects of geographic location and dietary habits on how isotopic signatures could distinguish birds from these two rearing systems. Finally, we assessed the performance and the main predictors of statistical models in classifying the rearing system based on isotopic values, and we applied our best model to estimate the origin of seized birds in different regions of Brazil.

Materials and methods

Sample collection

We analyzed 657 feathers of 58 non-migratory species from the Thraupidae family from Brazil (Figure 1; Table S1 and Table S2). We identified samples according to the species, dietary guild (following (Pigot *et al.*, 2020), location, collection date, and

origin (wild, captive, or unknown). Whenever possible, we also classified samples according to sex (female, male, or unknown), life stage (adult or subadult), and feather type (flight or body) (Table S1). The life stage was determined either directly from the information provided by the researchers who collected the samples or inferred based on the plumage and the estimated age of the individuals. Wing primaries or secondaries are more associated with a specific molting period, but if grown simultaneously, body and flight feathers are expected to have similar isotopic values (Kelly *et al.*, 2002; Silva, Costa, & Nardoto, 2024). Taxonomic classification follows the Brazilian Ornithological Records Committee (Pacheco *et al.*, 2021).

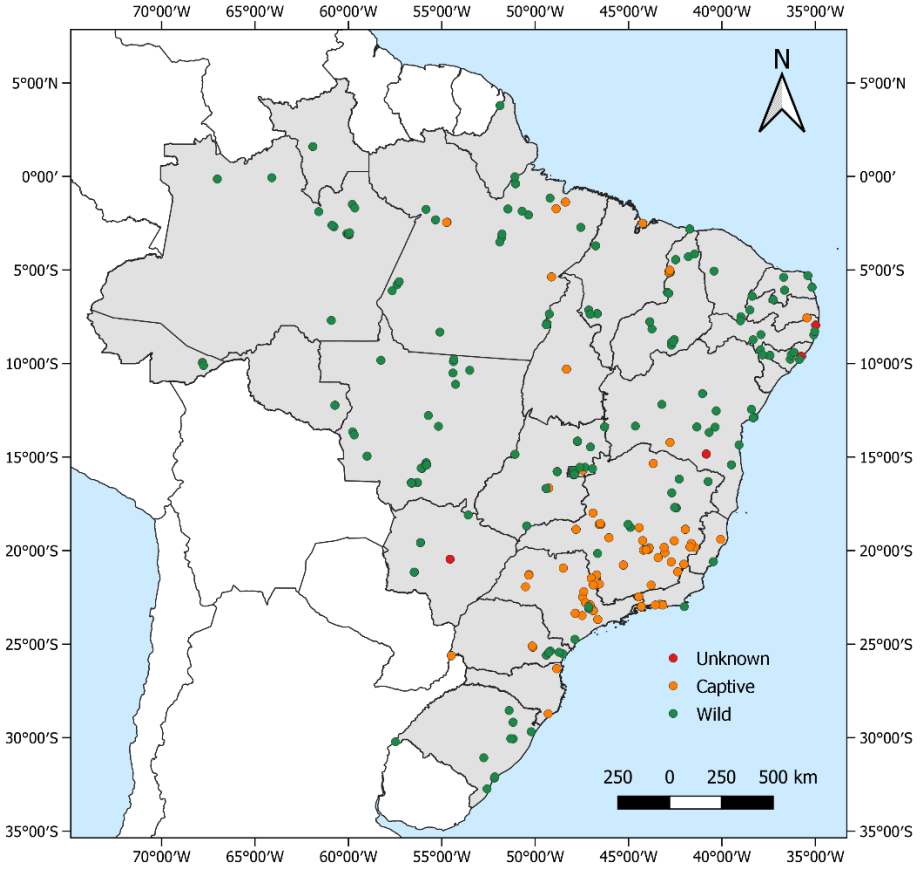

Figure 1. Feathers collected from wild (green circles), captive (orange circles), and unknown (red circles) birds from the Thraupidae family in Brazil.

Our captive dataset comprised 250 samples of 15 species from Brazil collected between 2017 and 2022 (Table S1; Table S2). Part of our captive samples were collected from a feather collection provided by the Stable Isotope Center at São Paulo State University (CIE/UNESP) supplied by a company that sexed birds from registered breeders. The remaining samples were obtained directly from legalized breeders or zoos

indicated by local environmental agencies. We considered samples from captivity when birds were born or kept in captivity for at least one year before the collection. When official ring numbers were available (n = 120), we searched for the life history of the birds in the online Brazilian passerine management system (SisPass) to identify information such as birth date, sex, and location changes. We also collected the body and flight feathers available of the same 23 individuals to assess possible differences between feather types, including the most trafficked species: *S. similis* (n = 8), *S. flaveola* (n = 5), *S. angolensis* (n = 7), *S. maximiliani* (n = 2), *V. jacarina* (n = 1).

Our dataset of wild birds comprised 341 samples of 54 species held in scientific ornithological collections in Brazil and from field campaigns in natural areas between 2007 and 2023 (Table S1). Of this dataset, 188 samples of 44 species were from a previous project that analyzed the $\delta^{2}H$ (Alquezar *et al.*, 2022), $\delta^{13}C$, and $\delta^{15}N$ of birds from the Thraupidae family in the Brazilian territory.

Finally, we collected feathers from 66 birds of 10 species in five governmental wildlife rescue and rehabilitation centers (Figure 1; Table S2) between 2022 and 2023. These centers are facilities where wild animals, seized by local authorities, are rehabilitated and subsequently released into the wild or transferred to legal breeders. Although these samples were collected from animals in captivity, their origin was classified as “unknown" since the time in captivity of seized animals is usually uncertain. Whenever possible, we accessed information about the animals' entry into the wildlife centers and the birds' life histories using SisPass.

Isotopic analysis of feathers

Feathers from the 188 individuals of the previous project had the $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ analyzed at the University of Western Ontario LSIS-AFAR laboratory. For all other samples, we analyzed the isotopic ratios of the four main elements $R(^{2}H/^{1}H)$, $R(^{13}C/^{12}C)$, $R(^{15}N/^{14}N)$, and $R(^{18}O/^{16}O)$ at the Stable Isotopes Center (CIE) of São Paulo State University (UNESP, Brazil). Feathers were soaked in a 2:1 chloroform:methanol solution overnight, drained, washed with water, and dried at 50 °C for 48h. For $R(^{2}H/^{1}H)$ and $R(^{18}O/^{16}O)$, feathers were weighed (~ 250 μg) into silver capsules and analyzed in an isotope ratio mass spectrometry system with high-temperature conversion elemental analyzer (Flash HT – Delta V Advantage, Thermo Scientific). For $R(^{13}C/^{12}C)$ e $R(^{15}N/^{14}N)$, feathers were weighed (~ 600 μg) into tin capsules and analyzed in an isotope ratio mass spectrometry system with combustion elemental analyzer (Flash 2000 – Delta V Advantage, Thermo Scientific).

Stable isotope ratios were expressed as relative differences of isotope ratio ($\delta^{i}E$) from standard ratios $R(^{i}E/^{j}E)_{standard}$ in parts per mil (‰):

$$\delta^{i}E_{(‰)} = \frac{R\left(\ {}^{i}E/\ {}^{j}E\right)_{sample}}{R\left(\ {}^{i}E/\ {}^{j}E\right)_{standard}} - 1$$

Where $R(^{i}E/^{j}E)_{sample}$ is the isotopic ratio of sample, ^{i}E is the rare isotope, and ^{j}E is the abundant isotope. $\delta^{13}C$ was reported relative to the standard Vienna Pee Dee Belemnite (VPDB), $\delta^{15}N$ was reported relative to atmospheric N_2 (AIR), $\delta^{2}H$ and $\delta^{18}O$ were reported relative to the Vienna Standard Mean Ocean Water (VSMOW). For internal quality control of analyses, during each cycle of readings (at the beginning and the end of each batch: 45-sample sequential analyses), a standard sample was used to ensure the accuracy of quantifications. The standard samples were calibrated against USGS and IAEA-certified reference materials (KHS, CBS, USGS42 and USGS43). The standard uncertainty for the analyses was ±0.9 ‰ for $\delta^{2}H$; ±0.40 ‰ for $\delta^{18}O$; ±0.10‰ for $\delta^{13}C$ and ±0.15‰ $\delta^{15}N$.

Statistical analyses

Exploratory analysis:

As we included samples analyzed in two laboratories, we first examined whether there were isotopic differences between them. We compared the $\delta^{13}C$ and $\delta^{2}H$ sub-samples of 13 individuals of the same species (*Sicalis citrina*) analyzed in both laboratories using Wilcoxon tests and performed the necessary transformations using linear regression. Isotopic ratio differed between laboratories for $\delta^{2}H$ ($V = 91$; $p < 0.001$), but nor for $\delta^{13}C$ ($V = 25$; $p = 0.17$). Since the $\delta^{2}H$ values obtained from the two laboratories are highly correlated ($S = 18$; $p < 0.001$; $\rho = 0.98$), we performed linear regression to derive the relationship between the $\delta^{2}H$ values from the University of Western Ontario LSIS-AFAR laboratory (Alquezar *et al.*, 2022) and those obtained at São Paulo State University CIE laboratory. Using the regression parameters, we transformed the $\delta^{2}H$ values of the 188 feathers analyzed at the LSIS-AFAR laboratory to predict the equivalent values that would have been obtained if these feathers had been analyzed at the CIE laboratory. This transformation ensured comparability between datasets. Table S1 and all the other tests used the transformed values.

To better understand how sample characteristics affected our dataset, we ran exploratory analyses evaluating whether isotopic values are affected by sex, life stage (adult or subadult), and feather type (body or flight). We used Mann-Whitney or Student's *t*-test to evaluate isotopic differences between sexes and life stages. Because there is a

bias in our captive samples, concentrated particularly in the country's southeast, we also carried out GLM models to assess differences in $\delta^{2}H$ and $\delta^{18}O$ according to the life stage controlling for latitude. We used only these two isotopes because they are mainly affected by geographical location. For feather type, we used PERMANOVA, as samples were not independent and did not meet the assumption of multivariate normality. Despite the risk of incurring Type I or Type II errors by performing multiple exploratory tests instead of model selection that considers all variables of interest simultaneously, we chose the former approach due to the large amount of missing data for "sex" and "life stage" variables.

Before each analysis, we tested normality using the Shapiro-Wilk test for each isotope within each compared category (e.g., $\delta^{13}C$ in adult captive individuals, $\delta^{13}C$ in subadult captive individuals, and so on). We tested the homoscedasticity using Bartlett tests.

Differences between wild and captive

We assessed the normality of the data distribution for each isotope in the wild and captive samples using Shapiro-Wilk test, and the homoscedasticity using Bartlett test. Given the unbalanced species composition between the two rearing systems (Table S2), we tested isotopic differences between wild and captive groups using quantile regression at the median ($\tau = 0.5$). This method was chosen because the data were neither normally distributed nor homoscedastic, allowing for robust estimation of differences while controlling for the effect of species as a fixed factor.

We also evaluated the influence of geographic location and dietary habits on how wild and captive birds differ isotopically. To assess the influence of geographic location, we used general linear models for each isotope, incorporating the interaction between latitude, longitude, and the rearing system (wild *vs*. captive). For dietary habits, we conducted a factorial Analysis of Variance (ANOVA) based on dietary guilds, as categorized by Pigot et al. (2020)

Classification models:

To include data from the 188 samples of wild birds from the previous project that did not have their oxygen isotopic values analyzed, we first imputed the $\delta^{18}O$ missing values using the missForest package (Stekhoven, 2022)., and using $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, dietary guild, biome, latitude and longitude as predictors.

To assess the accuracy of $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ values in discriminating between wild and captive individuals, we initially selected widely used methods from the

literature. We compared the performance of various models on our dataset (Table S3). We developed predictive models based on general linear model (GLM), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), K-nearest neighbor (KNN), classification trees, and Random Forest (RF), with the environment (wild or captive) as the response variable and δ^2H, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ values as predictor variables. Additionally, we tested a novel approach with a hierarchical clustering analysis, followed by a classification model. Four clusters were defined based on $\delta^{13}C$, $\delta^{15}N$, δ^2H, and $\delta^{18}O$, along with latitude, longitude, and dietary guilds. The clusters were incorporated as predictor variables in a Random Forest classification model. Model accuracy was evaluated using the repeated 10-fold cross validation technique. Finally, we used our best-performing model to classify samples of unknown origin collected from seized birds in five Brazilian wildlife rescue and rehabilitation centers.

All statistical analyses were conducted in R, Version 3.6.2 (R Core Team, 2020).

Results

Exploratory analysis

There was no influence of sex on any of the isotopes analyzed in either the captive or wild groups (Table S4). There was an influence of life stage (adult or subadult) on δ^2H and $\delta^{18}O$ in captive birds, but only when the geographical location was not controlled (Table S4). There was no influence of feather type on the isotopic values using samples from the same 23 captive individuals ($F = 0.92$; $p = 0.46$).

Differences between wild and captive birds

Feathers from wild and captive birds differed significantly in $\delta^{13}C$, $\delta^{15}N$, and δ^2H, but not in $\delta^{18}O$, as determined by quantile regression at the median ($\tau = 0.5$) while controlling for the effect of species (Table 1; Figure 2). The standard deviation was higher for $\delta^{13}C$, $\delta^{15}N$, and δ^2H and lower for $\delta^{18}O$ of wild animals compared with captive animals.

Table 1. Mean and standard deviation of $\delta^{13}C$, $\delta^{15}N$, δ^2H and $\delta^{18}O$ of birds in the wild and captive; and quantile regression results at the median ($\tau = 0.5$) comparing isotopic ratios between the two rearing systems while controlling for the effect of species .

	$\delta^{13}C$	**$\delta^{15}N$**	**δ^2H**	**$\delta^{18}O$**
Wild	-20.3 ± 5.49‰	8.21 ±2.95‰	-26.5 ± 17.6‰	16.3 ± 2.38‰
Captive	-16.6 ± 2.96‰	5.91 ± 1.56‰	-46.8 ± 11.9‰	14.8 ± 4.09‰
Wild *vs.* Captive	$t = 2.81$	$t = 3.39$	$t = -6.7$	$t = -0.41$

	$p = 0.005$	$p < 0.001$	$p < 0.001$	$p = 0.68$

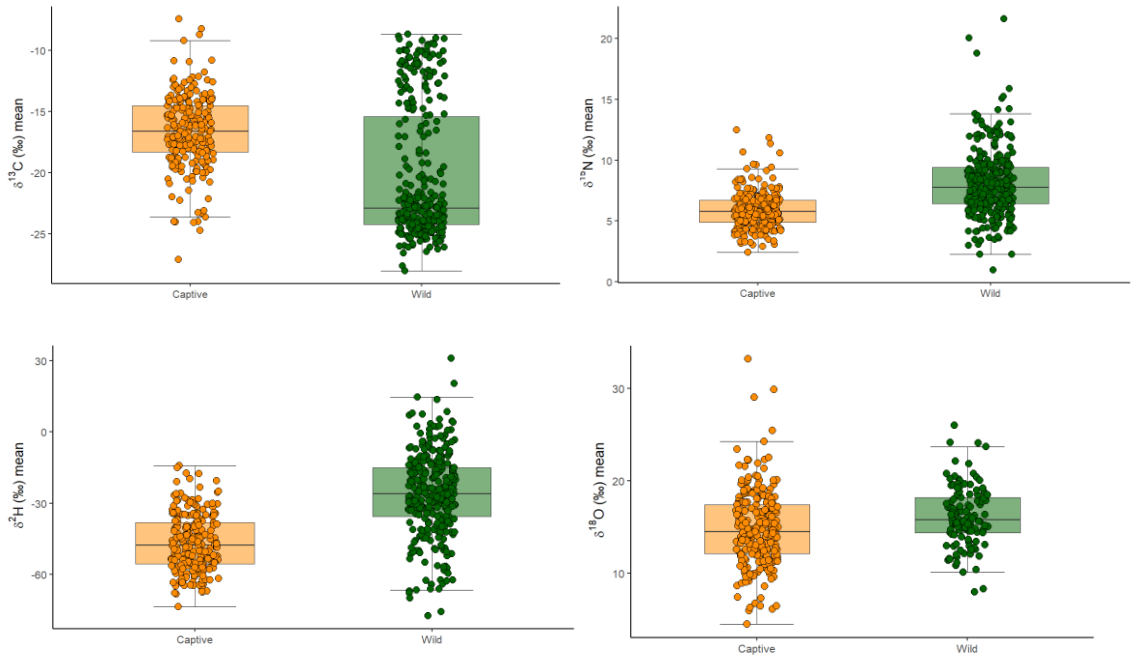

Figure 2. Differences in $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$ of wild and captive birds from Brazil.

The longitude and latitude influenced how $\delta^{13}C$ differentiates between wild and captive animals. The $\delta^{13}C$ of wild animals increased in regions of lower latitude and higher longitude, while the $\delta^{13}C$ of captive animals had the opposite tendency. The $\delta^{2}H$ was also influenced by longitude. While both captive and wild animals showed an increase in $\delta^{2}H$ with longitude, this trend was more pronounced in wild animals. The other isotopes increased with latitude and longitude, irrespective of the environment (Figure S1; Table S4).

Dietary guilds influenced how all isotopes differentiate the two groups (Figure S2; Table S5). Nonetheless, the $\delta^{15}N$ and $\delta^{2}H$ values were higher in wild animals in all dietary guilds. The $\delta^{13}C$ was generally higher in captive animals, except in granivores. The $\delta^{18}O$ presented higher values in captive frugivores, wild granivores, and invertivores.

Classification models:

The accuracy of the different classification models using $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ to predict the rearing system (wild or captive) ranged from 0.78 to 0.92 (Figure 3; Table S3). The Random Forest model performed best, using $\delta^{13}C$ as the main isotope to distinguish between the two environments, followed by $\delta^{2}H$ (Figure 3).

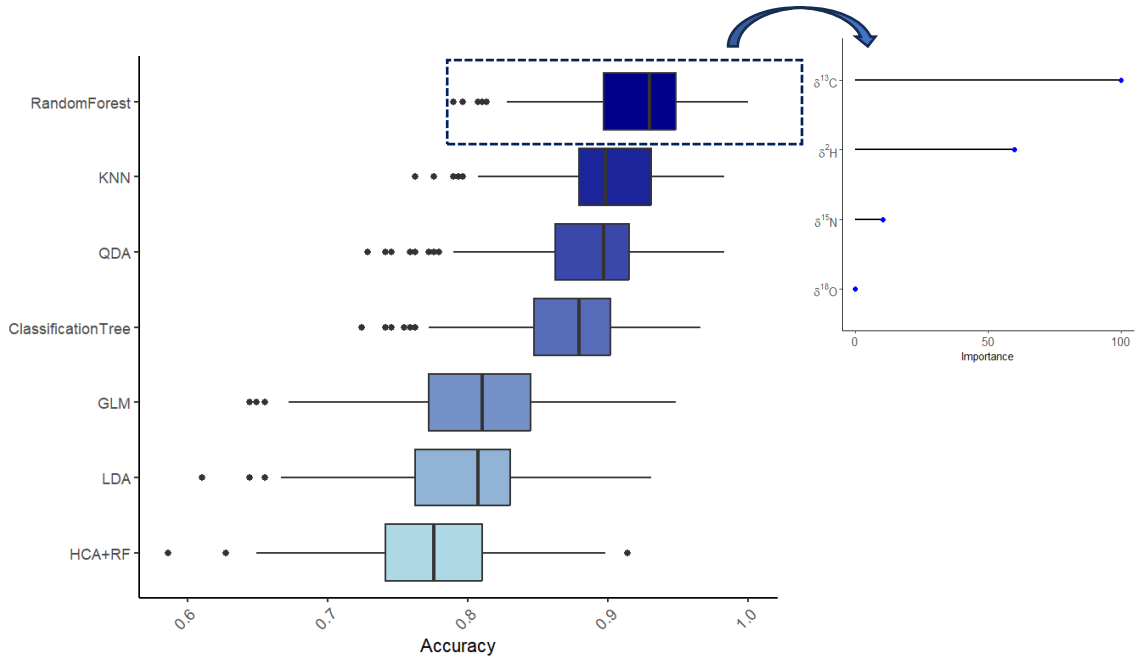

Figure 3. Left: The accuracy of different classification models using 10-fold cross-validation with 100 repetitions. Right: the main predictors of the classification models using Random Forest.

The linear models, such as GLM, LDA, and, to a lesser extent, QDA, may perform suboptimally with non-linear, non-normal, or heteroscedastic data. In contrast, the non-parametric approaches, KNN and classification tree, allowed for more flexible decision boundaries but occasionally lacked stability with certain distributions. RF consistently outperformed other models' predictive accuracy due to its ensemble approach, which reduced overfitting and enhanced robustness. Incorporating clustering with RF further sought to include other relevant variables in the classification model, such as latitude, longitude, and food guild. We chose the Random Forest classification model due to its superior accuracy in predicting wild and captive conditions. 95.16% and 89.09% of the test samples were correctly classified as wild and captive, respectively (Table S6).

Of the unknown samples, 62.12% were classified as captive and 37.88% as wild birds (Figure 4).

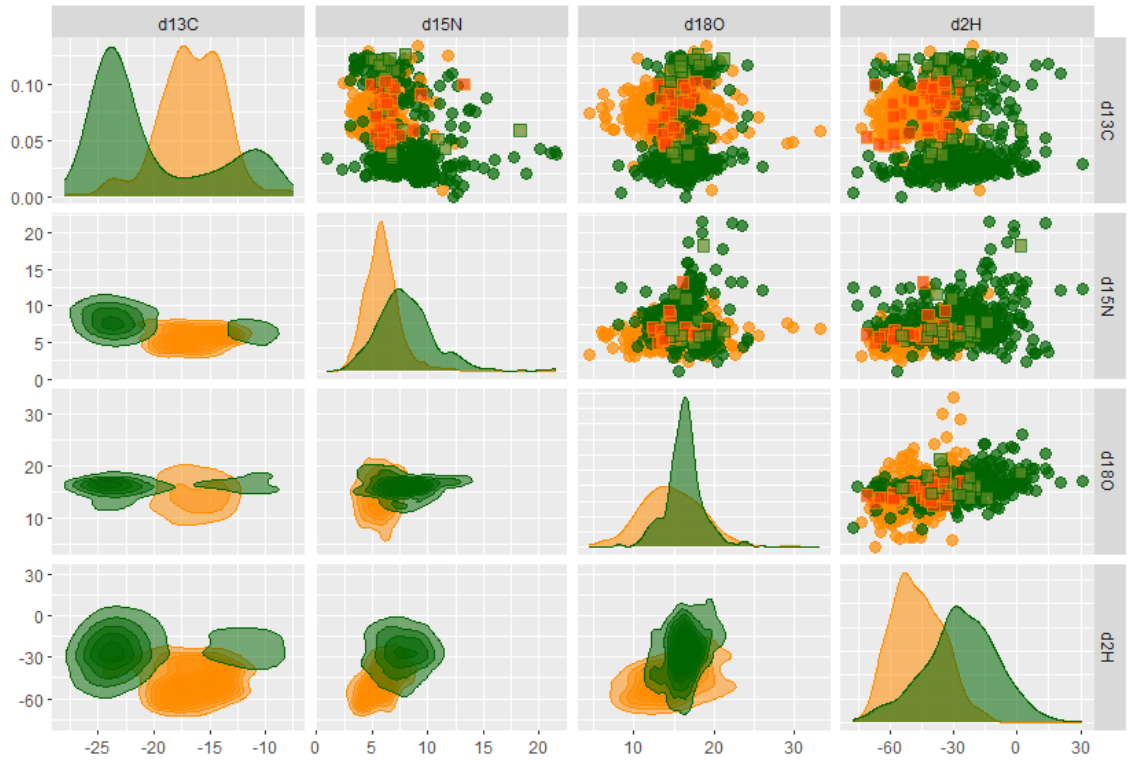

Figure 4. Diagonal and bottom: density distribution of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ for the wild (green) and captive (orange) samples. Top: bi-plot of the known wild (green circle) and captive (orange circle) samples and those classified by our best model using Random Forest as wild (green square) or captive (orange square).

Discussion

Our results demonstrate that stable isotopes can effectively distinguish between wild and captive birds in Brazil, even within a complex dataset encompassing over 50 species across this continent-sizes country. In our study, we emphasize the utility of combining $\delta^{13}C$ and $\delta^{2}H$ stable isotopes as the most effective pairing to distinguish these two groups. We encourage future studies to incorporate these isotopes alongside the traditional $\delta^{13}C$ and $\delta^{15}N$ pairing for this purpose. The high accuracy of our classification models suggests that stable isotopes could serve as a valuable complementary method for identifying the rearing origin of traded birds in Brazil, adding to the detection and prevention of illegal wildlife trade.

Differences between wild and captive birds

Except for $\delta^{18}O$, isotopes were effective in differentiating wild from captive birds. Most studies using stable isotopes to differentiate wild and captive animals focus on $\delta^{13}C$ and $\delta^{15}N$ of one or a few species, mainly on a local scale (Brasileiro *et al.*, 2023).

Our results suggest that the isotopic methodology could have a broader application in identifying the rearing system origin of wild birds, even if it involves a great diversity of species, large scales, and high variability of ecosystems, such as the Brazilian territory. These findings have potential applications in combating wildlife trafficking by providing a scientific basis for identifying the origin of seized animals. However, the methodology has limitations, particularly the potential loss of the original isotopic signature if the tissue undergoes complete turnover in a new environment, such as feathers after the last molt.

In line with previous findings (reviewed in Brasileiro et al., 2023), the wild birds had lower $\delta^{13}C$ and higher $\delta^{15}N$ than the captive ones, reflecting differences in the diet in these two environments. The $\delta^{15}N$ was higher in wild birds regardless of the geographic location and dietary trophic niche, suggesting consistent access to higher trophic level food items compared to those provided to captive individuals. On the other hand, the geographic location and the dietary guild influenced how $\delta^{13}C$ distinguished wild and captive birds. The $\delta^{13}C$ differed especially in regions of high latitude and low longitude, i.e., the Amazon rainforest region. The Amazon rainforest has the lowest leaf $\delta^{13}C$ in Brazil (Powell, Yoo, & Still, 2012; Martinelli *et al.*, 2021), leading to particularly lower $\delta^{13}C$ values in wild birds in this region.

Regarding the dietary guild, the $\delta^{13}C$ was higher in wild granivores than in captive ones, in contrast to the general trend. Despite possible regional differences, the food provided for bird keepers in Brazil typically consists of seeds (e.g., birdseed and millet), fruits, and commercial feed, usually based on corn and, in lower proportion, other grains such as rice, wheat, and oats. This combination, including C4 (e.g., corn and millet) and C3 (e.g., birdseed, rice, wheat, and oat) sources, leads to intermediate $\delta^{13}C$ isotopic values in captive birds, lower than wild granivores but higher than all other groups, which should access a higher proportion and variety of C3 items in the natural environment (Costa *et al.* in review; Santos *et al.* in review)

The $\delta^{2}H$ values were higher in the wild birds, regardless of the geographic location and the dietary guild. Less marked, the $\delta^{18}O$ also tended to be higher in wild birds, except for frugivores. These differences may be due to multiple reasons, such as the access to isotopically distinct water sources in the two environments (tap water for captive birds and rainwater or surface water bodies for wild birds). Also, due to the *ad libitum* water supply in captivity, the proportion of drinking water over the diet water influx is probably higher in this environment than in wild animals, leading to lower $\delta^{2}H$ and $\delta^{18}O$ values in captivity (Magozzi *et al.*, 2019).

Classification models

Previous studies used different statistical approaches to classify wild and captive animals according to their isotope signatures (Anderson, Hobbie, & Smith, 2010; van Schingen *et al.*, 2016; Castelli & Reed, 2017; Natusch *et al.*, 2017; Brandis *et al.*, 2018; Farabegoli *et al.*, 2018; Pereira *et al.*, 2019; Hill *et al.*, 2020; Hutchinson & Roberts, 2020; Andersson *et al.*, 2021; Hopkins *et al.*, 2022). The comparison between the seven main approaches revealed that all had accuracy above 0.8, reinforcing that isotopes can be a good predictor of the rearing system using distinct statistical methods. For our dataset, Random Forest had the best performance with 0.92 accuracy. Although all the isotopes differed significantly between wild and captive birds, $\delta^{13}C$ was the main predictor, followed by $\delta^{2}H$, while $\delta^{15}N$ had little and $\delta^{18}O$ negligible participation in the model.

The importance of $\delta^{13}C$ in our model aligns with most studies, where $\delta^{13}C$ played a significant role in distinguishing between wild and captive animals (Reviewed in Brasileiro *et al.*, 2023). Despite the influence of geographical location or trophic niche, the differences in $\delta^{13}C$ between wild and captive animals seem to be more significant than the intra-environment variation. The participation of $\delta^{2}H$ highlights the under-explored potential of this isotope to differentiate wild and captive animals. Although $\delta^{2}H$ has been applicable in differentiating wild and captive animals in a few studies (Alexander *et al.*, 2019; Jiguet, Kardynal, & Hobson, 2019), it has not been included in previous classification models for the origin of the rearing system, either because the isotope was not analyzed or because it did not appear as relevant for the classification (Castelli & Reed, 2017; Natusch *et al.*, 2017). Here, the consistent access to different sources and proportions of drinking water influx by wild and captive animals are probably the main reasons for the importance of the H in the classification model, suggesting that complex and varied dataset, including large territorial extensions and species diversity, it can perform better than the traditionally used $\delta^{15}N$.

Studies using stable isotopes to differentiate between wild and captive animals are typically based on the premise that these two rearing systems provide access to distinct diets (Dempson & Power, 2004; Kays & Feranec, 2011; Chaguri *et al.*, 2017; Natusch *et al.*, 2017). Such research often focuses on the combination of $\delta^{13}C$ and $\delta^{15}N$, as these elements are key components of organism tissues, derived almost entirely from diet, and reflect both the primary energy source and trophic level (DeNiro & Epstein, 1977; Fry, 2008). In contrast, multiple sources, including water, food, and atmospheric O_2, contribute to $\delta^{2}H$ and $\delta^{18}O$ in animal tissues. Despite this complexity, the mechanisms of

fractionation for these isotopes in relation to diet have become better-understood differentiation (e.g., Daniel Bryant & Froelich, 1995; Magozzi et al., 2019), highlighting their potential in research focused on diet.

Our classification model using Random Forest revealed that most samples of unknown origin collected from seized birds in the Brazilian governmental wildlife centers have captive signatures, suggesting these individuals were born or made their last molt in captivity, including the rehabilitation centers. However, 37.88% were classified as wild, including at least seven individuals with official metal rings and from all five centers collected (for eight individuals classified as wild, we had no information on whether they were ringed). The trade of songbirds in Brazil is permitted but restricted to captive-born birds, each carrying a closed, official metal ring. Thus, birds with these rings that displayed wild isotopic signatures were probably laundered through the illegal wildlife trade. These findings emphasize the importance of using complementary methods to traditional external markers to identify the origin of wild animals bred and marketed as captive.

Our study demonstrates the power of stable isotope analysis to reliably distinguish between wild and captive songbirds in Brazil, even within a diverse dataset spanning multiple species and ecological zones. Geographical location and dietary guild can influence the isotopic ratios of wild animals; however, the differences between the two rearing systems were more pronounced than variations within each environment. The $\delta^{13}C$ and the $\delta^{2}H$ were the main isotopes used to classify the rearing system, suggesting that combining a dietary and a geolocator indicator isotope may be particularly effective in large-scale studies. Our study suggests the occurrence of songbird laundering in Brazil. Stable isotope analysis shows significant potential for detecting and preventing this illegal practice, supplementing conventional external markers. Our findings support conservation and law enforcement actions against illegal trade practices by providing an additional layer of verification for wildlife trade compliance. This approach contributes to building a strong scientific foundation for applying stable isotope analysis to wildlife crime investigations, offering authorities a more effective tool for regulating wildlife trade.

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SUPPORTING INFORMATION

Table S1. Dataset with the raw data used in this study.

Table S2. Number of samples from individuals of wild, captive and unknown origin per specie.

SPECIE	WILD	CAPTIVE	UNKNOWN	TOTAL
Coereba flaveola	12	0	0	12
Compsothraupis loricata	1	0	0	1
Conirostrum bicolor	4	0	0	4
Coryphospingus cucullatus	7	0	0	7
Coryphospingus pileatus	23	0	0	23
Dacnis cayana	53	0	0	53
Eucometis penicillata	3	0	0	3
Loriotus cristatus	6	0	0	6
Loriotus luctuosus	1	0	0	1
Maschalethraupis surinamus	16	0	0	16
Microspingus lateralis	1	0	0	1
Nemosia pileata	2	0	0	2
Neothraupis fasciata	6	0	0	6
Paroaria baeri	2	0	0	2
Paroaria capitata	6	0	0	6
Paroaria coronata	1	3	5	9
Paroaria dominicana	5	4	8	17
Paroaria gularis	2	0	0	2
Porphyrospiza caerulescens	1	0	0	1
Pyrrhocoma ruficeps	1	0	0	1
Ramphocelus bresilius	1	0	0	1
Ramphocelus carbo	16	0	0	16
Saltator aurantiirostris	1	0	0	1
Saltator coerulescens	2	0	0	2
Saltator maximus	6	4	0	10
Saltator similis	4	75	6	85
Schistochlamys melanopis	3	0	0	3

Schistochlamys ruficapillus	1	0	0	1
Sicalis citrina	3	0	0	3
Sicalis columbiana	2	0	0	2
Sicalis flaveola	36	31	9	76
Sicalis luteola	1	0	0	1
Sporophila albogularis	3	0	1	4
Sporophila angolensis	4	70	13	87
Sporophila bouvreuil	0	0	4	4
Sporophila caerulescens	4	8	11	23
Sporophila falcirostris	0	3	0	3
Sporophila frontalis	0	2	0	2
Sporophila lineola	0	6	0	6
Sporophila maximiliani	1	23	1	25
Sporophila nigricollis	12	5	8	25
Sporophila plumbea	1	5	0	6
Stilpinia cyanicollis	3	0	0	3
Stilpinia nigrocincta	1	0	0	1
Tachyphonus rufus	9	0	0	9
Tangara chilensis	2	0	0	2
Tangara cyanocephala	4	0	0	4
Tangara cyanoventris	1	0	0	1
Tangara desmaresti	1	0	0	1
Tangara mexicana	3	0	0	3
Tangara preciosa	2	0	0	2
Tersina viridis	1	0	0	1
Thlypopsis sordida	1	0	0	1
Thraupis episcopus	7	0	0	7
Thraupis palmarum	16	0	0	16
Thraupis sayaca	17	2	0	19
Trichothraupis melanops	1	0	0	1
Volatinia jacarina	18	9	2	29

Table S3. The average accuracy of the different models to differentiate wild and captive thraupids in Brazil. The classification models selected from previously published works are indicated.

Classification model	Accuracy	Selected from
General linear model (GLM)	0.80	Hopkins *et al.* 2022
Linear Discriminant Analysis (LDA)	0.80	Anderson *et al.* 2010; Natusch *et al.* 2017; Castelli and Reed 2017; Andersson *et al.*, 2021
Quadratic Discriminant Analysis (QDA)	0.89	Anderson *et al.* 2010; Farabegoli *et al.* 2018; Pereira *et al.* 2019;
K-nearest neighbor (KNN)	0.90	Van Schingen *et al.* 2016; Hutchinson and Roberts 2020
Classification tree	0.89	Hill *et al.* 2020
Random Forest	0.92	Brandis *et al.* 2018
Cluster + Random Forest	0.78	-

Table S4. Comparison of isotopic values per sex (female or male) and life stage (adult or subadult). Significative results are shown in red.

		$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{18}O$
Sex	Wild	$W = 526$; $p = 0.69$	$t = 0.03$; $p = 0.97$	$t = -0.98$; $p = 0.33$	$t = -0.91$; $p = 0.36$
	Captive	$W = 6378$; $p = 0.65$	$W = 6980$; $p = 0.46$	$t = 0.13$; $p = 0.90$	$W = 5803$; $p = 0.09$
Life-stage	Wild	$W = 166$; $p = 0.40$	$W = 231$; $p = 0.69$	$t = 0.98$; $p = 0.37$	$t = -0.27$; $p = 0.80$
	Captive	$W = 5399$; $p = 0.12$	$W = 5532$; $p = 0.05$	$t = 2.58$; $p = 0.01$	$W = 5790$; $p = 0.03$
	Captive*	-	-	$t = -1.48$; $p = 0.14$	$t = -0.97$; $p = 0.33$

*Mixed-effects models controlling for latitude

Table S5. Influence of geographic location (latitude and longitude) and trophic niche on the isotopic differentiation between wild and captive birds. Significative results are shown in red.

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{18}O$
Latitude	$t = -5.20$; $p < 0.01$	$t = 0.90$; $p = 0.37$	$t = -0.33$; $p = 0.74$	$t = -0.94$; $p = 0.35$
Longitude	$t = 4.16$; $p = 0.01$	$t = -0.20$; $p = 0.84$	$t = 2.25$; $p = 0.02$	$t = 1.41$; $p = 0.16$
Trophic niche	$F = 24.41$; $p < 0.01$	$F = 4.8$; $p < 0.01$	$F = 4.23$ $p < 0.01$	$F = 6.59$; $p < 0.01$

Table S6. Confusion matrix of the classification model using Random Forest.

	Observed	
Predicted	Wild	Captive
Wild	59	6
Captive	3	49

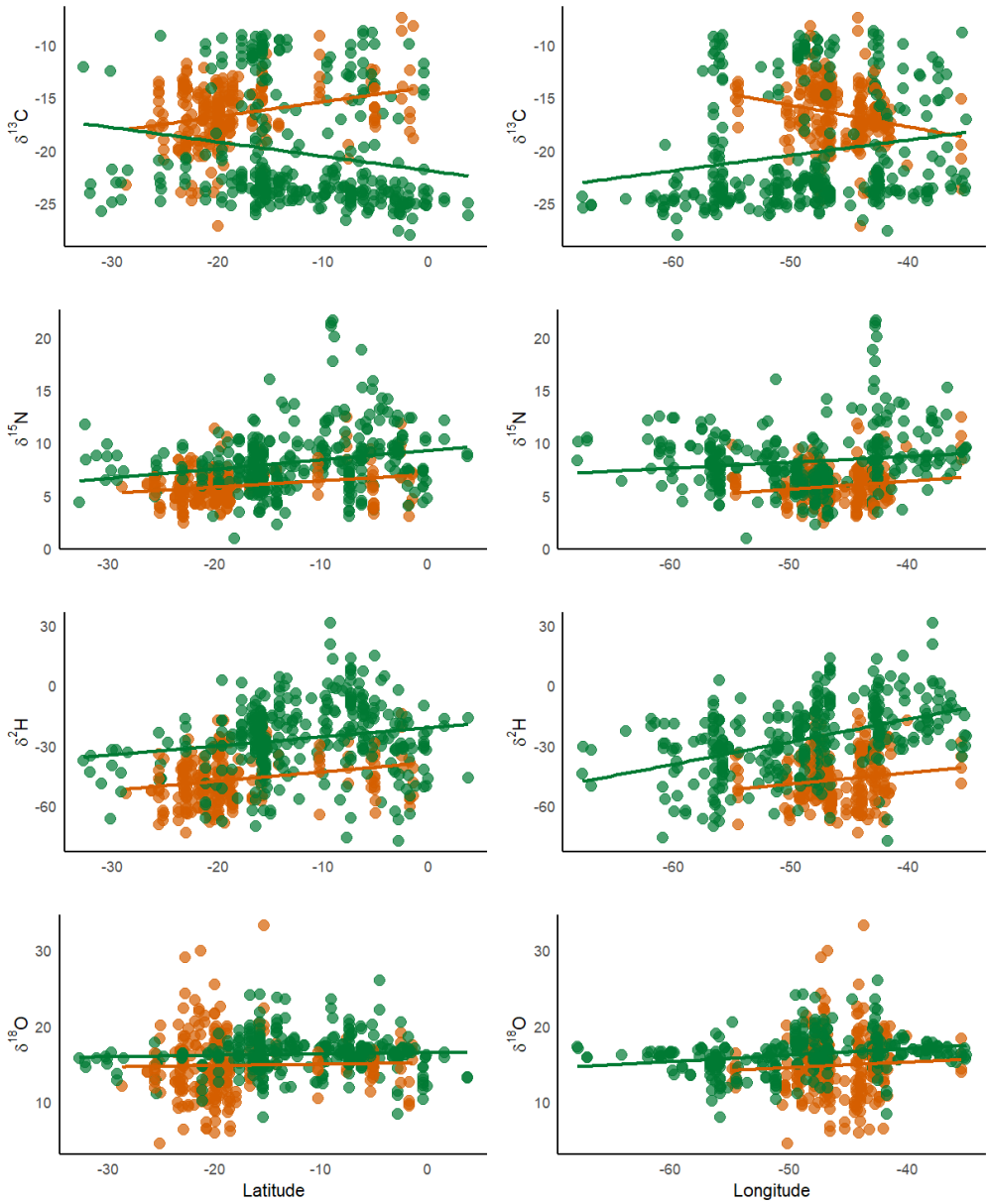


Figure S1. Variation of isotopic values in wild (green) and captive (orange) birds according to latitude and longitude. The lines represent the predicted relationships between latitude, longitude, and each isotope based on a generalized linear model (GLM).

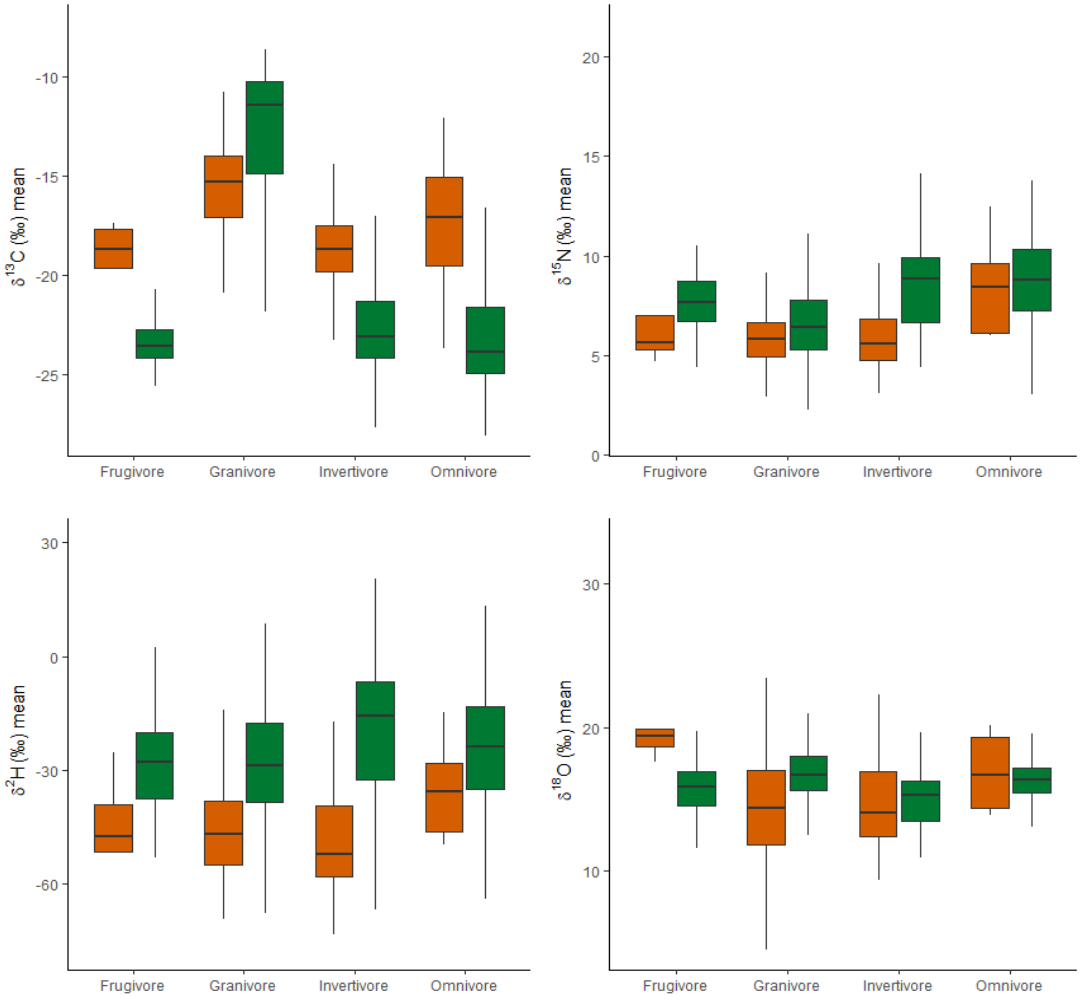


Figure S2. Wild *versus* captive per dietary guilds (following Pigot *et al.*, 2002).

CAPÍTULO 3 – CONNECTING GEOGRAPHY AND REARING SYSTEMS: ISOSCAPES OF WILD AND CAPTIVE SONGBIRS

Connecting geography and rearing system: isoscapes of wild and captive songbirds.

Abstract

Stable isotopes are valuable tools for understanding ecological processes and addressing wildlife trafficking. This study aimed to create high-resolution isoscapes of $\delta^{2}H$ and $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ for feathers of wild and captive birds in Brazil. We used isotopic data from feathers and environmental sources (precipitation, tap water, soil, and vegetation) to investigate spatial isotopic patterns and model their predictive power using Random Forest and Universal Kriging. The study revealed significant spatial isotopic variation across Brazil, influenced by environmental factors such as latitude, longitude, and dietary guilds. Random Forest models outperformed Universal Kriging for most isotopes, particularly $\delta^{2}H$ and $\delta^{13}C$, highlighting their ability to capture complex interactions between environmental variables and isotopic composition. However, the predictive power of some models, such as $\delta^{18}O$ for wild birds, was relatively low due to limited sample sizes and the intrinsic variability of this isotope. Future research should focus on increasing sample sizes and exploring isotopic differences across dietary guilds further. The generated isoscapes have practical applications in wildlife conservation and forensic investigations. For wild birds, $\delta^{13}C$ and $\delta^{15}N$ isoscapes can identify isotopic differences across biomes, reflecting dietary and environmental variability. For captive birds, $\delta^{2}H$ and $\delta^{18}O$ isoscapes provide a foundation for understanding how isotopic signatures relate to local water sources and feeding practices. These tools can help trace the geographic origin of seized animals, offering critical insights for conservation strategies and efforts to combat illegal wildlife trade. By integrating isotopic analyses with spatial modeling, this study contributes to the growing field of wildlife forensics, providing a framework for applying isotopic tools to conservation challenges. Enhanced isoscapes with greater accuracy and resolution will allow for improved identification of regions of origin, better guidance for reintroduction programs, and more effective disruption of wildlife trafficking networks.

INTRODUCTION

The illegal wildlife trade (IWT) significantly impacts on natural populations and is one of the main drivers of defaunation (Dirzo et al. 2014). However, this activity is extremely difficult to control, and it is often challenging to determine the provenance of seized animals (Destro et al. 2012; Destro, De Marco, e Terribile 2020). The legal and illegal wildlife trade in Brazil predominantly targets songbirds, affecting approximately 1.5 million birds annually (Costa et al. 2018; Destro et al. 2012; Develey 2021). As endogenous markers, isotopic analyses are a powerful tool for identifying the geographic origin and movement of trafficked animals and distinguishing between wild-caught and captive-bred specimens (Lyons & Natusch 2015; Vander Zanden et al. 2018; Brasileiro et al. 2023).

The isotopic signatures of wild and captive songbirds of the Thraupidae family differ markedly in Brazil (Brasileiro et al. submitted). The Thraupidae family includes many of Brazil's most trafficked songbirds, and representatives inhabit a wide range of terrestrial environments across the country (Pacheco et al. 2021). Developing isoscapes for both wild and captive birds is therefore critical not only for refining forensic investigations but also for guiding the reintroduction of confiscated animals and shedding light on the dynamics of the illegal bird trade. By identifying both the origin of birds (wild or captive and the geographic provenance), isotopic tools can provide valuable evidence to support law enforcement, enhance conservation strategies, and improve our understanding of the illegal wildlife trade networks.

Globally, numerous relevant isoscapes have been developed, including those for hydrogen and oxygen from precipitation water (Bowen, Wassenaar, Hobson 2005; Terzer et al. 2013; Terzer-Wassmuth et al. 2021), carbon from soil and vegetation (Powell, Yoo, e Still 2012; Wang et al. 2024), and nitrogen from soil (Amundson et al. 2003). These isoscapes have proven instrumental in advancing our understanding of ecosystem processes and have facilitated novel applications, such as tracking animal movements, reconstructing diets, and identifying habitat use across ecosystems (Vander Zanden et al. 2018; Hobson & Wassenaar 2019).

However, these isoscapes are often limited by broad spatial scales that may overlook finer variability. For example, Bowen et al. (2005) proposed models for generating hydrogen and oxygen isoscapes from bird feathers for forensic applications based on global precipitation isotope ratios. While significant, this model has higher

accuracy in regions with more pronounced natural isotopic variations, such as the United States and Europe. In tropical regions like South America, isotopic variation is less pronounced, limiting the model's applicability.

In recent years, significant progress has been made in developing isoscapes tailored to Brazil, which have provided considerable refinement over previous global models. Notable examples include a soil $\delta^{15}N$ isoscape for South America (Sena-Souza et al., 2020) and a soil $\delta^{13}C$ isoscape for the Cerrado (Neves et al. 2021), $\delta^{18}O$ model of mammal hair in the Cerrado and Pantanal (Costa, 2019) and a $\delta^{2}H$ isoscape for feathers of wild Thraupidae in Brazil (Alquezar et al. 2022). Developing more precise, high-resolution isoscapes and incorporating species-specific baselines can significantly enhance the applicability of stable isotope tools. Integrating multiple isotopes into these analyses allows researchers to gain deeper insights into the interactions between species and their environments and the ecological processes driving these patterns. Such advancements enable nuanced ecological inferences and provide essential insights into species' interactions with their environments (Hobson & Wassenaar 2019). (Bowen, Wassenaar, Hobson 2005; Ziegler et al. 2016; Vander Zanden et al. 2018; Alquezar et al. 2022).

Developing isoscapes for bird feathers requires a clear understanding of the relationship between isotopic signatures in tissues and those in the environment. Previous studies have demonstrated correlations between environmental isotopes and animal tissues, particularly for $\delta^{2}H$ (Hobson et al. 2009; Van Wilgenburg, et al. 2012; Hobson, Soto, et al. 2012;), but also for $\delta^{18}O$ (Hobson and Koehler, 2015), $\delta^{13}C$ (Haveles, Fox, & Fox-Dobbs, 2019; Diniz-Reis et al., 2024), and $\delta^{15}N$ (Koehler et al., 2019; Diniz-Reis et al., 2024). This relationship is especially relevant for wild birds since isotopic composition often reflects local environmental conditions. However, captive birds, which may be fed with commercial diets sourced from geographically distant areas, exhibit distinct isotopic patterns. Consequently, an isoscape based solely on wild bird feathers may not accurately represent the isotopic composition of captive birds. This discrepancy is supported by findings from the second chapter of this thesis, which identified significant isotopic differences between wild and captive Thraupidae birds for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$.

The primary objective of this study is to develop high-resolution isoscapes with broad geographic coverage to trace the origin of individual birds more effectively. While

these isoscapes are primarily designed to map the geographic provenance of wild birds, their precision and accuracy are critical for enhancing their forensic and ecological applications. By incorporating species-specific baselines and addressing the isotopic variability between wild and captive birds, these isoscapes provide a nuanced understanding of the isotopic interplay between tissues and the environment. This dual approach—focusing on both broad-scale geographic coverage and isotopic differentiation between wild and captive birds—enhances the utility of isotopic tools for tracking wildlife, informing conservation strategies, and combating the illegal wildlife trade.

METHODS

Feather collection

We analyzed 631 feathers of 57 non-migratory species from the Thraupidae family from Brazil (Figure 1). We identified samples according to the species, foraging guild (following Pigot *et al.*, 2020), location, collection date, and origin (wild, captive, or unknown). Whenever possible, we also classified samples according to sex (female, male, or unknown), life stage (adult or subadult), and feather type (flight or body) (Table 1S). Wing primaries or secondaries are more associated with a specific molting period, but if grown simultaneously, body and flight feathers are expected to have similar isotopic values (Costa *et al.*, 2022; Silva, Costa, & Nardoto, 2024). Taxonomic classification follows the Brazilian Ornithological Records Committee (Pacheco et al. 2021).

The captive samples were collected from a feather bank provided by the Stable Isotope Center at São Paulo State University (CIE/UNESP) and directly from legalized breeders or zoos. Samples were considered from captivity when birds were born or kept in captivity for at least one year before the collection. When possible and necessary, we researched the life history of the birds in the online Brazilian passerine management system (SisPass) to identify information such as birth date, sex, and location changes. Feathers from 41 birds seized by environmental agencies were also added to these data and classified as captive using a previous model (Brasileiro et al, submitted), totaling 290 captive samples (Figure 1A).

Our wild feathers dataset comprised samples from birds held in scientific ornithological collections in Brazil and from field campaigns in natural areas between 2007 and 2023. We also used the isotopic data from a previous project (Alquezar et al. 2022) that analyzed the $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ of 188 wild birds from the Thraupidae family,

comprising 153 samples for $\delta^{18}O$ and 341 samples for $\delta^{13}C$ and $\delta^{15}N$ collected between 2016 and 2018 (Figure 1B).

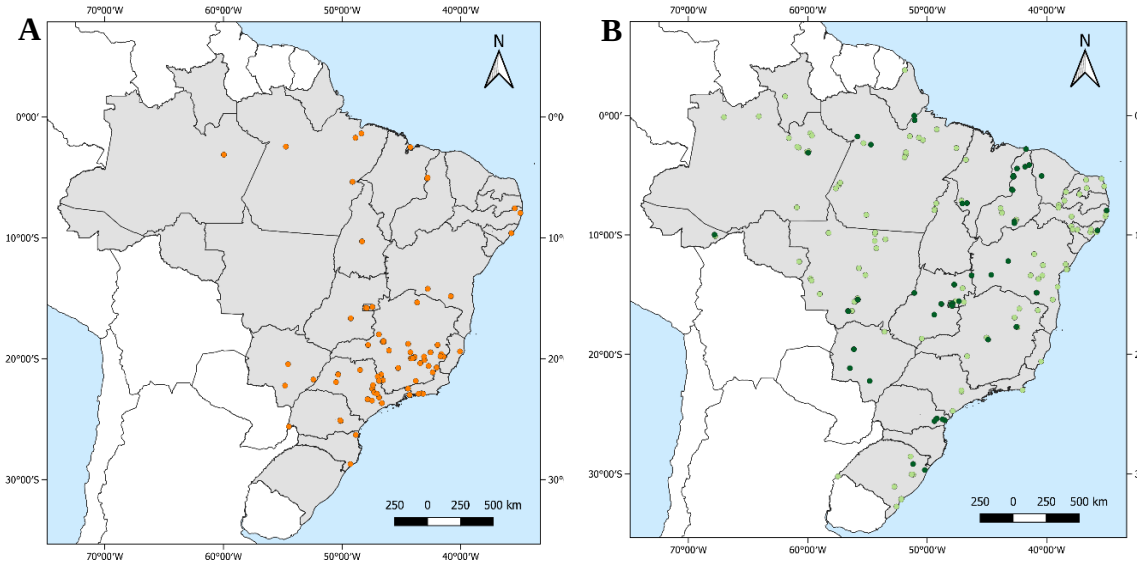


Figure 1. Feathers collected from captive (A) and wild (B) birds. Each point indicates a sampled location. For wild animals, dark green circles are the samples where $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ were analyzed and light green circles, samples from Alquezar *et al.*, 2022, where only $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ were analyzed.

Isotopic analysis of feathers

Feathers from the 188 individuals of the previous project mentioned above had the $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ analyzed at the University of Western Ontario LSIS-AFAR laboratory. For all other samples, we analyzed the isotopic ratios of the four main elements $R(^{2}H/^{1}H)$, $R(^{13}C/^{12}C)$, $R(^{15}N/^{14}N)$, and $R(^{18}O/^{16}O)$ at the Stable Isotopes Center (CIE) of São Paulo State University (UNESP, Brazil). Feathers were soaked in a 2:1 chloroform:methanol solution overnight, drained, washed with water, and dried at 50 °C for 48h. For $R(^{2}H/^{1}H)$ and $R(^{18}O/^{16}O)$, feathers were weighed (~ 250 µg) into silver capsules and analyzed in an isotope ratio mass spectrometry system with high-temperature conversion elemental analyzer (Flash HT – Delta V Advantage, Thermo Scientific). For $R(^{13}C/^{12}C)$ e $R(^{15}N/^{14}N)$, feathers were weighed (~ 600 µg) into tin capsules and analyzed in an isotope ratio mass spectrometry system with combustion elemental analyzer (Flash 2000 – Delta V Advantage, Thermo Scientific).

Stable isotope ratios were expressed as relative differences of isotope ratio ($\delta^{i}E$) from standard ratios $R(^{i}E/^{j}E)_{standard}$ in parts per mil (‰):

$$\delta^{i}E_{(‰)} = \frac{R\left({}^{i}E/{}^{j}E\right)_{sample}}{R\left({}^{i}E/{}^{j}E\right)_{standard}} - 1$$

Where $R({}^{i}E/{}^{j}E)_{sample}$ is the isotopic ratio of sample, ${}^{i}E$ is the rare isotope, and ${}^{j}E$ is the abundant isotope. $\delta^{13}C$ was reported relative to the standard Vienna Pee Dee Belemnite (VPDB), $\delta^{15}N$ was reported relative to atmospheric N_2 (AIR), $\delta^{2}H$ and $\delta^{18}O$ were reported relative to the Vienna Standard Mean Ocean Water (VSMOW). For internal quality control of analyses, during each cycle of readings (at the beginning and the end of each batch: 45-sample sequential analyses), a standard sample was used to ensure the accuracy of quantifications. The standard samples were calibrated against USGS and IAEA-certified reference materials (KHS, CBS, USGS42 and USGS43). The standard uncertainty for the analyses was ±0.9 ‰ for $\delta^{2}H$; ±0.40 ‰ for $\delta^{18}O$; ±0.10‰ for $\delta^{13}C$ and ±0.15‰ $\delta^{15}N$.

Exploratory analyses

Before each analysis, we evaluated normality using the Shapiro-Wilk test for each isotope in the wild and captive samples separately. We tested the homoscedasticity using Bartlett tests. We used parametric analysis for normally distributed data and non-parametric tests for non-normally distributed data. All statistical analyses were performed in R, Version 3.6.2 (R Core Team, 2020).

As we included samples analyzed in two laboratories, we first examined whether there were isotopic differences between them. We compared the $\delta^{13}C$ and $\delta^{2}H$ sub-samples of 13 individuals of the same species (*Sicalis citrina*) analyzed in both laboratories using Wilcoxon tests and performed the necessary transformations using linear regression. Isotopic ratio differed between laboratories for $\delta^{2}H$ ($V = 91$; $p < 0.001$), but nor for $\delta^{13}C$ ($V = 25$; $p = 0.17$). Since the $\delta^{2}H$ values obtained from the two laboratories are highly correlated ($S = 18$; $p < 0.001$; $\rho = 0.98$), we performed linear regression to derive the relationship between the $\delta^{2}H$ values from the University of Western Ontario LSIS-AFAR laboratory (Alquezar *et al.*, 2022) and those obtained at São Paulo State University CIE laboratory. Using the regression parameters, we transformed the $\delta^{2}H$ values of the 188 feathers analyzed at the LSIS-AFAR laboratory to predict the equivalent values that would have been obtained if these feathers had been analyzed at the CIE laboratory. This transformation ensured comparability between datasets. All subsequent analyses used the transformed values.

To better understand how sample characteristics influenced our dataset, we conducted exploratory analyses to evaluate whether isotopic values were affected by sex, life stage (adult or subadult), and feather type (body or flight) in Chapter Two. No significant influence of feather type, sex, or life stage was observed when geographical location was controlled for across any of the isotopes analyzed in captive or wild groups (see Chapter Two for details).

In the present analysis, we included biome for wild samples, Brazilian regions for captive samples, and dietary guild, latitude, and longitude for both groups. We expect that the isotopic variation across the different Brazilian biomes (Martinelli et al., 2021; Alquezar et al., 2022) may be reflected in animal tissues. Isotopic variation is also expected according to the dietary guild of wild animals, particularly for $\delta^{13}C$ and $\delta^{15}N$. Although it is not clear whether such variations occur in captive birds, as they typically lack access to natural resources, isotopic differences may arise due to the water and local resources provided, and the diet offered to different species. Dietary guild was tested using ANOVA when data were normally distributed or Kruskal-Wallis tests, if data were not normally distributed, followed by Tukey's HSD or Dunn tests, respectively. Latitude and longitude were analyzed through simple linear models.

Subsequently, we performed a linear model selection for each isotope in the two environments to explore the importance of variables in explaining the measured isotopic values. We evaluated the models' Variance Inflation Factor (VIF) using the '*car'* package and removed highly correlated variables. The best models were summarized using the "dredge" and "model average" functions from the '*MuMIn'* package, ranking them by increasing Akaike's Information Criterion (AICc) and considering models within $\Delta AIC < 2$ as competitive (Burnham and Anderson, 2002).

We applied Moran's I tests, considering the five nearest neighbors for $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ in both captive and wild bird groups to assess whether the data exhibited spatial autocorrelation.

Isoscape modeling

We organized the geographical coordinates and isotopic values by aggregating samples located within close distances ($< 0.04^{o}$) and calculating the isotopic mean for these nearby samples. For $\delta^{2}H$ and $\delta^{18}O$ of captive birds, as well as $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ of wild birds, we compared two different statistical approaches: Universal Kriging and

Random Forest analysis. For $\delta^{13}C$ and $\delta^{15}N$ of captive birds, we compared Random Forest with Ordinary Kriging, as no additional predictive variables were available.

Universal Kriging is a geostatistical interpolation method that combines spatial correlation (modeled through a variogram) with a deterministic trend derived from auxiliary variables. In contrast, Ordinary Kriging assumes a constant mean across the study area and relies solely on spatial autocorrelation to predict values. Random Forest is a machine-learning algorithm based on decision trees, aggregated into a single prediction to reduce noise and increase accuracy.

The models were validated using 10-fold cross-validation on a training subset comprising 80% of the samples. The remaining 20% of the dataset was reserved as an independent test subset to evaluate model performance on unseen data. We compared the performance of each method through cross-validation by calculating the Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the coefficient of determination (R^2). The scripts were adapted from Sena-Souza et al. (2020), Alquezar et al. (2022) and de Oliveira Mascarenhas et al. (2022).

Captive birds isoscapes

Because δ^2H and $\delta^{18}O$ of consumed water are incorporated into animal tissues, we first performed linear regressions between the δ^2H and $\delta^{18}O$ values of feathers from captive birds (δ^2H_f and $\delta^{18}O_f$), precipitation (δ^2H_p and $\delta^{18}O_p$), and tap water (δ^2H_t and $\delta^{18}O_t$). In temperate regions, the isotopic correlation between precipitation and animals' tissues is typically stronger when using "growing season" values, representing precipitation isotopic values during months with temperatures above 0º C. As temperatures in Brazil are rarely below 0º C, Alquezar *et al.* (2022) evaluated the relationship of δ^2H in feather and precipitation across different timeframes, finding the best fit for observed δ^2H_f using the timeframe of δ^2H_p from February to April (amount-weighted February-April precipitation δ^2H; $\delta^2H_{p(Feb\text{-}April)}$). Here, isotopic precipitation values for annual and $\delta^2H_{p(Feb\text{-}April)}$ amount-weighted averages were extracted from Bowen *et al*. (2005), Terzer *et al.* (2013), and Terzer-Wassmuth *et al*. (2021). Mean annual tap water values were derived from unpublished data from Sena-Souza et al. (Table 4S). Data of δ^2H_p from the growing season extracted from Terzer *et al.* (2013) and δ^2H_t from Sena-Souza (unpublish data) had similar fit to the δ^2H_f of captive birds, while $\delta^{18}O_t$ had the best fit to $\delta^{18}O_f$.

We did not have environmental isoscapes for $\delta^{13}C$, $\delta^{15}N$, so $\delta^{13}C$ and $\delta^{15}N$ isoscapes for captive birds were modeled based solely on the spatial distribution of the observed feather values.

Wild birds isoscapes

For $\delta^{2}H$ and $\delta^{18}O$ models, we first performed linear regressions between the feather isotopic values of wild birds and $\delta^{2}H$ and $\delta^{18}O$ of precipitation extracted from Bowen *et al.* (2005), Terzer *et al.* (2013), and Terzer-Wassmuth *et al.* (2021) (Table 4S). We used growing season precipitation data from Bowen *et al.* (2005) to create our $\delta^{18}O$ feather isoscape, as it best fit to the oxygen isotopic values of feathers. Additionally, we performed a linear regression between $\delta^{2}H$ values extracted from the feather isoscape developed by Alquezar *et al.* (2022) at the geographic coordinates of our wild bird sampling locations. Alquezar et al. (2022) developed a feather hydrogen isoscape for Brazil by integrating isotopic data and environmental variables to predict spatial patterns using Random Forest models. Our study includes 188 samples used by Alquezar *et al.* (2022) plus 154 new ones. Linear regression was conducted only for the new samples.

For the $\delta^{13}C$ feather isoscape, we used data from Powell *et al.* (2012), who modeled a $\delta^{13}C$ isoscape of vegetation for South America using remote sensing techniques. Their model was based on each land grid cell's C3/C4 plant composition. For the $\delta^{15}N$ feather isoscape, we used data from Sena-Souza *et al.* (2020), who developed a soil $\delta^{15}N$ isoscape for South America using Random Forest. Their model incorporated environmental variables such as climate (e.g., precipitation and temperature), vegetation (e.g., C3/C4 plant distributions), soil properties (e.g., organic matter content and nitrogen availability), and topography (e.g., elevation and slope) as covariates. We performed linear regressions between the $\delta^{13}C$ and $\delta^{15}N$ values of feathers and the corresponding $\delta^{13}C$ of vegetation and $\delta^{15}N$ of soil extracted from the modeled vegetation (Powel *et al.*, 2012) and soil (Sena-Souza *et al.*, 2020) isoscapes at the geographic coordinates of the wild bird sampling locations.

RESULTS

The number of samples, along with the mean, standard deviation, minimum, and maximum values for $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ in feathers from wild and captive birds, are presented in Table 1. The spatial distribution of raw data for both wild and captive individuals is displayed in Figures 2 and 3.

Table 1. Number of samples, mean, standard deviation, minimum and maximum values of isotopes of feathers from wild and captive birds.

Captive	n	Mean (‰)	SD (‰)	Min. (‰)	Max. (‰)
$\delta^{2}H$	290	-46.8	11.9	-73.57	-14.08
$\delta^{18}O$	290	14.8	4.09	4.53	33.15
$\delta^{13}C$	289	-16.6	2.96	-27.14	-7.42
$\delta^{15}N$	289	5.91	1.56	2.42	13.21
Wild	**n**	**Mean (‰)**	**SD (‰)**	**Min. (‰)**	**Max. (‰)**
$\delta^{2}H$	336	-26.5	17.6	-77.43	31.03
$\delta^{18}O$	148	16.3	2.38	8.02	25.97
$\delta^{13}C$	338	-20.3	5.49	-28.05	-8.66
$\delta^{15}N$	338	8.21	2.95	0.96	21.60

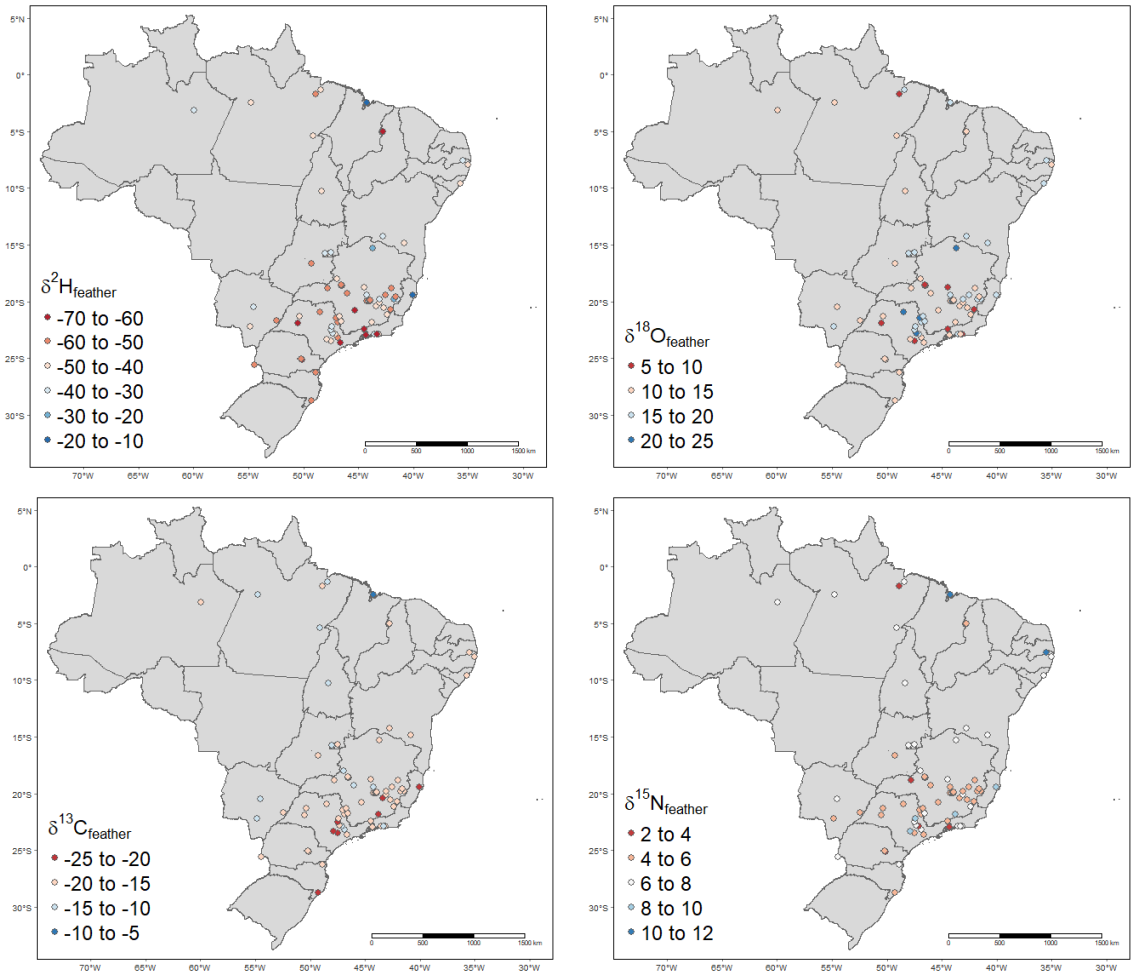


Figure 2. Raw observed $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ values of feathers from captive birds.

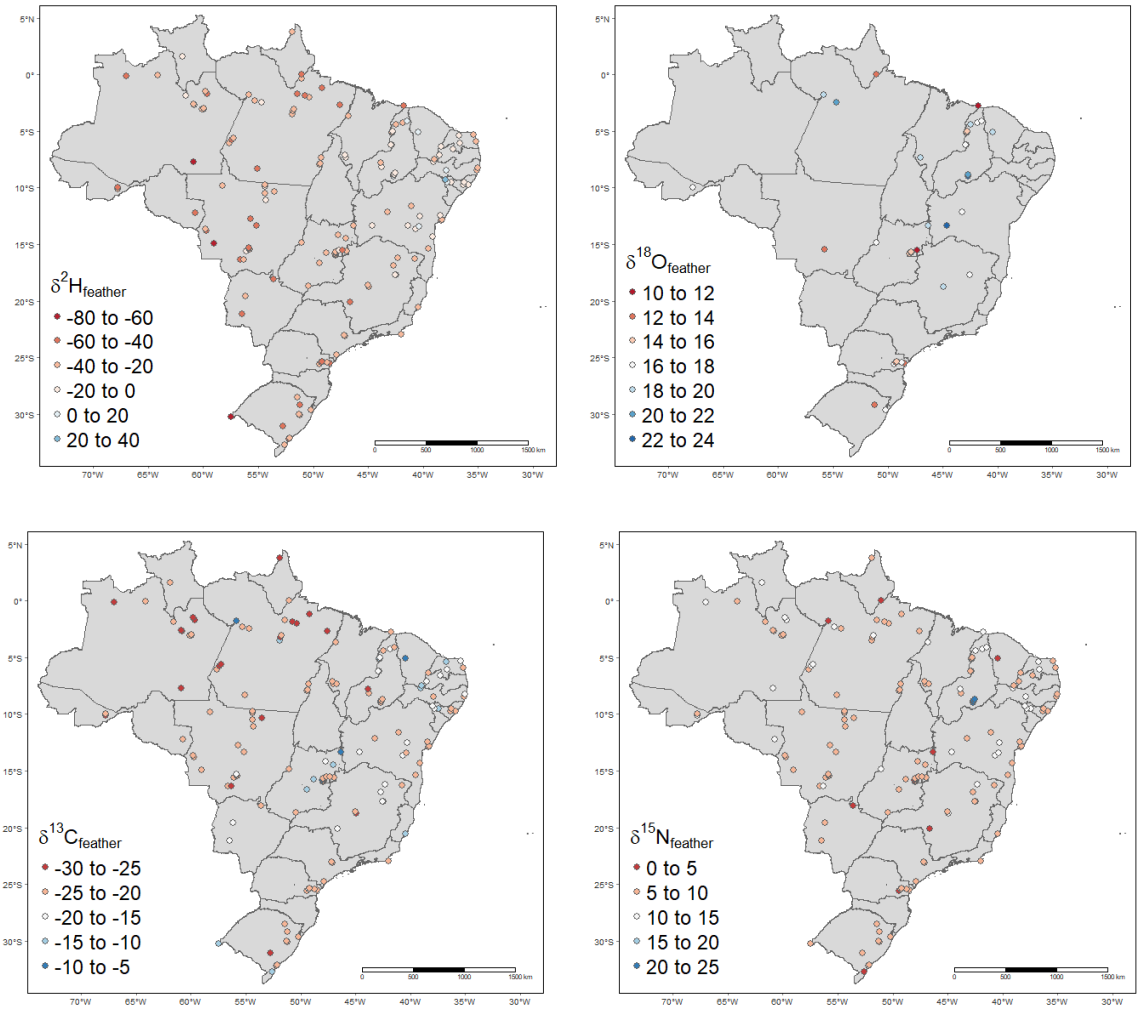


Figure 3. Raw observed δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ values of feathers from wild birds

Exploratory analysis

Except for δ^2H in captive birds, all isotopes varied according to the dietary guild in both rearing systems, with notable differences observed in granivores (Figure S2). In captive birds, all isotopes varied according to Brazilian regions, although post-hoc tests did not detect significant differences for δ^2H and $\delta^{18}O$ (Figure S3). In wild birds, isotopic values varied by Brazilian biomes, with δ^2H showing distinct patterns in the Caatinga, $\delta^{13}C$ in the Amazon Forest, and $\delta^{15}N$ in the Cerrado (Figure S4). Regarding geographic location, the isotopic values of captive birds were primarily influenced by latitude, whereas those of wild birds were more strongly affected by longitude (Figures 5S and 6S).

All the exploratory models included important covariates for explaining isotopic values in both wild and captive samples. In captive animals, latitude was included in all models with $\Delta AIC < 2$ for δ^2H, $\delta^{13}C$, and $\delta^{15}N$. Dietary guild was also included in all

selected models for $\delta^{13}C$ and $\delta^{15}N$ (Table 2S). Brazilian regions were removed as a covariate because they inflated the models (VIF > 10). In wild animals, dietary guild was included in all models with ΔAIC < 2 for all isotopes. The variables biome and longitude also appeared in most models, with biome being particularly important for $\delta^{13}C$ and $\delta^{15}N$ and longitude for $\delta^{2}H$ and $\delta^{18}O$ (Table S4).

Except for $\delta^{15}N$ in captive birds and $\delta^{18}O$ in wild birds, all isotopes exhibited spatial autocorrelation (Table 1).

Table 1. Moran tests for $\delta^{2}H$, $\delta^{18}O$ $\delta^{13}C$ and $\delta^{15}N$ of wild and captive samples.

Captive	**Moran test**	**p-value**
$\delta^{2}H$	$I = 2.44$	$p = 0.007$
$\delta^{18}O$	$I = 5.05$	$p < 0.001$
$\delta^{13}C$	$I = 3.62$	$p < 0.001$
$\delta^{15}N$	$I = -1.01$	$p = 0.84$
Wild		
$\delta^{2}H$	$I = 9.74$	$p < 0.001$
$\delta'^{8}O$	$I = 1.43$	$p = 0.077$
$\delta^{13}C$	$I = 4.55$	$p < 0.001$
$\delta^{15}N$	$I = 8.0198$	$p < 0.001$

Isoscape modeling

Captive birds isoscapes

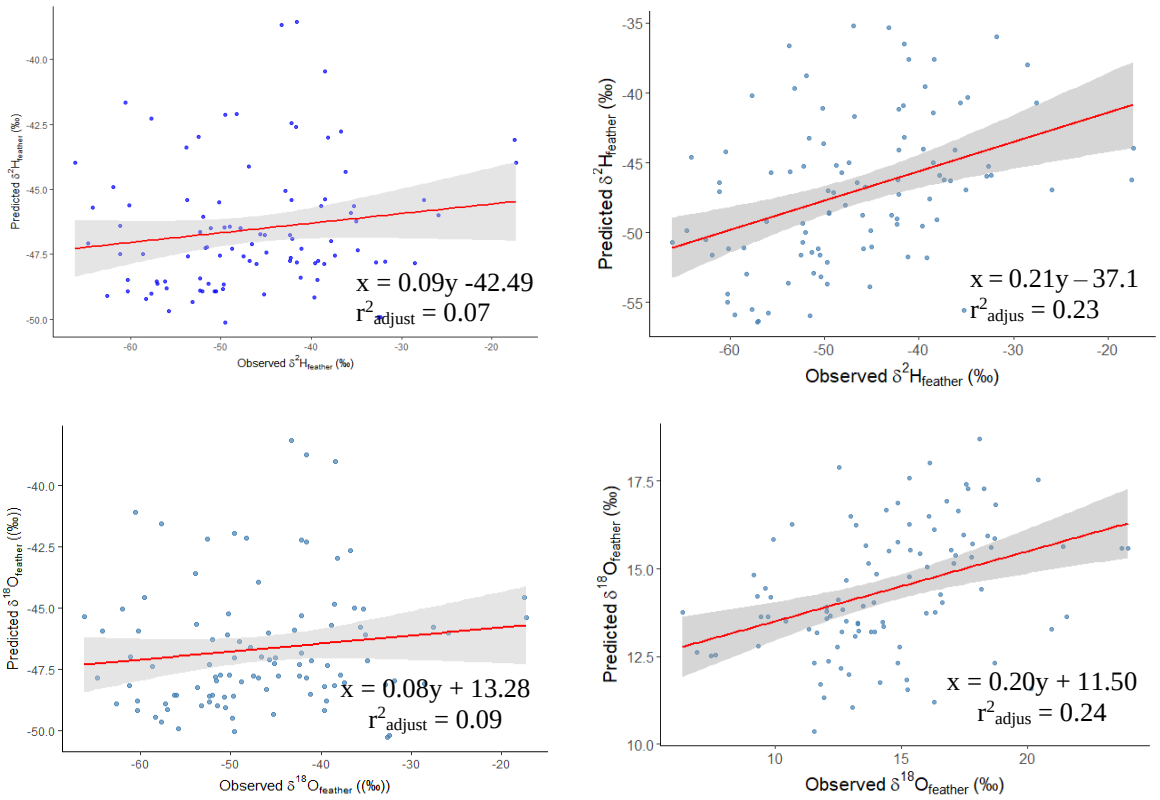
The Random Forest approach outperformed Universal Kriging in predicting $\delta^{2}H_f$ and $\delta^{18}O_f$ values for captive birds based on tap water (Table 2). Although both techniques produced comparable errors, the Universal Kriging explained only 7% ($F = 8.63$, $p = 0.004$) and 9% ($F = 11.44$, $p = 0.001$) of the observed variation in $\delta^{2}H$ and $\delta^{18}O$, respectively, while Random Forest explained around 23% in both cases ($\delta^{2}H$*:* $F = 16.54$, $p < 0.001$; $\delta^{18}O$*:* $F = 16.73$, $p < 0.001$). For both approaches, the slope of the regression line was significantly different from 1, and the intercept was different from 0 (Figure 4).

The Random Forest approach also better predicted $\delta^{13}C_f$ for captive birds, explaining 19% ($F = 12.07$; $p < 0.001$) of data variation, compared to 2% for $\delta^{13}C_f$ ($F = 0.02$; $p = 0.19$ using Kriging (Table 2; Figure 4). For predicting $\delta^{15}N_f$ values, the Kriging

performed slightly better than RF. However, it explained only 9% of the observed variation (F = 10.77; p = 0.001) (Table 2; Figure 4).

Table 2. Comparison of the performance metrics (Mean Absolute Error - MAE, Root Mean Square Error - RMSE, Adjusted R^2) and statistical significance (F-statistic and p-value) between Kriging and Random Forest models for δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ isoscapes of captive birds.

	Universal Kriging				**Random Forest**			
	δ^2H	**$\delta^{18}O$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**δ^2H**	**$\delta^{18}O$**	**$\delta^{13}C$**	**$\delta^{15}N$**
MAE	7.88	2.53	1.99	1.06	7.32	2.49	2.00	1.06
RMSE	9.93	3.31	2.68	1.45	9.28	3.22	2.55	1.38
$R^2_{ajustado}$	0.07	0.09	0.002	0.09	0.23	0.24	0.19	0.06
Statistic	F = 8.63 p = 0.004	F = 11.44 p = 0.001	F = 0.02 p = 0.19	F = 10.77 p = 0.001	F= 16.54; p < 0.001	F= 16.73; p < 0.001	F = 12.07; p < 0.001	F = 7.37 p = 0.008



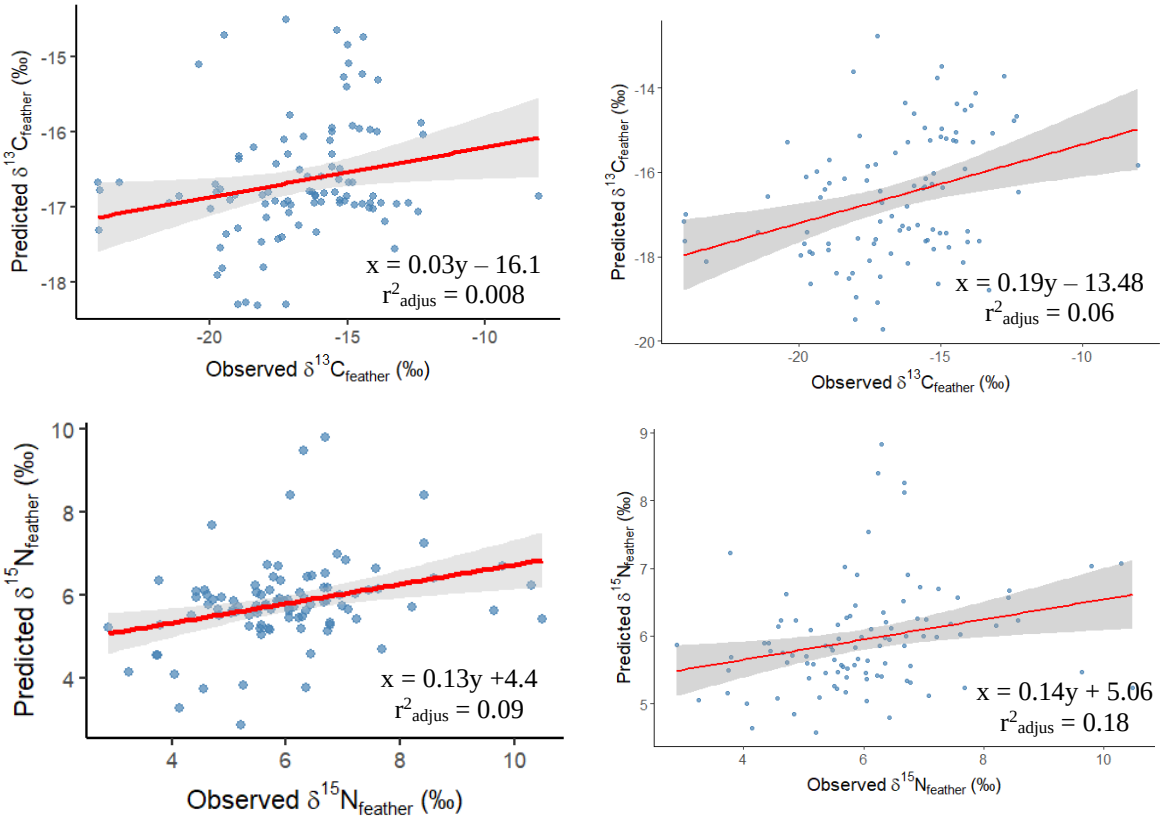
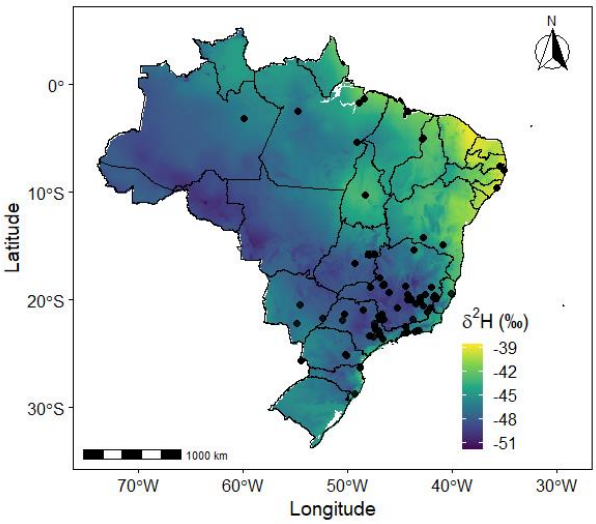
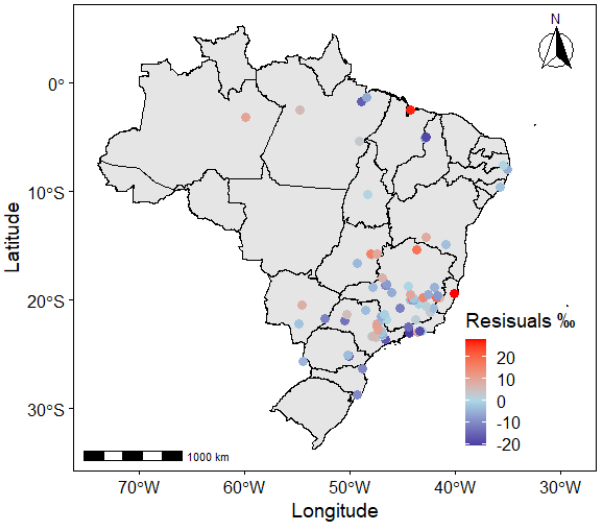


Figure 4. Observed vs. predicted δ^2H_f, $\delta^{18}O_f$, $\delta^{13}C_f$ and $\delta^{15}N_f$ values of captive birds using Kriging (left) and Random Forest (right). The solid red line represents the regression line, while the gray shaded area indicates the 95% confidence interval.

The isoscapes of δ^2H_f, $\delta^{18}O_f$, and $\delta^{13}C_f$ for captive birds, generated using Random Forest, as well as the isoscape of $\delta^{15}N_f$ using Ordinary Kriging, along with the spatial distribution of residuals, are presented in Figure 5. The predicted δ^2H_f values ranged from -54.88‰ to -33.32‰, with residuals ranging from -20.82‰ to 28.11‰. Predicted $\delta^{18}O_f$ values ranged from 15.07‰ to 15.33‰, with residuals ranging from -7.12‰ to 9.27‰. The predicted $\delta^{13}C_f$ values ranged from -27.71‰ to -13.27‰, with residuals ranging from -4.13‰ to 7.50‰. Finally, the predicted $\delta^{15}N_f$ values ranged from 3.67‰ to 10.48‰, with residuals ranging from -3.17‰ to 5.05‰.


N
0°
10°S
20°S
30°S
Latitude
δ^2H (‰)
-39
-42
-45
-48
-51
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude


N
0°
10°S
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30°S
Latitude
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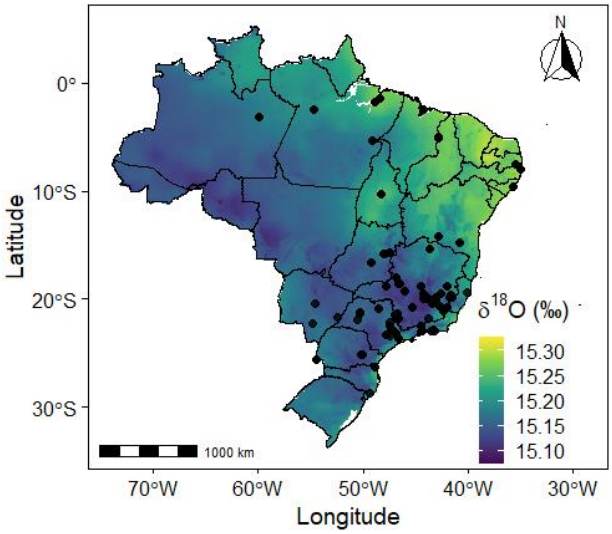
20
10
0
-10
-20
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude


N
0°
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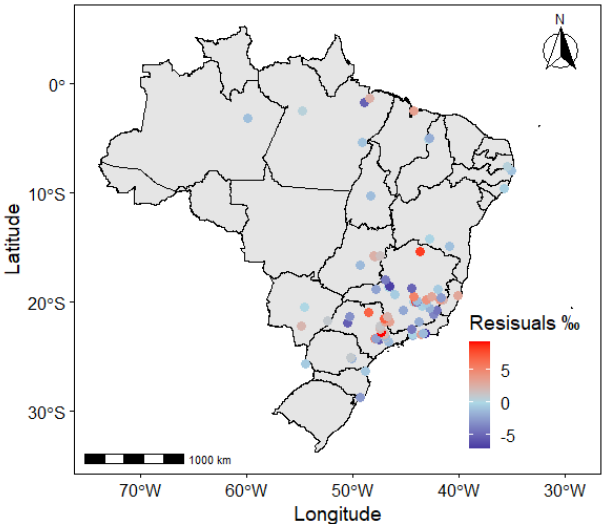
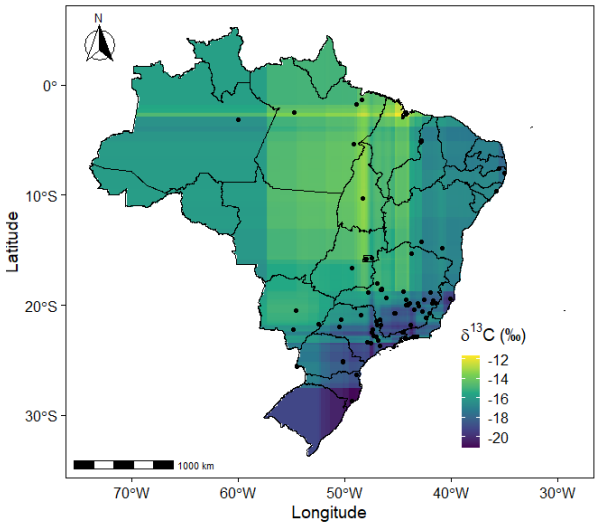
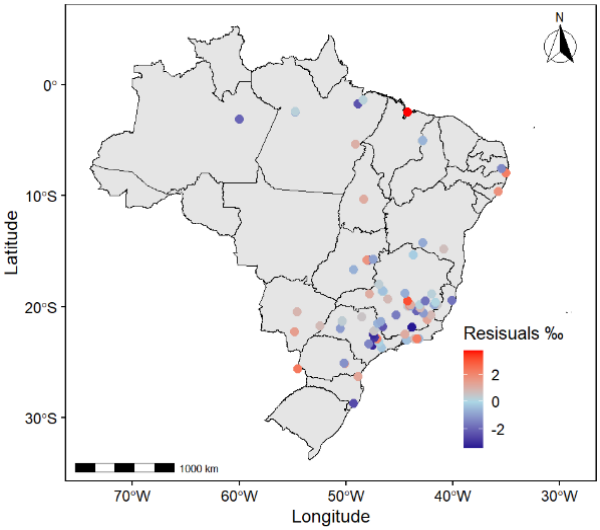
30°S
Latitude
δ^{18}O (‰)
15.30
15.25
15.20
15.15
15.10
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude


N
0°
10°S
20°S
30°S
Latitude
Resisuals ‰
5
0
-5
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude


N
0°
10°S
20°S
30°S
Latitude
δ^{13}C (‰)
-12
-14
-16
-18
-20
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude


N
0°
10°S
20°S
30°S
Latitude
Resisuals ‰
2
0
-2
1000 km
70°W
60°W
50°W
40°W
30°W
Longitude

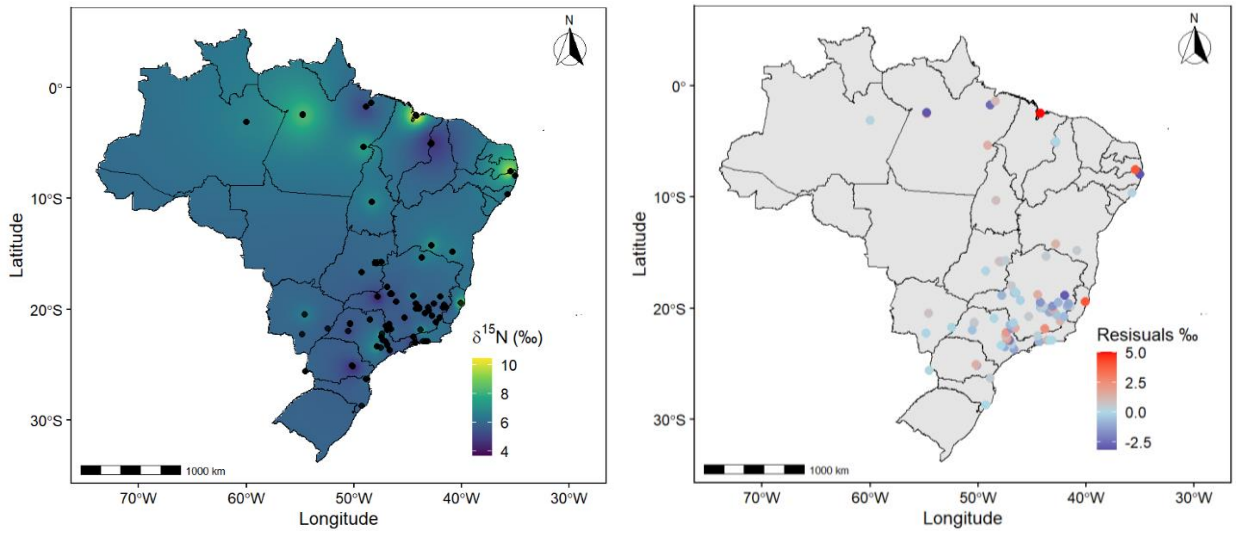


Figure 5. **Left**: Modeled δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ feather isoscape from captive birds for Brazil using Random Forest (δ^2H, $\delta^{18}O$ and $\delta^{13}C$) or Ordinary Kriging ($\delta^{15}N$). Black dots indicate sampling locations. **Right:** Residuals map for δ^2H and $\delta^{18}O$ predictions. Colors represent residual categories (‰) between observed and predicted δ^2 values and circle size reflects the absolute residual magnitude.

Wild birds isoscapes

Despite the higher error, the Random Forest approach performed slightly better in predicting $\delta^{13}C_f$ of wild birds using $\delta^{13}C$ of vegetation (Table 3). Random Forest explained 23% of the observed variation in $\delta^{13}C_f$ ($F = 13.42$, $p < 0.01$), compared to the Universal Kriging model, which explained 18% ($F = 8.63$, $p = 0.004$). Conversely, Universal Kriging performed better in predicting $\delta^{15}N_f$ of wild birds using $\delta^{15}N$ of soil (Table 3). The errors were lower with Universal Kriging, and the model explained 41% of the observed variation ($F = 11.44$, $p = 0.001$), while Random Forest explained 17% ($F = 13.42$, $p < 0.002$). For both approaches, the slope of the linear regression line was significantly different from 1, and the intercept was significantly different from 0 (Figure 6).

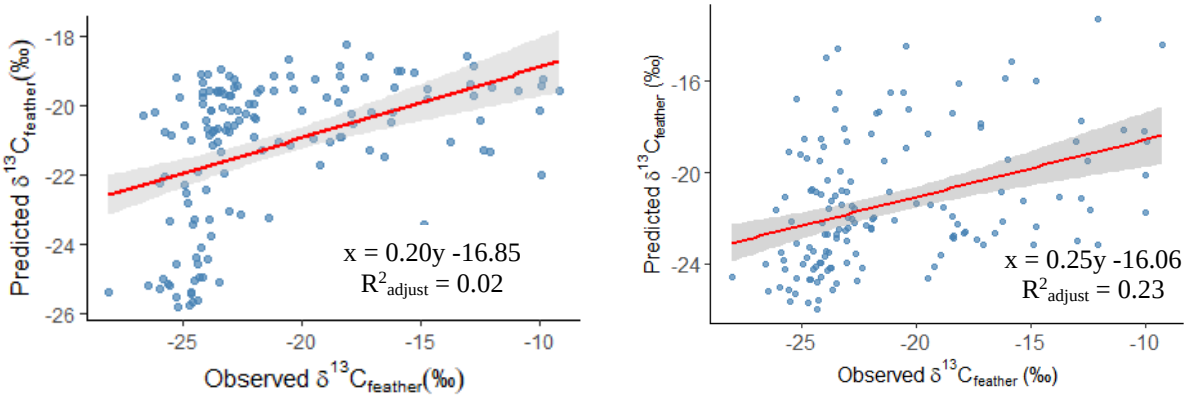
Universal Kriging had a higher error and lower fit than Random Forest in predicting $\delta^{18}O_f$ of wild birds. While the UK explained only 1% ($F = 2.86$, $p = 0.1$) of the observed variation in $\delta^{18}O_f$, RF explained 33% ($F = 4.75$, $p = 0.04$). However, in both models, the relationship was not statistically significant (Table 3; Figure 6).

We did not develop a new δ^2H isoscape for feathers from wild Thraupidae, as the regression analysis revealed a strong positive relationship between the δ^2H values

extracted from Alquezar et al. (2022) and the observed δ²H values for the new samples. The extracted values explained 70% of the variability in the observed δ²H (Figure 7).

Table 3. Comparison of the performance metrics (Mean Absolute Error - MAE, Root Mean Square Error - RMSE, Adjusted R^2) and statistical significance (F-statistic and p-value) between Universal Kriging and Random Forest models for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ isoscapes of wild birds.

	Universal Kriging			**Random Forest**		
	$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{18}O$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{18}O$**
MAE	3.08	1.76	2.23	7.32	2.37	2.19
RMSE	3.94	2.44	2.73	9.28	3.17	2.57
$R^2_{ajustado}$	0.18	0.41	0.04	0.23	0.17	0.33
Statistic	F = 8.63; p = 0.004	F = 11.44; p = 0.001	F =2.86; p = 0.1	F= 24.44; p < 0.001	F= 13.42; p < 0.001	F = 4.75; p = 0.04



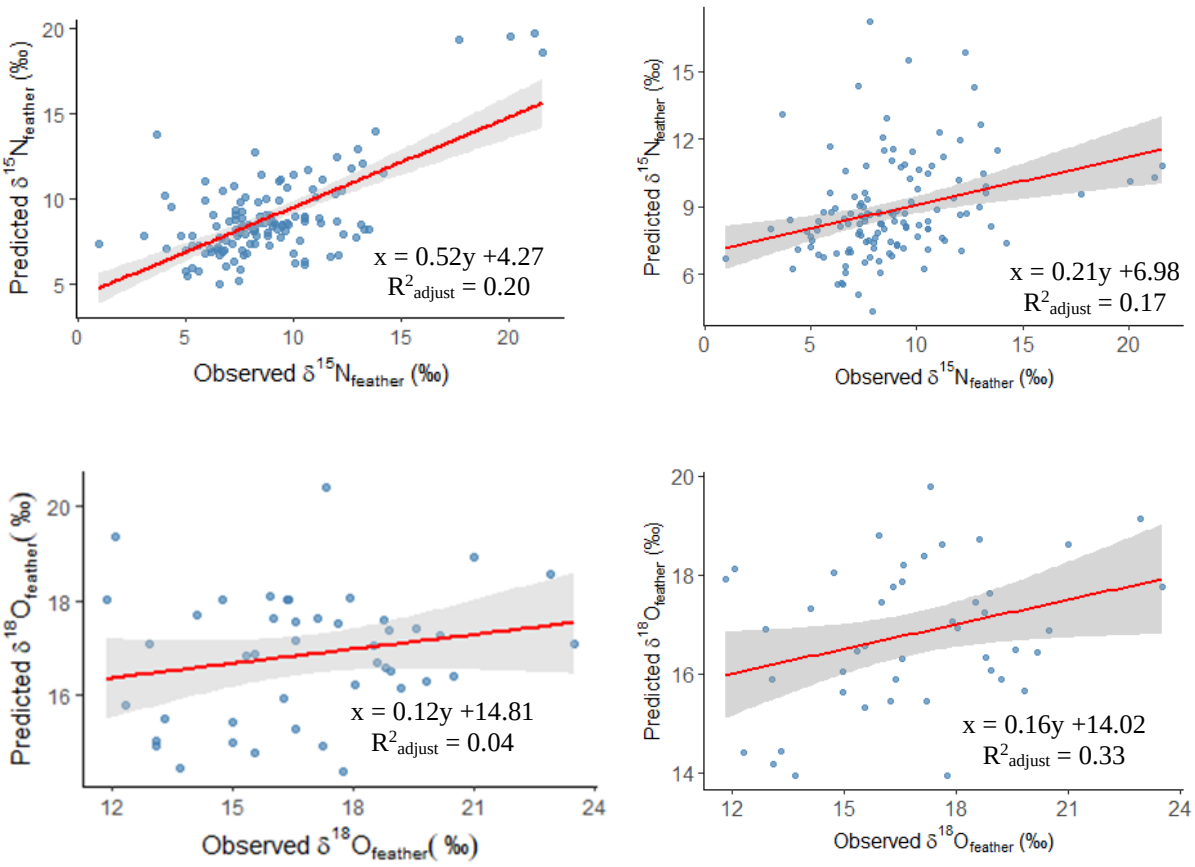


Figure 6. Observed vs. predicted δ^2C_f, $\delta^{15}N_f$ and $\delta^{18}O_f$ and values of wild bird using Universal Kriging (left) and Random Forest (right). The solid red line represents the regression line, while the gray shaded area indicates the 95% confidence interval.

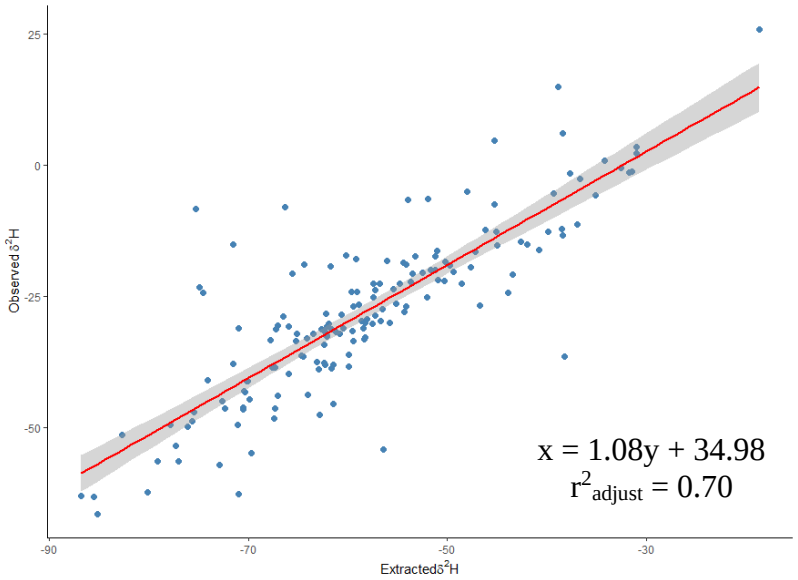
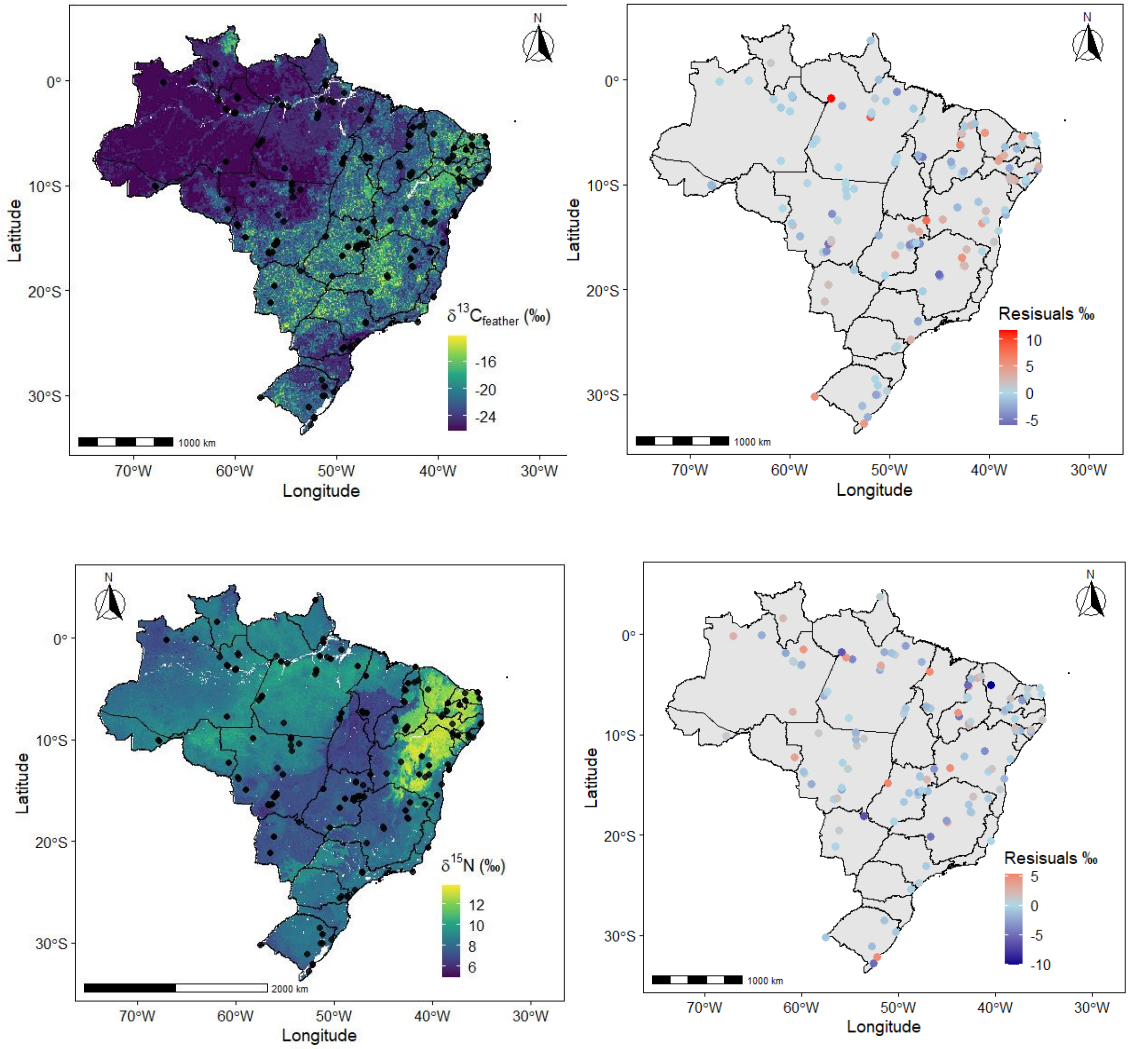


Figure 7. Relationship between observed feather δ²H values from new samples of Thraupidaes and δ²H values extracted from the isoscape model by Alquezar *et al*. (2022).

The red line represents the linear regression fit, while the gray shaded area indicates the 95% confidence interval.

The isoscapes of $\delta^{13}C_f$, $\delta^{15}N_f$, and $\delta^{18}O_f$ and the spatial distribution of residuals are shown in Figure 8. Random Forest was used to model $\delta^{13}C_f$ and $\delta^{18}O_f$, while Universal Kriging was applied for $\delta^{15}N_f$. The predicted $\delta^{13}C_f$ values ranged from -25.19‰ to -15.80 ‰ with residuals ranging from -6.29‰ to 12.05‰. The predicted $\delta^{15}N_f$ ranged from 4.94 ‰ to 13.77‰ with residuals ranging from -10.09‰ to 5.34‰. The predicted $\delta^{18}O_f$ values ranged from 13.72‰ to 19.21‰, with residuals ranging from -4.29‰ to 6.70‰.



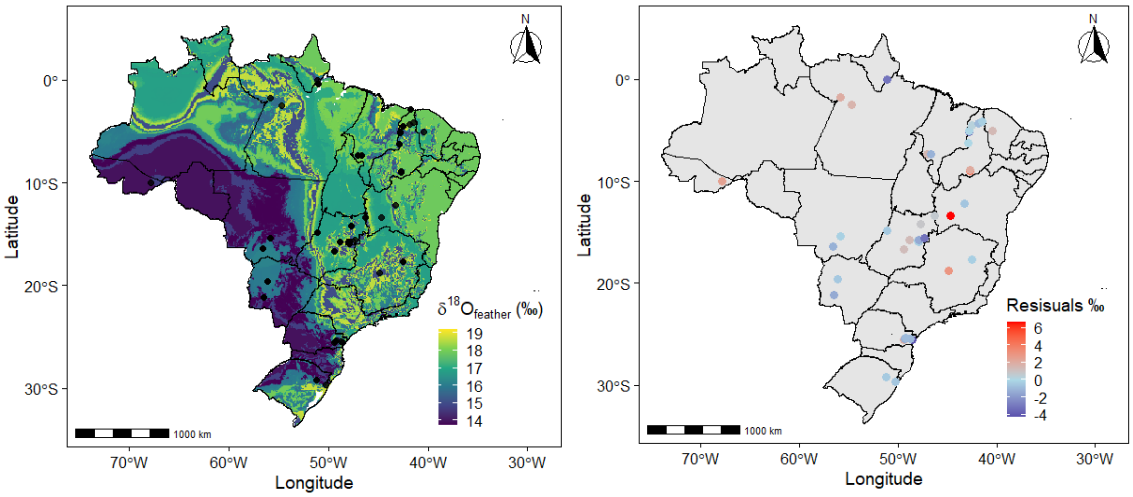


Figure 8. **Left**: Modeled $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ feather isoscape of wild birds for Brazil. Black dots indicate sampling locations. **Right:** Residuals map for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ predictions.

DISCUSSION

Here, we developed the first $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ isoscapes for captive animals based on feathers from Thraupidae individuals in Brazil. Additionally, we created the first $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ isoscapes for wild birds in Brazil.

Exploratory analysis

Dietary guild was an important factor influencing isotopic variability in captive and wild birds. Unsurprisingly, $\delta^{13}C$ and $\delta^{15}N$ differed among wild birds of different dietary guilds, as these isotopes primarily reflect the feeding ecology of animals. However, the influence of dietary guild was less obvious on $\delta^{2}H$ and $\delta^{18}O$ in wild resident birds, as these isotopes are expected to primarily reflect the isotopic composition of local precipitation. This finding may be explained by differences in the proportion of drinking water relative to dietary water intake among individuals from different dietary guilds, leading to variations in metabolic $\delta^{2}H$ and $\delta^{18}O$ values (Magozzi et al. 2019).

No prior information is available on the isotopic values of captive birds or their food in Brazil. However, our data suggest that bird-keepers are not providing the same diet to all birds. For instance, one of the main commercial bird feed suppliers offers distinct products for specific wild species, such as *Sicalis flaveola*, *Saltator similis*, *Sporophila angolensis*, and *Sporophila caerulescens*[1].

1. https://www.nutropica.com.br/produtos/passeriformes

Consistent with previous studies, the isotopic values of wild birds varied according to biome and were primarily influenced by longitude (Sena-Souza *et al.*, 2020; Alquezar *et al.*, 2022; Diniz-Reis *et al.*, 2024). Conversely, isotopic values in captive birds were mainly influenced by latitude. This result may reflect a distinct pattern of spatial variation between wild and captive birds. Still, it could also be attributed to a sampling bias, as the latitudinal gradient was better represented than the longitudinal one for captive birds.

All isotopes exhibited spatial autocorrelation except for $\delta^{18}O$ in wild birds and $\delta^{15}N$ in captive birds. The limited sample size likely influenced the $\delta^{18}O$ results, while the spatial variation observed in captive birds requires further investigation. Increasing the number of georeferenced and known-origin samples, particularly in under-sampled regions, is essential.

Isoscape modeling

The relationship between $\delta^{2}H$ and $\delta^{18}O$ in feathers and precipitation or tap water was weak, particularly for $\delta^{18}O$ in captive birds. The well-established relationship between $\delta^{2}H$ in keratinous tissues and precipitation observed in North America (Hobson et al. 2009; Hobson, Van Wilgenburg, et al. 2012; Hobson, Soto, et al. 2012) appears to be more challenging in Brazil, likely due to the complex climate and hydrology of tropical regions, as well as ecological and movement-related complexities of the studied species (Alquezar et al. 2022).

The relationship between $\delta^{18}O$ in animal tissues and precipitation seems to be even more intricate and less direct, likely influenced by the contribution of molecular oxygen (O_2) during amino acid metabolism (Hobson e Koehler 2015). Nevertheless, our results are consistent with the few studies that have examined the relationship between the isotopic composition of hydrogen and oxygen in precipitation and keratinous tissues of animals in Brazil (Alquezar *et al.*, 2022; Costa, 2019).

No previous study compares $\delta^{2}H$ and $\delta^{18}O$ values in animal tissues and tap water in Brazil's. However, Wolf *et al.*, 2013 reported a significant correlation between $\delta^{2}H$ in feathers and tap water for *Coturnix japonica* in Wyoming, USA, but no correlation for $\delta^{18}O$. These findings highlight the importance of further investigating the fractionation of $\delta^{18}O$ in animal tissues to enhance the application of this isotope in tracking animal movements through mechanistic approaches.

Although the predictive power of our δ^2H and $\delta^{18}O$ isoscapes for wild and captive birds was relatively low, the relationships between observed and predicted values were statistically significant (except for $\delta^{18}O$ in wild birds). These results are consistent with findings from other studies conducted in Brazil (e.g., Alquezar *et al.*, 2022; Costa, 2019; Mascarenhas *et al.,* 2021). The lack of statistical significance for $\delta^{18}O$ in wild birds was likely due to the limited number of samples available for this group. We anticipate that models' predictive power will improve as additional samples are incorporated into future analyses. While we did not develop a new δ^2H isoscape for feathers in this study, the model developed by Alquezar et al. (2022) was well-adjusted to our new samples, indicating that it can be reliably used to analyze our data.

Both the wild and captive δ^2H and $\delta^{18}O$ isoscapes of Thraupidae feathers displayed a longitudinal gradient, with higher values in northeastern Brazil and lower values in the western part of the country. However, the range of δ^2H_f values was greater in wild birds (-107‰ to +5‰; Alquezar et al., 2022) compared to the captive feather isoscape (-54‰ to -33.32‰), particularly in the northeast and southwest regions. Despite the limited number of $\delta^{18}O$ samples from wild birds, the range of the wild $\delta^{18}O_f$ isoscape was also broader (13.72‰ to 19.21‰) than that of captive birds (15.07‰ to 15.33‰). These findings suggest lower isotopic variability in captive animals and highlight the differences in isotopic values between wild and captive birds, particularly in regions with extreme δ^2H_f and $\delta^{18}O_f$ values.

Despite the complexity of factors influencing isotopic fractionation from the environment to animal tissues, there was a significant relationship between $\delta^{13}C$ and $\delta^{15}N$ in the feathers of wild birds and the $\delta^{13}C$ in vegetation and $\delta^{15}N$ in soil, respectively. Isoscapes of carbon and nitrogen isotopes are less common than those of oxygen and, especially, hydrogen. However, studies have demonstrated the potential of multi-isotopic approaches in determining the origin of animals (Hobson et al. 2012; Garcia-Perez et al. 2013; Hobson & Kardynal 2016).

The $\delta^{13}C$ isoscape for wild birds had a lower error and predictive power comparable to those of the δ^2H and $\delta^{18}O$ isoscapes but better or similar to other $\delta^{13}C$ isoscapes of keratinous tissues based on vegetation (Haveles, Fox, & Fox-Dobbs, 2019; Diniz-Reis *et al.,* 2024). In contrast, the $\delta^{15}N$ isoscape for wild birds demonstrated the highest predictive power in this study, reinforcing the potential of nitrogen isotopes as auxiliary tools in tracing geographical origin. The spatial distribution of feather $\delta^{13}C$

shows lower values in the Amazon region and higher values in the central region, while the spatial distribution of $\delta^{15}N$ shows lower values in the central region and higher values in the northeast. Similar trends are observed in $\delta^{13}C$ of vegetation (Powell, Yoo, e Still 2012), $\delta^{15}N$ of soil (Sena-Souza et al. 2020), and mammals' hair (Costa, 2019; Diniz-Reiz *et al.*, 2024).

The $\delta^{13}C$ and $\delta^{15}N$ isoscapes of captive animals exhibited less distinct spatial distribution patterns, with either no significant influence of geographical location or low predictive power. This was particularly evident for $\delta^{15}N$, where the isoscape map highlighted only localized differences around specific sampling points, suggesting that these variations are driven by site-specific factors rather than broader spatial distribution patterns. Conversely, these findings underscore the potential of $\delta^{13}C$ and $\delta^{15}N$ isotopes to distinguish between wild and captive animals across different regions, such as the Amazon and southern Brazil for $\delta^{13}C$, and the Caatinga and Cerrado for $\delta^{15}N$.

Except for $\delta^{15}N$ in wild and captive birds, Random Forest outperformed Universal and Ordinary Kriging. Random Forest, a machine learning approach, is particularly effective at capturing complex, non-linear relationships between variables and can handle high-dimensional datasets without requiring strict assumptions about data distribution. In contrast, Kriging, a geostatistical method, excels in situations where spatial autocorrelation is strong and the relationship between variables and spatial coordinates is linear or well-defined. The superior performance of Random Forest in this study likely reflects the complex, multi-factorial nature of isotopic variation. However, in cases with dense spatial sampling and strong spatial patterns, Kriging remains a robust and reliable choice.

Final considerations

Despite challenges related to the predictive power of some models, the isoscapes provide significant insights into the isotopic variation across Brazil and how we could use it to distinguish wild from captive birds. The Random Forest models demonstrated superior performance compared to Universal Kriging for most isotopes, emphasizing their potential in handling the complex interactions underlying isotopic fractionation.

Future research should prioritize increasing sample sizes, especially for captive birds and $\delta^{18}O$ in wild birds. Additionally, isotopic variability resulting from different guilds should be considered and recommended in future research. By enhancing the accuracy and resolution of isotopic isoscapes, these tools can be further developed to trace

the geographic origin of wild and captive birds. This approach provides critical insights for identifying regions of origin for seized animals, supporting conservation strategies, and combating illegal wildlife trade. Moreover, it can guide the reintroduction of animals into appropriate habitats and inform strategies to disrupt wildlife trafficking routes and hotspots.

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SUPPORTING INFORMATION

Table S1. Dataset with the raw data used in this study.

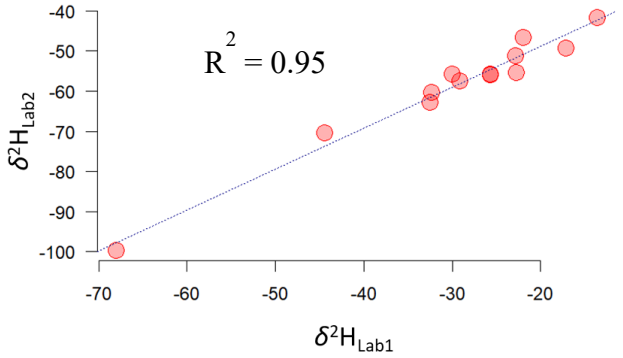

Figure 1S. Correlation between δ²H measured at the University of Western Ontario LSIS-AFAR (Lab_1) and São Paulo State University CIE laboratory (Lab_2). The dotted line represents the linear regression, with points indicating individual samples.

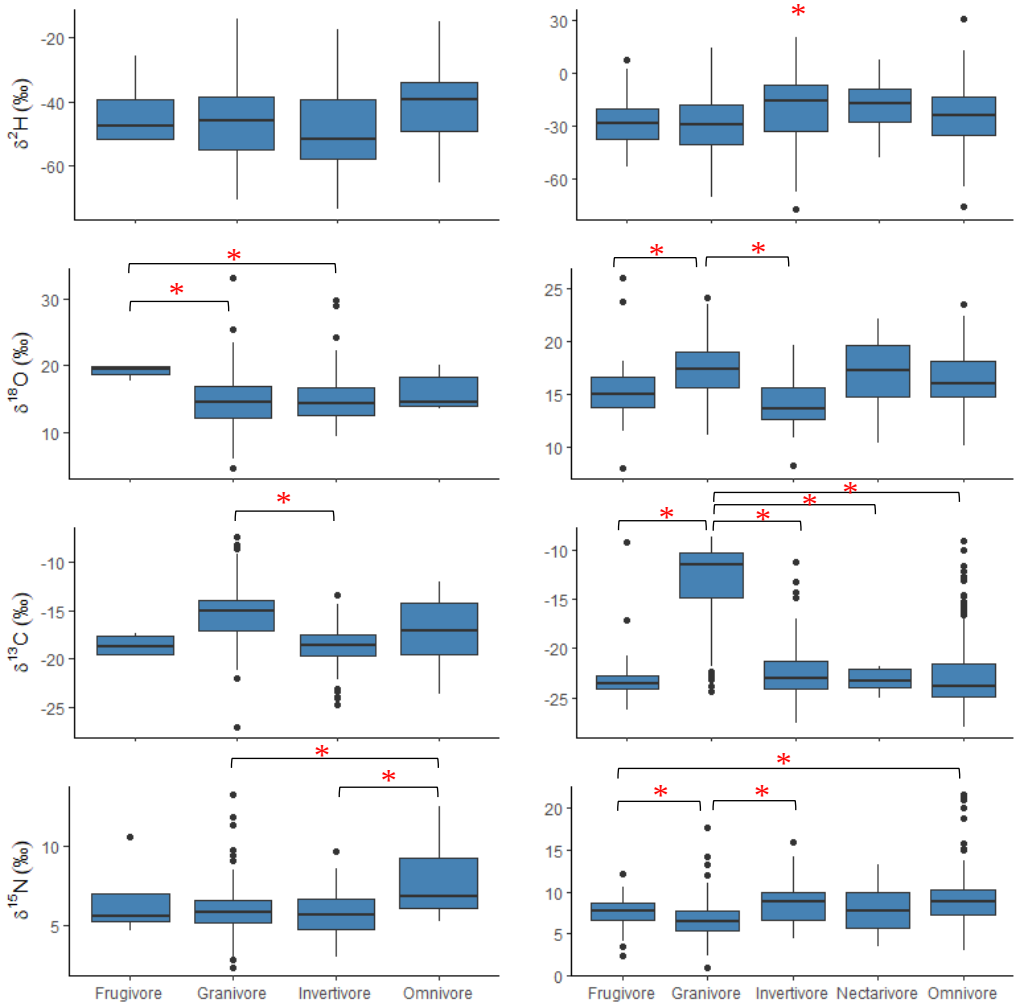

Figure 2S. Isotopic values of feathers from captive (left) and wild (right) birds in different dietary guilds. Differences are indicated by a red asterisk. δ²H of wild birds differed in ANOVA, but not in Tukey's test.

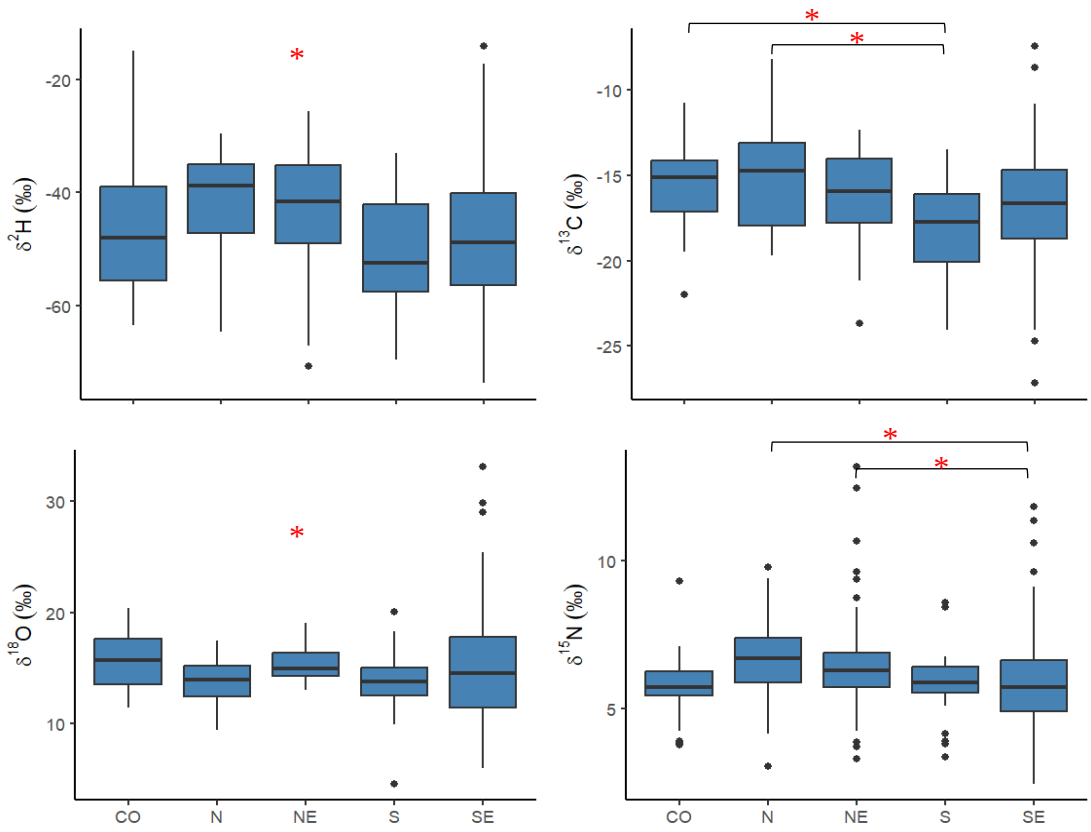

Figure 3S. Isotopic values of feathers from captive birds in the five Brazilian regions (CO = Central-Weast; N = North; NE = Northeast; S = South; SE = Southeast). Differences are indicated by a red asterisk. δ^2H and $\delta^{18}O$ of wild birds differed in Kruskal-Wallis, but not in Dunn tests.

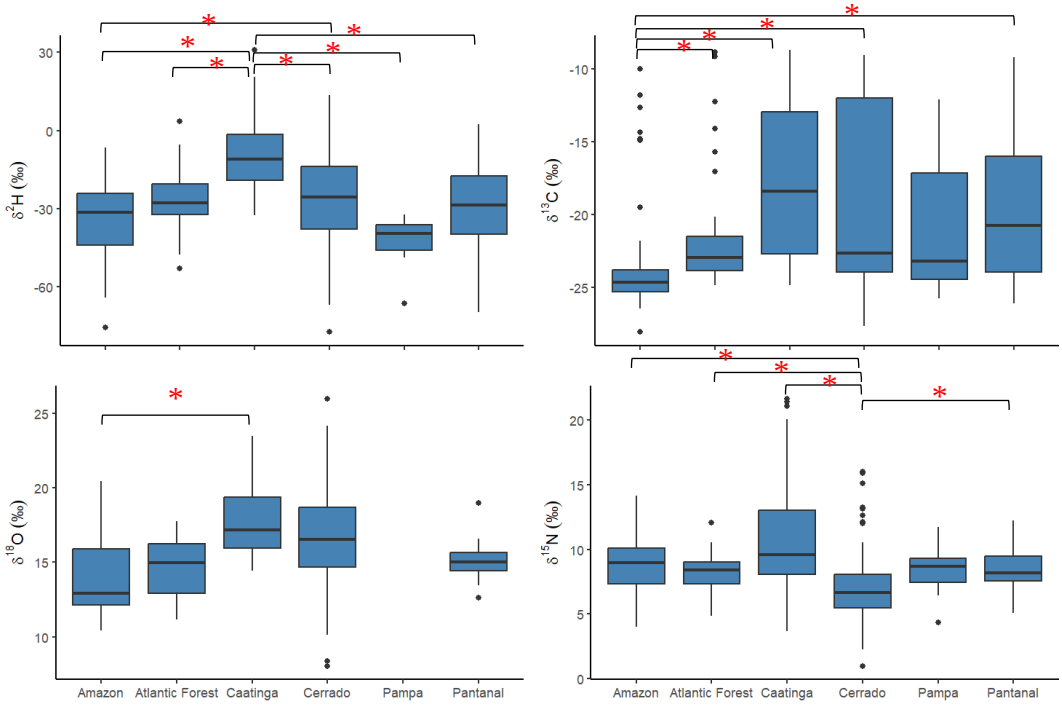

Figure 4S. Isotopic values of feathers from wild birds in the six Brazilian biomes. Differences are indicated by a red asterisk.

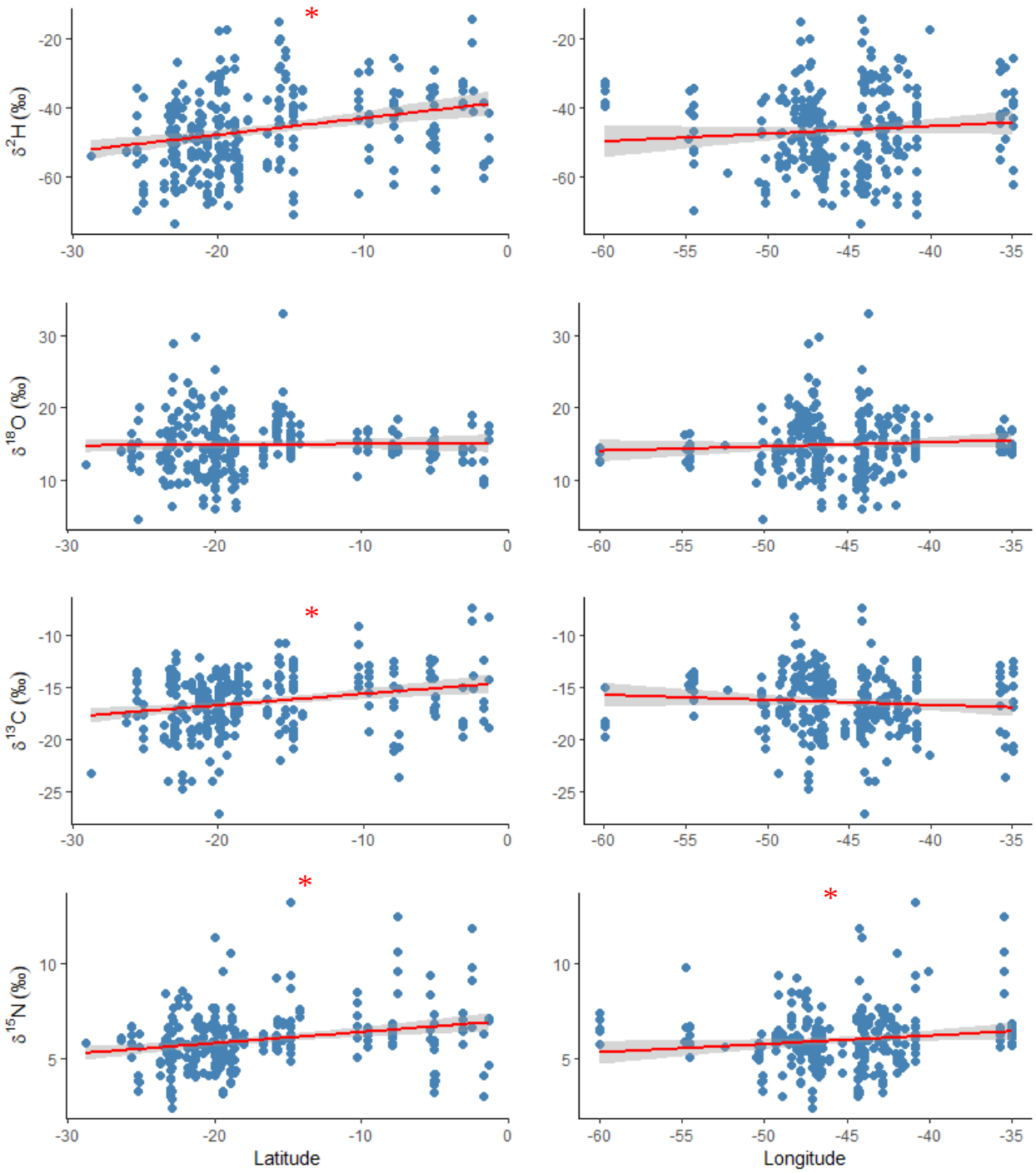

Figure 5S. Correlation between $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ of feathers from captive birds and geographic coordinates (latitude and longitude). The red line indicates the fitted linear regression model, and shaded areas represent 95% confidence intervals. Significant results are indicated by a red asterisk.

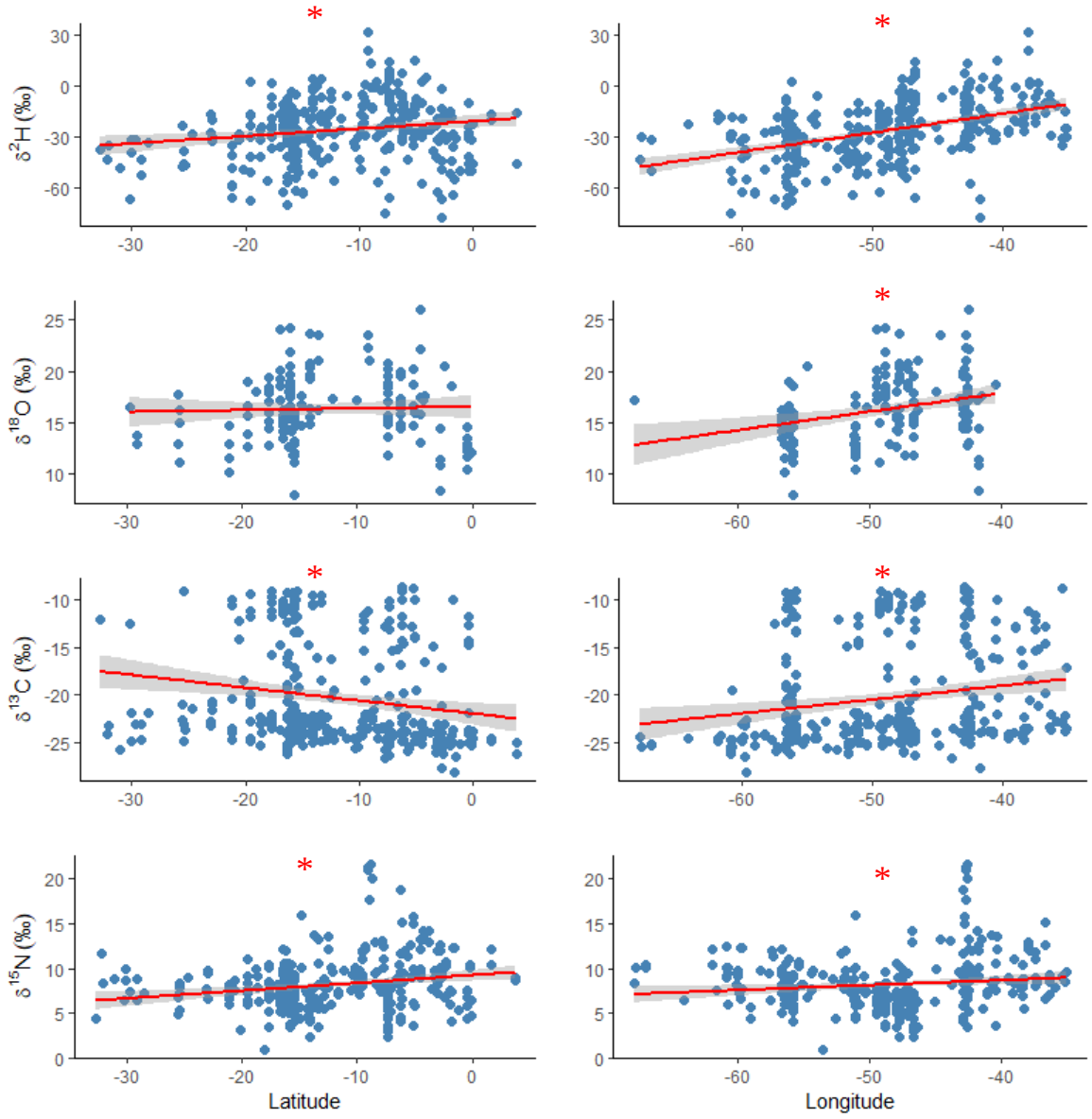

Figure 6S. Relationship between $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ of feathers from wild birds and geographic coordinates (latitude and longitude). The red line indicates the fitted linear regression model, and shaded areas represent 95% confidence intervals. Significant results are indicated by a red asterisk

Table 2S. Exploratory model selection results for observed $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ values of captive birds. Brazilian regions were removed from the model due they were highly correlated with other variables (VIF > 10). Showing the more competitive models and their degrees of freedom, AICc, ΔAIC and weight.

Full model: $\delta^{2}H$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Lat	3	2225.06	0.00	0.56
Lat + Long	4	2226.6	1.54	0.26
Full model: $\delta^{18}O$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild	4	1589.39	0.00	0.29
Long	3	1590.80	1.40	0.14
Guild + Long	6	1590.89	1.50	0.14
Full model: $\delta^{13}C$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Lat	6	1350.27	0.00	0.56
Guild + Lat + Long	7	1351.93	1.66	0.24
Full model: $\delta^{15}N$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Lat	6	1039.72	0.00	0.78
Guild + Lat + Long	7	1041.6	1.88	0.28

Table 3S. Exploratory model selection results for observed $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ values of wild birds. Showing the more competitive models and their degrees of freedom, AICc, ΔAIC and weight.

Full model: $\delta^{2}H$ ~ Guild + Biome + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Biome + Long	12	2759.84	0.00	0.56
Guild + Biome + Lat + Long	13	2760.36	0.52	0.44
Full model: $\delta^{18}O$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Long	7	752.42	0.00	0.7
Guild + Lat + Long	8	754.07	1.65	0.3
Full model: $\delta^{13}C$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Biome + Lat + Long	13	1798.22	0.00	0.34
Guild + Biome + Long	12	1798.26	0.04	0.34
Guild + Biome	11	1799.02	0.80	0.23
Full model: $\delta^{15}N$ ~ Guild + Latitude + Longitude				
Selected models	**df**	**AICc**	**ΔAIC**	**Weight**
Guild + Biome	11	1590.74	0.00	0.32
Guild + Biome + Lat	12	1590.83	0.09	0.31
Guild + Biome + Long	12	1591.27	0.53	0.25
Guild + Biome + Lat + Long	13	1592.54	1.80	0.13

Table 4S. Linear regression results between δ^2H and $\delta^{18}O$ of precipitation and tap water and the δ^2H and $\delta^{18}O$ feather of captive and wild birds with each associated $r^2_{ajusted}$ and *p* value.

Reference	Origin	Period	Captive		Wild	
			δ^2H	**$\delta^{18}O$**	**δ^2H**	**$\delta^{18}O$**
Bowen, 2005	Precipitation	Annual	r^2 = 0.012; p = 0.12	r^2 = -0.001; p = 0.36	r^2 = 0.13; p < 0.001	r^2 = 0.031; p = 0.02
Bowen, 2005	Precipitation	Growing season	r^2 = 0.038; p = 0.02	r^2 = -0.008; p = 0.17	r^2 = 0.20; p < 0.001	r^2 = 0.14; p < 0.001
Terzer, 2013	Precipitation	Annual	r^2 = 0.037; p = 0.02	r^2 = -0.002; p = 0.40	r^2 = 0.03; p < 0.001	r^2 = 0.01; p = 0.06
Terzer, 2013	Precipitation	Growing season	r^2 = 0.048; p < 0.001	r^2 = 0.006; p = 0.57	r^2 = 0.02; p = 0.005	r^2 = 0.006; p = 0.17
Tezer-Wassmuth 2021	Precipitation	Annual	r^2 = 0.044; p = 0.01	r^2 = -0.003; p = 0.44	r^2 = 0.10; p < 0.001	r^2 = 0.009; p = 0.13
Sena-Souza, unpublished	Tap water	Annual	r^2 = 0.047; p = 0.02	r^2 = -0.01; p = 0.09	-	-

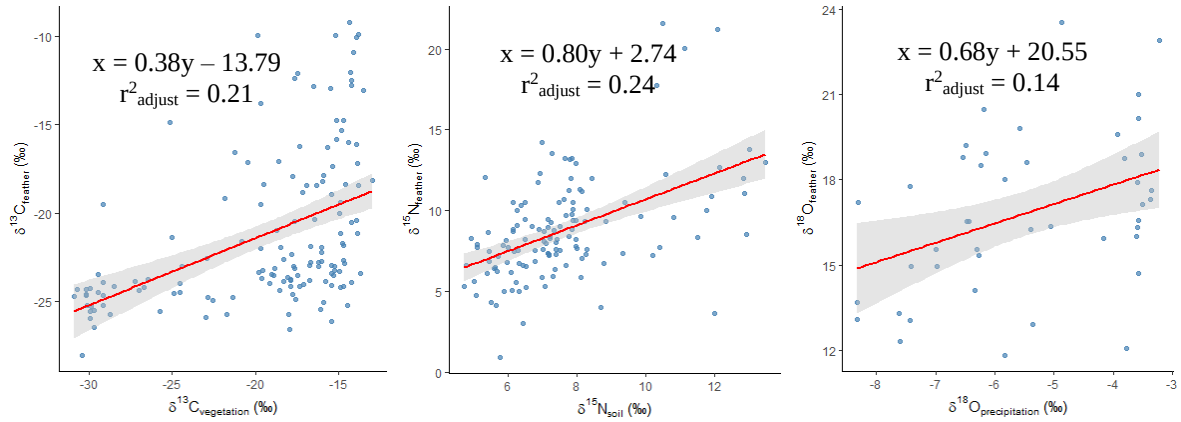

Figure 7S. Relationships between isotopic values in feathers of wild birds and their respective sources ($\delta^{13}C$ of vegetation, $\delta^{15}N$ of soil and $\delta^{18}O$ of precipitation). The red line indicates the fitted linear regression model, and shaded areas represent 95% confidence intervals.