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NÍVEL DE DESIDRATAÇÃO VOLUNTÁRIA DE DOIS LAGARTOS DE FORRAGEAMENTOS DISTINTOS EM UMA ÁREA DE CAATINGA DO ESTADO DE PERNAMBUCO, BRASIL

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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal, da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Mestre em Biologia Animal.

Área de concentração: Ecologia

Orientador: Prof. Dr. Pedro M. Sales Nunes

Coorientador: Prof. Dr. Carlos A. Navas Iannini

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"Nada na vida deve ser temido,
somente compreendido. Agora é hora de
compreender mais para temer menos."

Curie, Marie

RESUMO

Lagartos possuem alta sensibilidade a temperaturas extremas por serem animais que dependem do ambiente para regular sua temperatura corpórea. E nos répteis, a exposição a condições desidratantes durante a atividade está relacionada, principalmente, aos habitats e microhabitats ocupados. Como forma de alcançar um equilíbrio hídrico, os répteis podem beber água distribuída no ambiente ou ingerir uma refeição. Aqui, objetivamos entender se lagartos da Caatinga estão expostos à algum nível de déficit hídrico, além de caracterizar a dieta das espécies visando discutir sobre possíveis influências da umidade do conteúdo estomacal no equilíbrio hídrico dessas espécies. Submetemos os indivíduos, coletados em campo e pesados, a uma hidratação induzida de duas horas. Logo após essa hidratação e com a estabilização do peso, usamos a diferença das massas corporais em campo e pós experimento para o cálculo do balanço hídrico de campo. Os espécimes foram eutanasiados e seus conteúdos estomacais retirados para pesagem. Em laboratório, os itens ingeridos foram identificados. Concluímos que o item mais frequente na dieta de *Ameivula ocellifera* foi Isoptera e na dieta de *Tropidurus cocorobensis*, Formicidae (Hymenoptera). A sobreposição de nicho trófico entre as espécies foi de aproximadamente 0.20, embora existam muitos itens consumidos em comum. O entendimento da ecologia alimentar dessas espécies forneceu base para discutirmos o segundo capítulo, onde observamos que *A. ocellifera* consome uma dieta proporcionalmente mais úmida em comparação com *T. cocorobensis*, o que pode ser um reflexo dos principais itens ingeridos e da amplitude de sua dieta devido ao seu modo de forragear. A ingestão de itens também pode ser uma estratégia para contornar a falta de água disponível no ambiente. Os experimentos de hidratação mostram que o balanço hídrico de campo de *A. ocellifera* foi mais crítico que o de *T. cocorobensis*, o que pode refletir diversos aspectos fisiológicos, ecológicos, evolutivos e a possível influência do modo de forrageamento. O balanço hídrico de campo de *T. cocorobensis* está significativamente relacionado à temperatura do solo, o que pode ser explicado por uma estratégia utilizada para reduzir a perda de água. Os resultados aqui expostos são importantes para o entendimento de como a ecologia alimentar de lagartos pode estar relacionada ao status hídrico e nos traz importantes novidades acerca da exposição de lagartos da Caatinga à um déficit hídrico.

Palavras chave: Nicho Ecológico; Ecofisiologia; Desidratação; Dieta; Ecologia Alimentar

ABSTRACT

Lizards have a high sensitivity to extreme temperatures as they are animals that depend on the environment to regulate their body temperature. Moreover, in reptiles, exposure to dehydrating conditions is mainly related to occupied habitats and microhabitats. Reptiles can drink water distributed in the environment or eat a meal to achieve a water balance. Here, we aim to understand if Caatinga lizards are exposed to some level of water deficit and characterize the diet of the species to discuss possible influences of the moisture content of the stomach on the water balance of these species. We subjected the individuals, collected in the field and weighed, to induced hydration of two hours. Soon after this hydration and with the weight stabilization, we used the difference of body masses in the field and after the experiment to calculate the field water balance. The specimens were euthanized and their stomach contents removed for weighing. In the laboratory, the ingested items were identified. We concluded that in *Ameivula ocellifera* diet, the most frequent item was Isoptera, and in the diet of *Tropidurus cocorobensis*, Formicidae (Hymenoptera). The trophic niche overlap between species was approximately 0.20, although there are many items consumed in common. Understanding the food ecology of these species provided the basis for discussing the second chapter. We observed that A. ocellifera consumes a proportionally more humid diet than T. cocorobensis, which may reflect the main items ingested and the breadth of their diet due to their mode of foraging. The ingestion of items can also be a strategy to overcome the lack of water available in the environment. The hydration experiments showed that the field water balance of *A. ocellifera* was more critical than that of *T. cocorobensis*, which may reflect several physiological, ecological, evolutionary aspects, and we highlight the possible influence of the foraging mode. The field water balance of *T. cocorobensis* is significantly related to soil temperature, which can be explained by a strategy used to reduce water loss. The results presented here are important for understanding how the food ecology of lizards can be related to water status and bring us important news about the exposure of Caatinga lizards to a water deficit.

Keywords: Dehydration; Diet; Ecological Niche; Ecophysiology; Food Ecology

SUMÁRIO

1. INTRODUÇÃO

Os lagartos possuem uma alta sensibilidade a temperaturas extremas, principalmente por serem animais que dependem do ambiente para regular sua temperatura corpórea e, consequentemente, realizarem suas funções básicas (Huey, 1982; Huey et al., 2010). Apesar dos desafios climáticos presentes em regiões com marcada sazonalidade, linhagens de lagartos têm ocupado ambientes com essa característica, como as florestas tropicais sazonalmente secas do semiárido. Em lagartos, a exposição a condições desidratantes durante a atividade está relacionada, principalmente, aos habitats e microhabitats ocupados (Heatwole e Veron, 1977) e às suas atividades termorregulatórias (Foley e Spotila, 1978). Sendo assim, os ambientes áridos e desérticos, onde os organismos são expostos à alto estresse hídrico, podem ser considerados importantes forças seletivas (Waldschmidt e Tracy, 1983).

Uma das características de ambientes semiáridos é a sazonalidade bastante marcada, com uma estação seca e outra chuvosa, que se reflete nas diferentes temperaturas e intensidade da radiação solar ao longo do ano. No Brasil, podemos observar essa característica climática no sertão semiárido, na região ecológica da Caatinga (Ab'Saber, 1977; Prado, 2003; IBGE. 2004). A floresta tropical sazonalmente seca da Caatinga apresenta extremos climáticos, com alta radiação solar, alta temperatura média anual, baixas taxas de umidade e precipitações baixas e limitadas a determinadas épocas (Reis, 1976). Essa região é caracterizada por um déficit hídrico e umidade relativa baixa (Andrade et al., 2018), com uma média de 773 mm de precipitação por ano e 70% da mesma sendo restrita a um mês do ano (Andrade et al., 2010; Andrade et al., 2018). Além disso, a Caatinga apresenta apenas uma estação seca e outra chuvosa, com alternação de anos de estiagem e anos de inundação (Andrade et al., 2018). Essa sazonalidade destacada, observada também em desertos, influencia diferentes adaptações morfológicas e ecológicas em animais e plantas (Leal et al, 2003).

A influência dessa sazonalidade climática da Caatinga na ecologia de lagartos tem sido bastante explorada na literatura recente (Kolodiuk et al., 2009; Ribeiro et al., 2011; Sales et al., 2011; Ferreira et al., 2017). Em algumas espécies simpátricas, como *Tropidurus hispidus* e *T. semitaeniatus*, pode-se observar mudanças nos comportamentos de forrageio de acordo com as estações climáticas, coexistindo em condições limitantes durante a estação de déficit hídrico (Kolodiuk et al., 2009). Interferências na composição da dieta também já são bem documentadas. Ferreira et al. (2017) concluiram, após analisar a dieta de seis espécies de lagartos na Caatinga, que durante a estação seca há mudanças na "escolha" de presas mais consumidas em comparação com a estação chuvosa. Sales et al. (2011) observaram que indivíduos do lagarto *Ameiva ameiva* (Teiidae) em uma área de Caatinga no estado do Rio Grande do Norte possuem mais especializações individuais em suas dietas durante a estação seca, fazendo com que a largura de nicho trófico dessa população fosse maior nessa estação do que na chuvosa, onde eles se especializariam em comer larvas e pupas. A influência da sazonalidade também foi observada em ciclos reprodutivos das espécies *T. hispidus* e *T. semitaeniatus*, nos quais a atividade reprodutiva das fêmeas foi influenciada pelas precipitações, sugerindo que esses ciclos reprodutivos estão relacionados às condições microclimáticas da Caatinga (Ribeiro et al., 2012). Além disso, ambientes desérticos e sazonalmente secos podem proporcionar uma limitação de água livre por períodos longos, impondo consideráveis restrições hídricas aos répteis (Henen et al., 1998).

Essas restrições desencadeiam um estresse hídrico nos lagartos, que respondem fisiologicamente de diversas formas, eventualmente com impactos sobre seu desempenho fisiológico ou ecológico em diversos aspectos fundamentais da sua história natural como reprodução, forrageamento e termorregulação (Huey et al., 2010). Sannolo e Carretero (2019) puderam concluir que a desidratação afeta negativamente a termorregulação de lagartos do gênero *Podarcis* (Lacertidae), nos quais indivíduos desidratados diminuíram consideravelmente suas temperaturas corporais preferidas e passaram mais tempo utilizando refúgios, afetando também o uso de espaço pelas espécies. Consequências da desidratação também foram observadas na capacidade de corrida de lagartos, onde indivíduos da espécie *Uta stansburiana* (Phrynosomatidae) com déficit hídrico mostraram uma redução em sua capacidade de locomoção (Wilson et al., 1989). No âmbito fisiológico, a desidratação aumenta mecanismos de defesa com reatividade ao estresse e as concentrações plasmáticas de glicocorticóides, além de gerar uma possível supressão da função imunológica dos lagartos (Moeller et al., 2017).

Além das situações mencionadas, respostas na alimentação também são documentadas, por exemplo, na seleção das presas que serão ingeridas (Nagy, 1973). Lagartos, em sua maioria, possuem uma dieta composta primordialmente por artrópodes (Silva e Araújo, 2008), ingerindo principalmente insetos. Algumas espécies são estritamente herbívoras, como *Iguana iguana* (Iguanidae) (Van Sluys, 1993), e outras podem ser consideradas onívoras por conta do alto consumo volumétrico de material vegetal, como algumas espécies da família Tropiduridae (Cooper e Vitt, 2002). O estudo da composição dietética de lagartos pode responder diversas questões acerca da ecologia trófica, principalmente sobre o modo de forrageamento e consequentemente o uso de energia e nutrientes para o lagarto realizar suas funções necessárias (Pough, 1973). Além disso, o consumo de uma refeição pode estar intimamente relacionado à uma forma de contornar o déficit hídrico que algumas espécies sofrem em ambientes desérticos (Cooper 1985; Znari e Nagy, 1997; Henen et al., 1998; Ostrowski et al., 2002), como parece ser o caso em lagartos do deserto, nos quais a ingestão de itens alimentares é alta por fornecer uma maior quantidade de água (Nagy, 1973).

No desempenho de funções básicas do organismo dos animais, a alimentação fornece a energia necessária e, complementarmente, a ingestão de água supriria as necessidades de hidratação. Porém, em grande parte das situações em ambientes naturais, a água livre pode ser escassa e a água na dieta (água adquirida através dos itens alimentares predados) pode representar papel importante na hidratação (Golightly e Ohmart, 1984; Nagy e Medica, 1986). Nesse sentido, algumas espécies, principalmente as encontradas em ambientes desérticos e sazonalmente secos, tendem a modificar suas dietas, procurando por presas capazes de fornecer mais água (Cooper 1985; Znari e Nagy, 1997; Henen et al., 1998; Ostrowski et al., 2002). Porém, pode-se observar que em algumas espécies de lagartos e serpentes desérticas (e.g., *Heloderma suspectum* [Helodermatidae], *Crotalus atrox* [Viperidae]) o consumo de uma refeição não é efetivo para corrigir o balanço hídrico (Wright et al., 2013; Murphy e DeNardo, 2019). Por outro lado, a tartaruga do deserto *Gopherus agassizii* (Testudinidae) depende totalmente da água obtida através da dieta para complementar sua hidratação e não gerar um déficit hídrico (Henen et al., 1998).

Nesse sentido, algumas espécies, principalmente as que dependem da alimentação para um equilíbrio hídrico, podem sofrer influências da desidratação na busca por presas através do forrageamento (Kotler et al., 1998). Complementarmente, o modo de forrageamento pode estar relacionado a diferenças fisiológicas, morfológicas, ambientais e ecológicas (Huey e Pianka, 1981; Cooper, 2005). Em lagartos, a busca por alimento através do forrageamento geralmente ocorre por forrageamento do tipo senta-espera ou ativo. Os lagartos de forrageamento senta-espera não exploram tanto o ambiente na procura por alimento e se aproveitam das presas mais abundantes e mais ativas; por outro lado, os lagartos de forrageamento ativo exploram o ambiente na procura por presas (Huey e Pianka, 1981). Algumas situações interferem e podem influenciar modificações no forrageamento (Huey e Pianka, 1981), como, por exemplo, os ambientes sazonalmente secos, que podem fornecer uma influência sazonal no comportamento de forrageamento de lagartos (Colli et al., 1997; Kolodiuk et al., 2010; Ribeiro et al., 2011). Kolodiuk et al. (2009) puderam observar que duas espécies de lagartos tropidurídeos com o mesmo tipo de forrageamento senta-e-espera apresentaram semelhanças consideráveis no seu comportamento de forrageamento durante a estação seca, algo não observado durante a estação chuvosa, onde as espécies diferem em quase todos os parâmetros de busca por presas.

Nesse contexto, o presente estudo procurou relacionar o balanço hídrico de campo com a ecologia alimentar, principalmente com foco no modo de forrageamento de lagartos. Temos como hipótese principal que espécies de lagartos com forrageamento ativo se expõem mais na procura de presas e, como consequência, possuiriam um status de desidratação maior. De forma contrastante, espécies com forrageamento do tipo senta-e-espera, que utilizam mais refúgios e não se movimentam tanto, apresentariam um status de desidratação menor. Por outro lado, lagartos ativos poderiam contar com uma dieta mais variada entre seus itens consumidos, buscando aquelas presas capazes de fornecer mais água, compensando a maior desidratação causada pela atividade de forrageamento. Para testar essas hipóteses, escolhemos duas espécies modelos de lagartos presentes na Caatinga: *Ameivula ocellifera* (Spix, 1825) e *Tropidurus cocorobensis* Rodrigues, 1987. A primeira é uma espécie de lagarto da família Teiidae, de forrageamento do tipo ativo e encontrada em ambientes de Restinga, Cerrado e Caatinga (Menezes et al., 2006; Sales e Freire, 2015). A segunda espécie pertence à família Tropiduridae, é classificada como forrageadora do tipo senta-e-espera e possui uma distribuição relictual na Caatinga (Rodrigues, 1987). As duas espécies são heliófilas e compartilham o micro-habitat arenoso.

Além disso, a perda de água é bastante explorada em lagartos do deserto da Austrália, como os das famílias Agamidae, Gekkonidae e Scincidae, com foco na perda de água através da evaporação (Dawson et al., 1966) e os resultados apresentados para estes estudos poderiam ser extrapolados para a desidratação na natureza. Entretanto, ainda não se conhece o status de desidratação voluntária (perda de água não induzida, em ambiente natural) desse grupo de répteis. Após todo o contexto apresentado, lagartos brasileiros que habitam a Caatinga, domínio fitogeográfico do semiárido brasileiro, se encaixam como potenciais modelos para se definir os níveis de perda de água voluntária. Complementarmente, se torna importante compreender o grau de exposição à desidratação de lagartos com estratégias de forrageamento distintas em regiões que permitam a avaliação da influência das condições climáticas. Essa comparação com enfoque no forrageamento tem potencial de revelar adaptações fisiológicas diferentes e, como consequência, fomentar avanços nas investigações sobre as consequências das mudanças climáticas na Caatinga e outras regiões do planeta com condições semelhantes.

Nesse sentido, o presente trabalho teve como objetivos: 1) Definir o balanço hídrico de campo de indivíduos adultos de duas espécies de lagartos (*Ameivula ocellifera* e *Tropidurus cocorobensis*) em uma área de Caatinga; 2) Comparar o balanço hídrico de campo de lagartos da espécie *Ameivula ocellifera* (forrageador ativo) e da espécie *Tropidurus cocorobensis* (forrageador senta-e-espera), compreendendo se o modo de forrageamento é um possível influenciador; 3) Elucidar e entender melhor a hipótese de que quanto maior a temperatura corpórea, mais crítico o balanço hídrico de campo; 4) Procurar possíveis preditores de um crítico balanço hídrico de campo em lagartos ao relacionar dados abióticos (temperatura do ar, umidade, temperatura do solo) com o balanço hídrico de campo dos espécimes; 5) Averiguar a quantidade de água que o conteúdo estomacal é capaz de fornecer às espécies.

Este trabalho, desenvolvido e agora apresentado como dissertação junto ao PPGBA-UFPE, foi proposto como um projeto de mestrado e, com seus resultados promissores e mais questionamentos advindos, propusemos ampliá-lo a projeto de doutorado, o que foi aprovado pelo PPGBA-UFPE e pela FACEPE, principal agência financiadora deste projeto. Para a continuidade deste projeto e desenvolvimento da tese de doutorado, investigaremos a influência de um crítico balanço hídrico de campo na ecologia trófica e térmica de lagartos da mesma área de Caatinga, através do projeto intitulado: "A INFLUÊNCIA DA DESIDRATAÇÃO NA ECOLOGIA ALIMENTAR E TÉRMICA DE TRÊS LAGARTOS DA CAATINGA DO ESTADO DE PERNAMBUCO, BRASIL".

O presente estudo será apresentado através de uma divisão em dois capítulos, cada um correspondente a um manuscrito produto dos dados obtidos durante o projeto de dissertação. O primeiro capítulo intitulado: "DIET COMPOSITION AND TROPHIC NICHE OVERLAP OF *AMEIVULA OCELLIFERA* (SPIX, 1825) (SQUAMATA: TEIIDAE) AND *TROPIDURUS COCOROBENSIS* RODRIGUES, 1987 (SQUAMATA: TROPIDURIDAE), SYMPATRIC SPECIES WITH DIFFERENT FORAGING MODES IN CAATINGA", fornece informações gerais acerca da ecologia alimentar das duas espécies modelos do projeto (*Ameivula ocellifera* e *Tropidurus cocorobensis*), informações essas que serão valiosas para a discussão do segundo capítulo. Este segundo capítulo é intitulado: "FIELD WATER BALANCE OF TWO LIZARDS WITH DIFFERENT FORAGING MODES DURING THE DRY SEASON IN CAATINGA", e traz informações sobre o balanço hídrico de campo das duas espécies de lagartos, abrangendo os dados acerca da quantidade de água fornecida pelo conteúdo estomacal dos espécimes. Os capítulos seguem as formatações e regras das revistas South American Journal of Herpetology (Capítulo 1) e Journal of Experimental Biology (Capítulo 2).

2. CAPÍTULO I – DIET COMPOSITION AND TROPHIC NICHE OVERLAP OF AMEIVULA OCELLIFERA (SPIX, 1825) (SQUAMATA: TEIIDAE) AND TROPIDURUS COCOROBENSIS RODRIGUES, 1987 (SQUAMATA: TROPIDURIDAE), SYMPATRIC SPECIES WITH DIFFERENT FORAGING MODES, IN CAATINGA

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Abstract. Lizards' diet can be influenced by several factors, such as age, physiological aspects, food availability, behavior, and foraging mode. The latter can work as an important predictor of the type of prey to be consumed. Furthermore, the characterization of the lizards' diet provides information about trophic interactions. This study analyzed and categorized the diet of the species *Ameivula ocellifera* and *Tropidurus cocorobensis,* both psammophiles and coexisting in an area of Caatinga in Pernambuco, Brazil, but with different foraging modes. We sampled 84 individuals during the dry season in the Catimbau National Park. Lizard stomachs were analyzed, with food items quantified in the frequency of occurrence, number, volume, and relative importance index. In addition, we investigated the trophic niche overlap between species using the Pianka index. The most frequent item in *A. ocellifera* diet was Isoptera, while in the *T. cocorobensis* diet it was Formicidae (Hymenoptera). Considering the prey volume, the main item for *A. ocellifera* was still Isoptera, while for *T. cocorobensis* it was plant matter. The trophic niche overlap between the species was approximately 0.20. Although there are many consumed items in common, the way these items are used is quite divergent, such as the consumption of plant material by both species, but much more important for T. cocorobensis species than the active foraging species. Our results indicate that despite sharing the same space and consuming the same prey, the species present peculiarities in the composition of their diets. Moreover, these divergences can be explained by several factors in the environment and even in the evolutionary history of each species, which are from different families and not evolutionarily close.

Keywords. Diet; Niche Overlap; Foraging Mode; Plant consumption; Competition

Resumo. A dieta dos lagartos pode ser influenciada por diversos fatores, tais como idade, aspectos fisiológicos, disponibilidade alimentar, comportamento e modo de forrageamento. Este último pode funcionar como um importante preditor do tipo de presa a ser consumida. Além disso, a caracterização da dieta dos lagartos nos fornece informações sobre as interações tróficas. Este estudo analisou e categorizou a dieta das espécies *Ameivula ocellifera* e *Tropidurus cocorobensis*, ambas psammófilas e coexistentes em uma área da Caatinga de Pernambuco, Brasil, mas com diferentes modos de forrageamento. Amostramos 84 indivíduos durante a estação seca no Parque Nacional do Catimbau. Foram analisados estômagos de lagartos, com itens alimentares quantificados em frequência de ocorrência, número, volume e índice de importância relativa. Além disso, investigamos a sobreposição de nicho trófico entre as espécies usando o índice de Pianka. Na dieta de *A. ocellifera*, o item mais frequente foi Isoptera e na dieta de *T. cocorobensis*, Formicidae (Hymenoptera). Considerando o volume da presa, as posições mudam um pouco: o principal item para *A. ocellifera* ainda é Isoptera e para *T. cocorobensis* era matéria vegetal. A sobreposição de nicho trófico entre as espécies foi de aproximadamente 0,20, embora existam muitos itens consumidos em comum, a forma como esses itens são utilizados é bastante divergente, como o consumo de material vegetal, item presente na dieta das duas espécies, mas muito mais importante para as espécies de *T. cocorobensis* em comparação com a espécie de forrageamento ativo. Nossos resultados indicam que apesar de compartilharem o mesmo espaço e consumirem a mesma presa, as espécies apresentam peculiaridades na composição de suas dietas. Essas divergências podem ser explicadas por diversos fatores do meio ambiente e até mesmo da história evolutiva de cada espécie, que são de famílias diferentes e não evolutivamente próximas.

INTRODUCTION

The diet composition of lizard species is an important source of information on the trophic interactions between these reptiles, other animals and plants, and the environment (Duffield and Bull, 1998). In addition, diet can inform about environmental diversification and population differences, for example, induced by food availability (Perez-Cembranos et al., 2016), local traits as vegetation cover in the study area (Rodríguez et al., 2008), climatic seasonality (Ferreira et al., 2017), age (Whitfield and Donnelly, 2006; Toyama et al., 2018), sex (Barden and Shine, 1994, Teixeira-Filho et al., 2003), predation (Hawlena and Pérez-Mellado, 2009), physiology (Kohl et al., 2016), behavior (Vanhooydonck at al., 2007) and foraging mode (Bergallo and Rocha, 1994). This last-mentioned factor may influence energy expenditure, morphology, life-history traits, and prey selection (Huey and Pianka, 1981; Verwaijen and Van Damme, 2007). Huey and Pianka (1981) observed that small to medium sit-and-wait foraging lizards ingest abundant preys that move a lot, such as ants, apparently because lizards with this foraging mode find this prey more often. In contrast, small to medium active foraging lizards ingest proportionally more sedentary and conglomerated prey, such as insect larvae and termites, as well as large and often inaccessible prey for sit-and-wait lizards (e.g., scorpions).

Environmental factors may also influence the dietary patterns of lizards (Griffiths and Christian, 1996; Whitfield and Donnelly, 2006; Perez-Cembranos et al., 2016), especially in regions with pronounced seasonality, as the Brazilian Caatinga. Caatinga is an ecoregion with high average temperatures (25-30 ° C), particularly during the long dry season, and presents rainfall regime restricted to three months of the year (annual average 773 mm) (Andrade et al., 2018). This type of seasonality affects the trophic ecology of lizards, particularly by reducing the diversity of items ingested during the dry season, thus generating physiological challenges to be overcome (Vasconcellos et al., 2010; Sannolo and Carretero, 2019; Oliveira et al., *in prep.*). Consequently, the trophic niches of lizard species in this environment are dynamic, concerning intraspecific width and niche overlap (Ribeiro and Freire, 2011; Ferreira et al., 2017; Oliveira et al., *in prep.*).

Sharing dietary resources with sympatric species is an important element when considering trophic niches (Huey and Pianka, 1977; Sutherland, 2011; Sousa et al., 2017), particularly if involving competition. Hypothetically, niche overlap between two or more sympatric species must be limited in coexisting taxa (Pianka, 1974). For example, in desertic regions, congeneric and fossorial lizards, when in sympatry, tend to eat prey of different sizes than those reported in allopatry, apparently segregating trophic niches and reducing competition (Pianka, 1973). According to this niche-overlap theory, sympatric species must differ in at least one of the niche dimensions (spatial, temporal, or trophic) to make their coexistence feasible (Pianka, 1974).

In this study, we explore the diet as a tool to understand trophic overlap in *Ameivula ocellifera* (Spix, 1825) and *Tropidurus cocorobensis* Rodrigues, 1987, two frequently sympatric lizard species in the Brazilian Caatinga (Rodrigues, 1996; Menezes et al., 2011; Pedrosa et al., 2014). Herein we characterize their diet composition aiming to determine and quantify the food items shared and the overlap of trophic niche, considering their distinct foraging strategies. *Ameivula ocellifera* is considered a small to medium size (SVL Males = ~ 100 mm and SVL Females = ~ 80 mm) heliophile lizard, with active foraging mode (Menezes et al., 2006; Zanchi-Silva et al., 2014; Sales and Freire, 2015), and diet based on small arthropods, mainly insects, with predation of termites and larvae very common (Sales and Freire, 2015; Ferreira et al., 2017). *Tropidurus cocorobensis* is considered a small heliophile and a sit-and-wait foraging lizard, restricted to sandy environments (Rodrigues, 1987). It is considered psammophile (Rodrigues, 2003) with preferences for ants and plants (Oliveira et al., *in prep*).

MATERIAL AND METHODS

Study Area

The study was carried out in the Catimbau National Park (hereafter PARNA Catimbau), a conservation unit inserted in the Caatinga ecoregion, between the geographical coordinates 08°24"S; 37°09'W and 08°36'S; 37°14'W, in the state of Pernambuco, Brazil. The PARNA Catimbau presents about 62,000 ha, with an altitude varying between 700 and 1000 meters a.s.l (PROJETO RADAMBRASIL, 1983), and is located in a transition zone between the Brazilian mesoregions known as Agreste and Sertão. The climate in the region is considered semi-arid hot (BSh) according to the Köppen classification, with 600 mm average annual precipitation and 26 °C average annual temperature (Gomes et al., 2006). The PARNA Catimbau area is covered by outcrops of sand conglomerates and sandy soil (PROJETO RADAMBRASIL, 1983), and the vegetation is typical of the Caatinga, predominantly xeromorphic, with families such as Cactaceae, Euphorbiaceae, Mymosaceae, and Fabaceae (Gomes et al, 2006). In general, the landscapes of PARNA Catimbau follow the characteristics of other places in the Caatinga: there are dry plants without their leaves and a warm and dry environment during the dry season, while during the rainy season, the plants bloom fully, their leaves sprout and some flooded areas can be formed due to rainwater.

Data Collection

The specimens of *Ameivula ocellifera* and *Tropidurus cocorobensis* were collected during two expeditions in the PARNA Catimbau, in November 2020 and January 2021, during the dry season in the same collection area (Fig.1). Animals were captured with pitfall traps (Cechin and Martins, 2000; Foster, 2012) and by lasso ("noosing" in Fitzgerald, 2012). There is no evidence of significant differences between these methods in the stomach content of lizards captured (Costa et al., 2008).

Adult males and females were collected, with an average size of 66.43 mm for *Ameivula ocellifera* and 71.32 mm for *Tropidurus cocorobensis*. Thirty-one individuals of *A. ocellifera* (15 in the 1st and 15 in the 2nd expedition) and 53 individuals of *T. cocorobensis* (30 in the 1st and 23 in the 2nd expedition) were collected. All collections were authorized by the Brazilian environmental organs (permit SISBIO #73617), and experimental procedures were approved by the Ethics Committee on Animal Use (CEUA-UFPE process 0004/2020). All collected specimens were deposited in the Herpetological Collection of the Federal University of Pernambuco (CHUFPE). Each specimen had its stomach and intestine removed in the laboratory and the contents analyzed in a stereomicroscope. The ingested items were identified at the order level and, specifically for Hymenoptera (Formicidae), at the family level. The measurements of food items (maximum length and width) were made with a digital caliper (precision of 0.1 mm).

Data Analysis

We calculated the frequency of occurrence, number (individuals per stomach), and volume (in mm^3 per stomach) for each item ingested. The volume was estimated using the ellipsoid formula, using length and width, according to Dunham (1983):

$$\frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right)$$

In addition, we calculated the relative importance index (IX) (Howard et al., 1999) for each type of ingested prey. The calculation considered nX representing the average number of times that item X is repeated within the stomachs, fX the number of stomachs in which item X was found, and vX, the average volume of food item X in mm^3. N, F, and V correspond, respectively, to the general sum of the number, frequency, and volume of all prey items grouped:

$$Ix = \frac{\left[\left(\frac{nX}{N}\right) + \left(\frac{vX}{V}\right) + \left(\frac{fX}{F}\right)\right]}{3}$$

We also calculated the food niche overlap according to the Pianka index (1974), considering the volume of each item of prey. In the formula, "pij" corresponds to the proportion of items in the diet of *Ameivula ocellifera* and "pik", the proportion of items in the diet of *Tropidurus cocorobensis*:

$$Oik = \frac{\sum pij * pik}{\sqrt{\sum pij^2 * pik^2}}$$

RESULTS

The specimens of *Ameivula ocellifera* and *Tropidurus cocorobensis* sampled in PARNA Catimbau ingested mainly insects (Table 1). The diversity of ingested items was very similar, with 17 different items ingested by *A. ocellifera* and 18 by *T. cocorobensis*. The most frequent item in the diet of *A. ocellifera* was Isoptera (24.66%) (Table 1; Fig. 2) and in the diet of *T. cocorobensis*, Formicidae (Hymenoptera) (28.75%) (Table 1; Fig. 2). Considering the volume of the items, the positions change slightly: the main item for *A. ocellifera* was still Isoptera (69.64%), and for *T. cocorobensis* was plant matter (83.45%) (Fig. 3).

Regarding the Relative Importance, Isoptera was the most important food item for *A. ocellifera*, with IX=53.49. The other items ingested by the species were quite distant from this value, being the second and third most important items with 14.75 (Blattodea) and 6.08 (Hymenoptera, except Formicidae) (Table 1, Fig. 4). For *T. cocorobensis*, plant matter presented the highest Ix (30.28), and Formicidae (Hymenoptera) and Isoptera presented the second and third, respectively (26.74 and 13.60). The trophic niche overlap between both species is 0.2037 (20.37%).

DISCUSSION

Ameivula ocellifera and *T. cocorobensis* mainly ingest a wide range of insects and a few different items such as arachnids and gastropods as complimentary items matter plant appears as a main item in *T. cocorobensis* (Tables 1 and 2). Although they are sympatric in the study area and have many diet items in common, the contributions of these items are sensibly different in diets (Table 1 and 2). Such differences were already noticed by Huey and Pianka (1981), who related active foraging predators tending to ingest more frequently sedentary prey, such as termites, whereas sit-and-wait foraging species usually capture more active and abundant prey, such as ants.

According to Pianka's niche overlap theory, significant ecological differences should occur in at least one of the three different niches (spatial, temporal, and trophic) that characterize the studied species (Pianka, 1974). If niche segregation is realized, competition should decrease, thus favoring the co-existence of different species (Pianka 1973, 1974). In this study, *A. ocellifera* and *T. cocorobensis* seem to share similar spatial (Pedrosa et al., 2014) and temporal niches, given that both are diurnal and heliophile lizards (Rodrigues, 1987; Menezes et al., 2011). Subtle differences regarding niche segregation are theoretically possible. However, we lack information on important variables such as the time of day for the peak in activity, time at which foraging starts, and others, which are only known for *A. ocellifera* (Menezes et al., 2011; Albuquerque et al., 2018). Considering that both species share many ecological similarities in spatial and temporal niches, then niche differentiation may be mainly trophic, as our results suggest for the populations at the PARNA Catimbau. It seems important that different foraging strategies characterize the observed species. The "sit-and-wait" *T. cocorobensis* eats a larger frequency of vagile prey in diet (i.e., ants), whereas the more active forager *A. ocellifera* ingests less vagile prey (i.e., termites). These results corroborate the already cited proposals by Huey and Pianka (1981).

Historical influences also need to be considered as an important factor in the differences of consumed items by both species (i.e., Number, frequency, and volume), which are nested in very distant clades within Squamata (Teiioidea and Iguania; Pyron et al., 2013; Simões and Pyron, 2021). In Iguania and particularly in Tropiduridae, the consumption of plant material is vastly documented (e.g., Fialho et al., 2000; Van Sluys et al., 2004; Kolodiuk et al., 2010; Garda et al., 2012; Siqueira et al., 2013; Verrastro and Ely, 2015; García-Rosales et al., 2020; Tan et al., 2020; Oliveira et al., in prep), and Tropiduridae are within the families with the highest percentages of omnivorous species and with the highest average percentages of plant matter in diet (Cooper and Vitt, 2002).

This high intake of plant material seems to be a result of historical heritage since although the dietary primitive condition in Iguania seems to be the carnivory, and the primitive condition of Tropiduridae is uncertain, the plant consumption is widespread in the crown groups of the family and it seems to be the condition in the root of the *torquatus* group in which *T. cocorobensis* is nested (Frost et al., 2001; Cooper and Vitt, 2002). On the other hand, the carnivory seems to be the primitive condition to Scincoidea, Teiidae, and the genus *Ameivula* (Cooper and Vitt, 2002). Considering the dietetic composition of lizard species in Brazil, it is possible to notice plant matter intake records in taxa within the family Teiidae (Menezes et al., 2006; Menezes et al., 2011; Sales et al., 2011; Sales and Freire, 2015). However, the consumption of plants in the family Teiidae is reduced compared with the observed in Tropiduridae (Cooper and Vitt, 2002).

Finally, the different responses to environmental variations in each species must also be considered when comparing the usage of food resources, especially in environments with marked seasonality, such as the Caatinga. In this environment, some species of *Tropidurus*, such as *T. cocorobensis*, *T. hispidus,* and *T. semitaeniatus*, vary their ingested items according to the different seasons, consuming mainly ants and termites and increasing the trophic niche overlap during the dry season, and becoming opportunist predators of arthropods in general during the rainy season (Ribeiro and Freire, 2011, Oliveira et al. in prep.). In some species of the genus *Ameivula*, seasonal changes in trophic ecology are not present, as in *Ameivula littoralis* in coastal sandbank environments, with reduced seasonality and constantly high air humidity and rainfall (Teixeira-Filho et al., 2003). On the other hand, in a population of *A. ocellifera* in the Caatinga, the seasonal change in diet was recorded in the first three most important items for the species during each season (insect larvae, termites, and orthopterans in rainy seasons, contrasting with spiders, hemipterans and insect larvae in the dry season) (Sales and Freire, 2015), what seems to reinforce the influence of the seasonality in the diet.

Despite the low overlap of trophic niche, *A. ocellifera* and *T. cocorobensis* consume similar food items in the Catimbau National Park and other populations of the same species, with slight variation in dietary composition (Menezes et al., 2011; Sales and Freire, 2015; Oliveira et al., *In press*). The predation of termites by *A. ocellifera* in the Caatinga and Cerrado is well documented (Menezes et al., 2011; Sales and Freire, 2015), but populations of this species in Amazonian savannahs consume mainly orthopterans (Mesquita and Colli, 2003). Specimens of *T. cocorobensis* consume several items in common with their congeners. Ants are the most ingested items, as in the diet of *T. hispidus* and *T. semitaeniatus* (Ribeiro and Freire, 2011; Oliveira et al., *In press*), which are also sympatric with *T. cocorobensis* and *A. ocellifera* in Catimbau National Park (Pedrosa et al., 2014; Oliveira et al., *In press*).

Our results demonstrate that even sharing the same food resources, the trophic niche overlap between the species is low. This pattern can be related to competition strategies, historical constraints, and/or the responses to environmental variations. We suggest that future studies consider these species' possible temporal niche overlap, with more information about the period of activity for the species *T. cocorobensis*. Our study contributes widely to the knowledge of resource sharing between sympatric species from different families of lizards in Caatinga.

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Table 1. Diet composition of *Ameivula ocellifera* and *Tropidurus cocorobensis* in Catimbau National Park, Pernambuco, Brazil. F represents the frequency of items in the diet, N represents the number, V the volume in mm³ and Ix the value of the relative importance.

	***Ameivula ocellifera* (N=31)**				***Tropidurus cocorobensis* (N=53)**			
Prey Types	F(%)	N(%)	V(%)	I.R	F(%)	N(%)	V(%)	I.R
Araneae	4 (5.48)	4 (0.37)	2131.92 (0.37)	3.71	3 (1.96)	3 (0.18)	6189.30 (0.35)	6.73
Blattodea	2 (2.74)	4 (0.37)	8701.40 (0.37)	14.75	1 (0.65)	1 (0.06)	121.78 (0.01)	0.59
Coleoptera	3 (4.11)	5 (0.47)	522.25 (0.47)	0.89	4 (2.61)	9 (0.54)	1422.00 (0.08)	1.51
Coleoptera (Larvae)	5 (6.85)	5 (0.47)	1236.75 (0.47)	1.89	2 (1.31)	13 (0.79)	787.54 (0.04)	0.87
Diptera	-	-	-	-	3 (1.96)	3 (0.18)	12.00 (0.01)	0.83
Gastropoda	4 (5.48)	4 (0.37)	214.84 (0.37)	0.49	1 (0.65)	1 (0.06)	8.67 (~0)	0.26
Hemiptera	-	-	-	-	2 (1.31)	3 (0.18)	103.50 (0.01)	0.60
Homoptera	1 (1.37)	1 (0.09)	11.23 (0.09)	0.10	3 (1.96)	4 (0.24)	49.40 (~0)	0.77
Hymenoptera								
Formicidae	11 (15.06)	33 (3.08)	2106.39 (3.08)	1.97	44 (28.75)	826 (49.97)	140915.60 (7.86)	26.74
Formicidae (Larvae)	-	-	-	-	3 (1.96)	3 (0.18)	984.30 (0.05)	1.67
Non Formicidae	10 (13.69)	14 (1.14)	8318.80 (1.31)	6.08	28 (18.30)	44 (2.66)	46040.72 (2.57)	10.04
Isoptera	18 (24.66)	957 (89.36)	83383.41 (69.64)	53.49	15 (9.80)	483 (29.22)	97290.69 (5.43)	13.60
Mantodea	-	-	-	-	2 (1.31)	2 (0.12)	649.22 (0.04)	1.42
Matter Plant	3 (4.11)	30 (2.80)	7365.90 (6.15)	4.36	36 (23.53)	251 (15.18)	1496251.16 (83.45)	30.28
Odonata	1 (1.37)	1 (0.09)	194.83 (0.16)	1.18	-	-	-	-
Orthoptera	3 (4.11)	5 (0.47)	2947.00 (2.46)	4.28	3 (1.96)	3 (0.18)	1591.47 (0.09)	2.26
Phasmatodea	1 (1.37)	1 (0.09)	6.69 (0.01)	0.07	1 (0.65)	2 (0.12)	15.14 (~0)	0.28
Pseudoscorpianida	2 (2.74)	2 (0.19)	4.32 (~0)	0.08	1 (0.65)	1 (0.06)	1.32 (~0)	0.24
Scolopendrida	1 (1.37)	1 (0.09)	183.84 (0.15)	1.11	-	-	-	-
Scorpiones	1 (1.37)	1 (0.09)	75.24 (0.06)	0.47	-	-	-	-
Unidentified Arthropod	3 (4.11)	3 (0.28)	2324.28 (1.94)	5.09	-	-	-	-
Vertebrate								
Squamata	-	-	-	-	1 (0.65)	1 (0.06)	363.63 (0.02)	1.30
Total		**1071**	**5942.68**			**1653**	**1792908.44**	

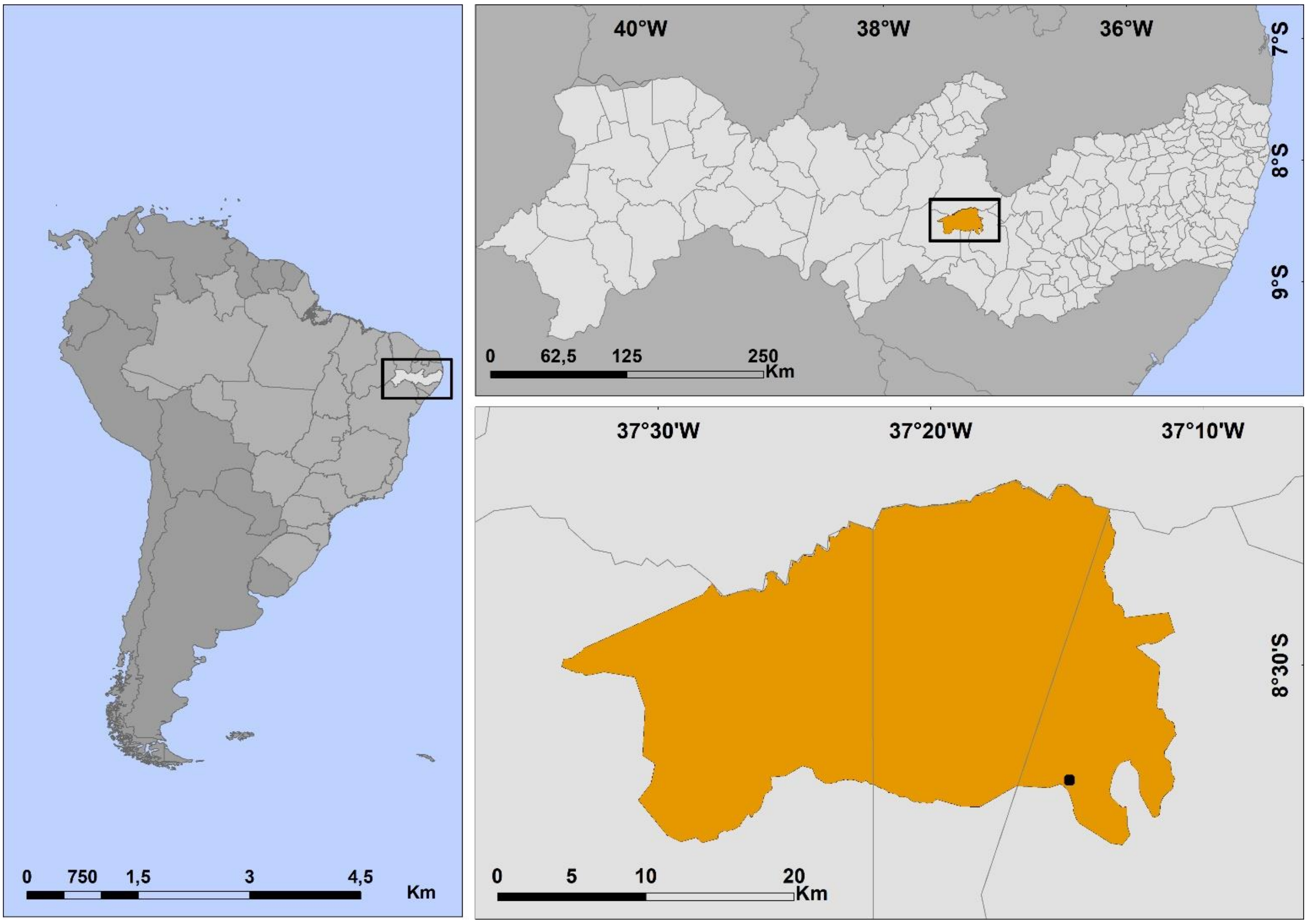

Figure 1. Map with the location of Catimbau National Park. The black dot represents the collection area.

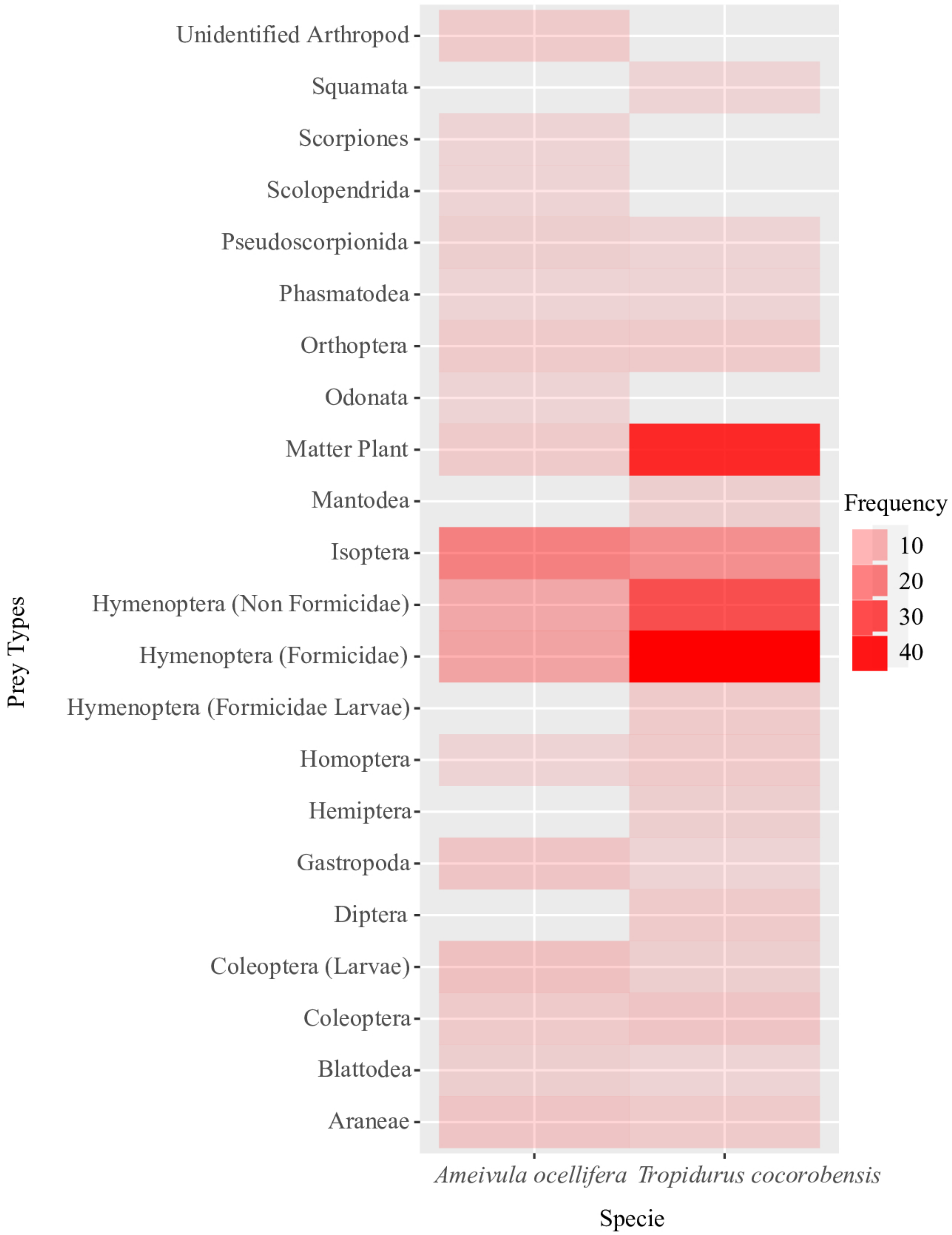

Figure 2. Heat map representing the frequencies of each item ingested by the species *Ameivula ocellifera* (first column) and *Tropidurus cocorobensis* (second column) collected at PARNA Catimbau. The darker the color, the higher the absolute value of the frequency.

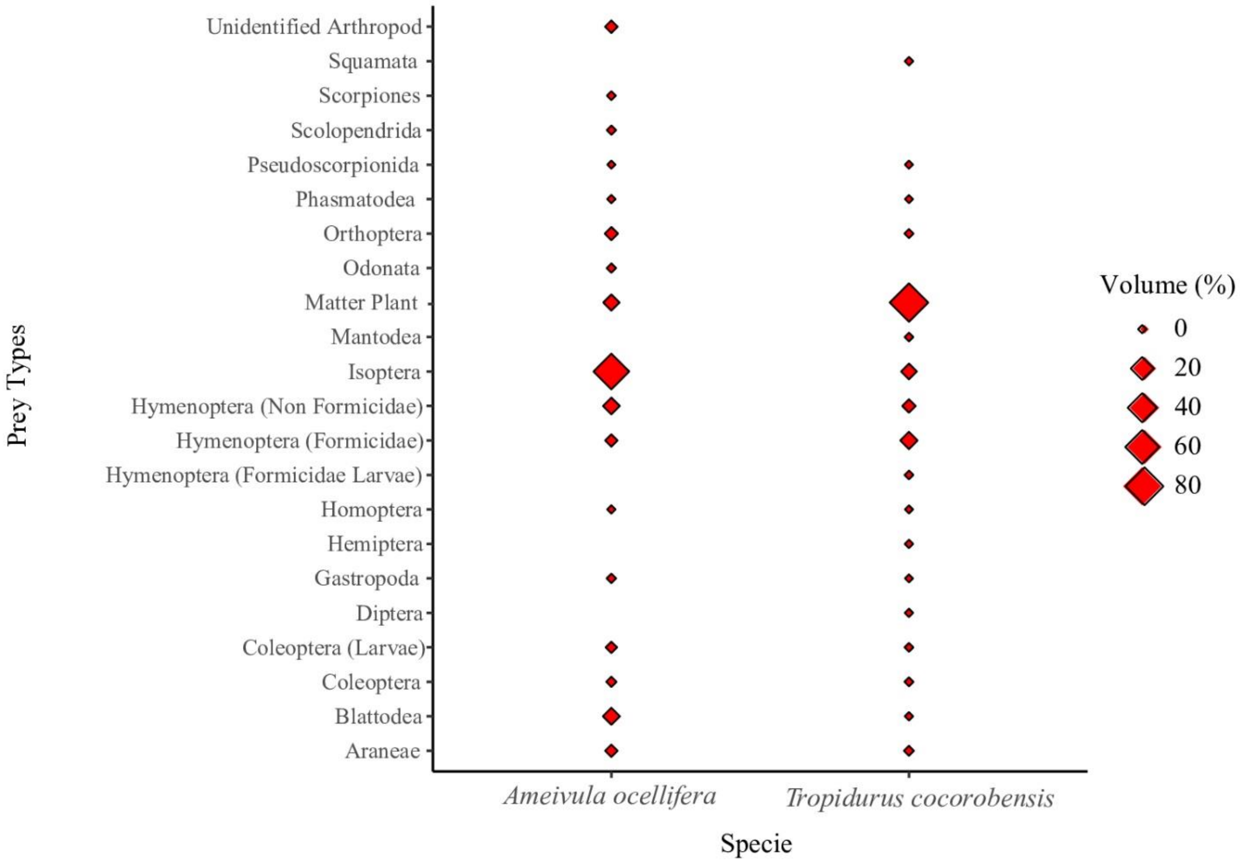

Figure 3. Graph representing the volume contribution (%) of each item ingested by the species *Ameivula ocellifera* (first column) and *Tropidurus cocorobensis* (second column) collected at Catimbau National Park. The larger the symbol, the greater the volume of the item.

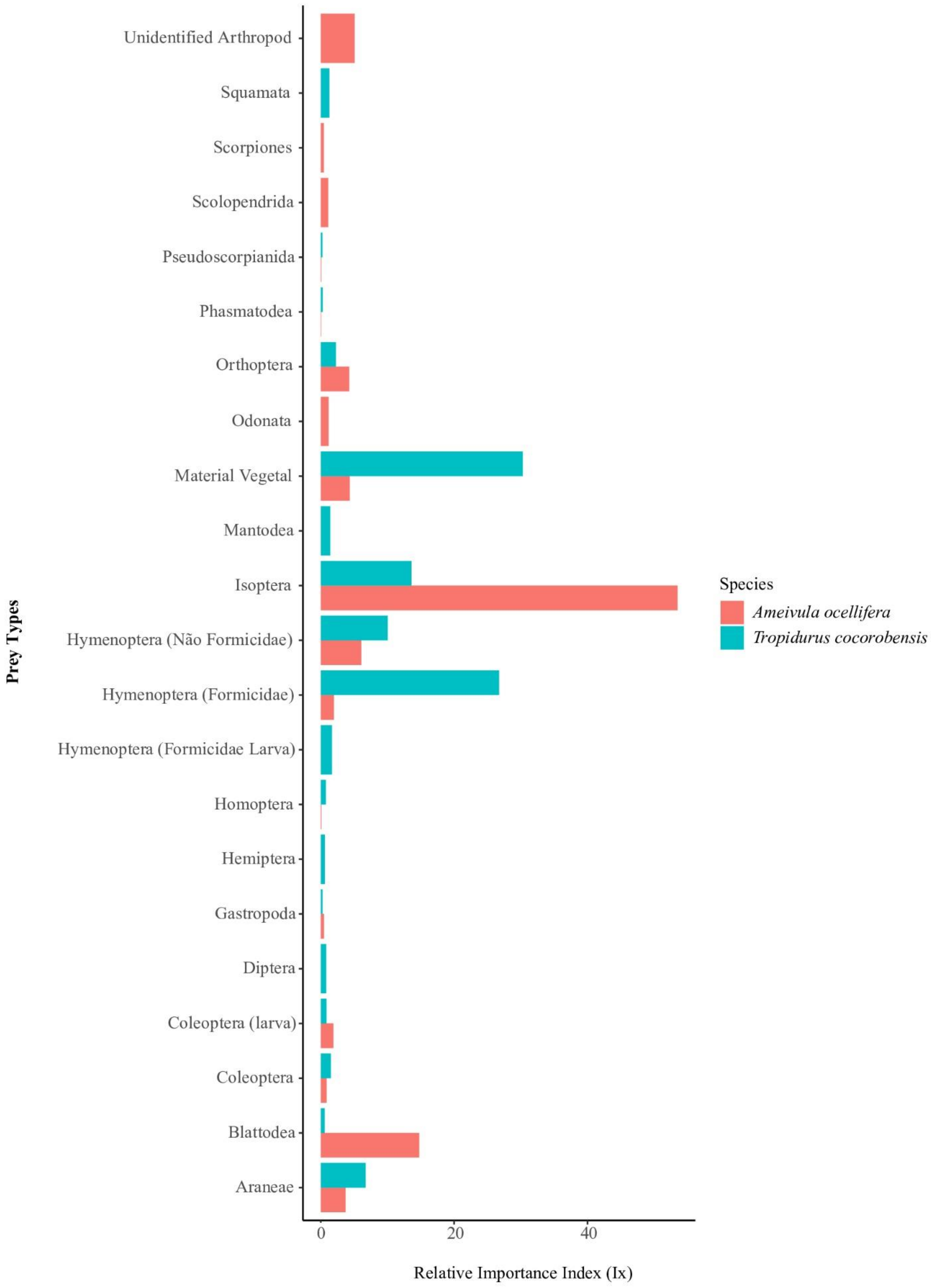

Figure 4. Bar chart representing the importance index corresponding to each item ingested by the species *Ameivula ocellifera* (first column) and *Tropidurus cocorobensis* (second column) collected in PARNA Catimbau.

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3. CAPÍTULO II – FIELD WATER BALANCE OF TWO LIZARDS SPECIES, WITH DIFFERENT FORAGING MODES, DURING THE DRY SEASON IN CAATINGA

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ABSTRACT

With possible climate change scenarios indicating an increase in aridity and a decrease in rainfall in some areas, it is important to understand how reptiles are exposed to a water imbalance in their natural environment considering their high sensitivity to extreme temperatures as they are animals that depend on the environment to regulate their body temperature. This study brings us pioneering information on the field water balance of *Ameivula ocellifera* and *Tropidurus cocorobensis* in Brazil's Caatinga, semi-arid region. We submitted specimens of both species to induced hydration for two hours and then calculated the dehydration status of the specimens by adding the weight to their initial mass. We also calculated the moisture present in the stomach contents of each species using the difference between the wet weight and the dry weight of these samples. Despite sharing the same environment, *A. ocellifera* absorbed comparatively more water during induced hydration. We deduce that they experience greater water imbalance relative to *T. cocorobensis,* assuming similar body mass, reflecting several physiological, ecological, evolutionary aspects. We highlight the possible influence of the foraging mode. The field water balance of *T. cocorobensis* is significantly related to soil temperature, which can be explained as a strategy used to reduce water loss. We also observed that *A. ocellifera* ingests proportionally more moist food than T. cocorobensis, which may reflect the main items ingested and the breadth of its diet due to its way of foraging. Understanding the field water balance of Caatinga reptiles becomes important to future considerations, as this group of vertebrates is more susceptible to temperature changes, increased aridity, and reduced rainfall.

KEY WORDS: Water Balance, Water in Diet, Teiidae, Tropiduridae

INTRODUCTION

Dietary shifts are important for lizards to avoid dehydration under water restriction conditions. Thus, how these shifts occur becomes a relevant question, particularly because of contrasting foraging strategies. In lizards, the search for food takes two distinct general forms: sit-and-wait and active foraging. Sit-and-wait foragers do not explore the environment nor cover large distances in search of food and take advantage of the most abundant and active prey items, whereas active foragers may cover large distances in an active search of food (Huey and Pianka 1981). Also, foraging mode is integrated with physiological, morphological, and ecological differences (Huey and Pianka, 1981), yet this behavior may respond to environmental conditions (Huey and Pianka, 1981) such as seasonality in water availability (Colli et al., 1997; Kolodiuk et al., 2010; Ribeiro et al., 2011). Kolodiuk et al. (2009) observed that two typical sit-and-wait tropidurid lizards are comparable in foraging behavior during the dry season but differ during the rainy season. Because foraging is flexible and influenced by environmental variation, it is important to analyze the consequences of seasonal climate change (Huey and Pianka, 1981; Kolodiuk et al., 2009) and understand responses to dehydrating conditions.

Changes in the diet composition of lizards have also been documented during the dry period in semi-arid environments such as the Caatinga, these changes mainly involve the diversification of the most consumed items (Ribeiro et al., 2011; Ferreira et al., 2017) and probably are related with the reduced availability of arthropods in this season (Vasconcellos et al., 2010; Nobre et al., 2012; Ferreira et al., 2017). However, other points such as the influence of dehydration on prey choice were never tested in this environment. Similar to the observed in the Caatinga, the diet of lizards is also affected by environmental conditions in desert environments, and water imbalance responses reflecting on the item selection are reported, such as lizards selecting items that provide more water and high consumption of vegetables (Nagy, 1973). In other reptiles, such as desert turtles, diet becomes important in extreme drought since eating a meal maintains the water balance (Henen et al., 1998). Thus, a water balance negatively affected by dry environments can influence aspects related to food ecology, such as the search for prey through foraging (Kotler et al., 1998). Furthermore, the mode of foraging may be related to physiological, morphological, environmental, and ecological differences (Huey and Pianka, 1981

Under given conditions, water restrictions in the environment may affect water balance and even generate water stress in lizards. These animals respond in different ways, eventually with changes in their physiological or ecological performance related to their reproduction, foraging, and thermoregulation, among other activities (Huey et al., 2010; Rozen-Rechels, 2018; Rozen-Rechels et al., 2020; Camacho et al., 2021). A water deficit can bring significant physiological changes and may imply in reduction of longevity and softer oxidative defenses, as observed by Dupoué et al. (2020a) in lizards of the species *Zootoca vivipara* subjected to water stress in the first year of age. Among other physiological influences, dehydration also affects the thermal ecology of lizards, which is directly related to thermoregulation, in addition to the area of use and locomotor capacity (Wilson et al., 1989). Results published by Sannolo and Carretero (2019) show that *Podarcis* lizards, when dehydrated, change their preferred body temperature, choosing to shelter in refuges and at lower temperatures.

Lizards representing several lineages are negatively affected by extreme temperatures given their need to regulate their body temperature within limits compatible with performing their basic functions (Huey, 1982). Whereas the term ‘extreme temperature’ is lineage-specific given differences in the field body temperature of lizards across lineages, the occurrence of some lizard lineages in environments considered ‘thermally extreme’ is intriguing. Those lineages must display characteristics compatible with environmental challenges, as is the case of lizards in hot arid and semiarid regions (Mesquita et al., 2018). In addition, the high seasonality of semi-arid environments, with a long dry season and a short rainy season, results in different temperatures and solar radiation over a year and, consequently, in the search and maintenance of hydration of the individuals who occupy these environments. In lizards, dehydration conditions depend on the nature of occupied habitats and microhabitats (Heatwole and Veron, 1977) and the profile of thermoregulatory activities (Foley and Spotila, 1978). In hot arid environments, where organisms are exposed to high temperatures and water restriction, important selective forces may be associated with water balance (Waldschmidt and Tracy, 1983). In this sense, understanding how much reptiles are exposed to water deficit becomes an important predictor of knowledge of ecological and physiological changes in species (Dupuoé et al., 2018, 2020b; Rozen-Rechels et al., 2018, 2021; Brusch et al., 2020; Le Galliard et al., 2021).

With all this in mind, it is reasonable to consider that lizards lose more water and are exposed to higher levels of dehydration as a direct consequence of the increase in average temperatures and solar irradiation resulting from global climate change in recent decades (Huey et al., 2010). However, little is known regarding the limits and effects of dehydration in these animals under natural conditions. Specifically, here we ask: if dietary shifts are important for lizards facing water deficit in the environment, and how sit-and-wait and active foragers differ; if active foraging by definition involves greater exposure to dehydrating conditions, whereas that exposure translates to dehydration carried out in the field; and if individual lizard characteristics and environmental conditions affect field water balance. To answer these questions, we studied two species of Caatinga lizards, *Ameivula ocellifera* (Spix, 1825) (Family Teiidae) and *Tropidurus cocorobensis* Rodrigues, 1987 (Family Tropiduridae), relying on stomachal contents as an expression of foraging behavior. We also analyze the water content of the diet and relate dietary variables with indicators of body condition, particularly field water balance status and body condition.

MATERIAL AND METHODS

Study Area

The study was carried out in the Catimbau National Park (hereafter PARNA Catimbau), a conservation unit inserted in the Caatinga ecoregion, between the geographical coordinates 08°24''S and 08°36'S and 37°09'W and 37°14'W, in the state of Pernambuco, Brazil. The park has about 62,000 ha, with an altitude varying between 700 and 1000 meters a.s.l (Projeto RadamBrasil, 1983), and is located in a transition zone between the Brazilian mesoregions known as Agreste and Sertão. The climate in the region is considered semi-arid hot (BSh) according to the Köppen classification, with 600 mm average annual precipitation and 26 °C average annual temperature (Gomes et al., 2006). The Park area is covered by sand conglomerates and sandy soil (Projeto RadamBrasil, 1983). The vegetation is typical of the Caatinga, predominantly xeromorphic, with families such as Cactaceae, Euphorbiaceae, Mymosaceae, and Fabaceae as the most common (Gomes et al., 2006). In general, the landscapes of PARNA follow the characteristics of other places in the Caatinga: there are dry plants during the dry season, without their leaves and a warm and dry environment. During the rainy season, the plants bloom fully, their leaves sprout, and some flooded areas can be formed due to rainwater. Specifically, at our collection point (Figure 1), the mean air temperature was 32.62 °C, and the mean relative humidity was 45.90% (data collected by the authors). At our collection point, there was also no puddle or water source nearby.

Natural History of the Species Compared

Ameivula ocellifera (Spix, 1825) is a species of lizard of the Teiidae family, active-type foraging and found in Restinga, Cerrado, and Caatinga environments (Menezes et al., 2006; Sales and Freire, 2015). Their diet is diversified among arthropods, mainly insects, and termites are the most consumed items (This study, Chapter 1; Sales and Freire, 2015). *A. ocellifera* is associated with the sandy microhabitat, and in the PARNA Catimbau, this species is sympatric to *Tropidurus cocorobensis* Rodrigues, 1987 (Pedrosa et al., 2014), the second model species in this study. *T. cocorobensis* is a heliophilic lizard belonging to the Tropiduridae family, classified as a sit-and-wait forager and has a relictual distribution in the Caatinga (Rodrigues, 1987), found only in the states of Alagoas, Bahia, and Pernambuco (Ribeiro et al., 2012). Like *A. ocellifera*, it is considered a psammophilous species (Rodrigues, 1987). *T. cocorobensis* mainly consumes ants and plant material, but its diet is diversified among other arthropods such as termites, beetles, and scorpions (This study, Chapter 1; Oliveira et al., *in prep*).

Data Collection

The specimens of *Ameivula ocellifera* and *Tropidurus cocorobensis* were collected during two expeditions in the PARNA Catimbau, in November 2020 and January 2021, both in the dry season and in the same collection area (Figure 1), with pitfall traps (Cechin and Martins, 2000; Foster, 2012) and capture by lasso ("noosing" in Fitzgerald, 2012). There is no evidence of significant differences between these methods in the stomach content of lizards captured (Costa et al., 2008).

The traps were opened during the morning and monitored by a person close to the trap throughout the activity period. Immediately after capturing a specimen of the target species, we removed it from the trap and measured its body temperature using a cloacal thermometer. We emphasize that it was important to measure body temperature as soon as the animal was captured, avoiding the influence of bucket temperature or insolation on the specimen's body temperature. When it was impossible to measure the temperature immediately, the specimens were disregarded and released without data collection. Air temperature and humidity data were measured at the exact place of collection using a Thermo-Hygrometer.

Adult specimens were collected and subjected to the field water balance experiment. We defined *T. cocorobensis* adults by the presence of black spots on the thighs in males and the snout-vent length in females (Rodrigues, 1987). For *A. ocellifera,* we used the snout-vent length as an age definer (to define adult males - 45.55 to 91.14 mm; to define adult females - 41.60 to 77.72 mm) (Sales and Freire, 2015). In total, 31 specimens of *A. ocellifera* (15 in the 1st and 15 in the 2nd expedition) and 53 specimens of *T. cocorobensis* (30 in the 1st and 23 in the 2nd expedition) were sampled. All lizards collected were killed using a lethal dose of 2% lidocaine hydrochloride. After killing, we made a ventral cut in each specimen to remove the stomach contents. After this procedure, the specimens were fixed in 10% formalin and then stored in 70% ethanol. Specimens collected for this study are deposited in the Herpetological Collection of the Federal University of Pernambuco (CHUFPE) (All vouchers in Appendix 1). Collections were allowed by the Brazilian environmental organs (permit SISBIO #73617), and experimental procedures were approved by the Ethics Committee on Animal Use (CEUA-UFPE process 0004/2020).

Field Water Balance

The collected specimens were immediately weighed on a precision digital scale. The first weighing of the specimen was carried out immediately after its collection, so we considered it the body mass in nature (BM1). After the first mass measurement, the lizard was subjected to an involuntary hydration procedure for two hours, keeping the specimen in a container with water up to half the belly in a shaded place. Then, the lizard was externally dried with the aid of absorbent paper and again subjected to weighing (BM2) and then to four more weighing every 15 minutes for one hour (BM3, BM4, BM5, BM6) (Preest, 1988; Tracy et al., 2014). In addition, we measured the snout-vent length (CRC) of all individuals to control results according to body size.

Moisture in stomach content samples

All stomach contents removed from the specimens were weighed while still wet on a precision balance (wet weight) and then preserved in a container with 70% alcohol. In the laboratory, they were dried in an oven at 60°C until their weights stabilized (Dry Weight).

Body Condition Index

It is necessary to highlight the influence of specimens' body size (SVL) and initial weight/in nature (BM1) on Field Water Balance to analyze better the specimens’ field water balance and the stomach’s moisture content. In this sense, we calculated the Body Condition Index through the residuals (Ri) of an ordinary least squares (OLS) regression using the log-transformed snout-vent length (SVL) and the log-transformed initial weight/in nature (BM1) of each specimen. Regressions were performed separately for each lizard species studied (Lagrue and Poulin, 2015; Warner et al., 2016; Gaston and Vaira, 2020). As the body condition index covers parameters of the body structure (here, snout-vent length and body mass in nature) of each individual studied, it becomes important for a fairer comparison between the species’ field water balance, as this status changes according to the size and weight of the specimen.

Data Analysis

For calculating field water balance, we used the difference of values from the initial weight in nature (BM1) to the mean of weights three and four (BM3, BM4; 15 and 30 minutes, respectively, after hydration) for each specimen. We used the average of these weights because the body mass of the specimens, in general, stabilized in this interval. We assumed that the greater the state of water loss in nature, the more water the specimen would absorb after induced hydration.

$$\text{Field Water Balance} = (BM3+BM4)/2 - BM1$$

For calculating moisture in stomach content samples, we use the subtraction: Wet Weight - Dry Weight. The dry weight was considered when the mass of the stomach contents stabilized after drying in the oven. This calculation was performed for all the stomachs of each specimen collected.

We performed a Shapiro-Wilk test to determine whether the data obtained presents normal distribution (Shapiro and Wilk, 1965). Knowing that the data present a non-normal distribution, we performed a non-parametric Wilcoxon Test to compare body masses before (BM1) and after induced hydration (Mean of BM3 and BM4) within species and the wet and dry weight of stomach contents (Gibbons, 1971). In addition, we used the non-parametric Mann Whitney Test to determine the Field Water Balance and Moisture in Stomach Content Samples among the studied lizard species (Gibbons, 1971). We also performed simple linear regressions using Field Water Balance and Moisture in stomach content samples as dependent variables of Body Condition Index, ambient temperature, ambient humidity, soil temperature, and body temperature. All tests and regressions were performed with 'rstatix' and 'psych' packages in R 3.5.1 (R Core Team, 2018). All graphics made to expose our results were developed with the 'ggplot2' package in R 3.5.1 (R Core Team, 2018).

RESULTS

Field Body Condition

Our results revealed *Ameivula ocellifera* with 0.27 grams (corresponding to 3.6%) and *Tropidurus cocorobensis* with 0.11 grams (1.6%) of average Field Water Balance (Table 1). According to the Wilcoxon test, the difference in weights before and after hydration (BM1 and BM3+BM4 Mean) is significant in both species (*A. ocellifera v-value* = 430, P <0.0001; *T. cocorobensis v-value* = 1060, P < 0.0001) (Table 1). The means of the field water balance between both species differ significantly (*w-value* = 893, P = 0.0006). *T. cocorobensis* has an average snout-vent length greater than *A. ocellifera*. Moreover, *A. ocellifera* has a mean body temperature higher than *T. cocorobensis* (Table 1). The means of the SVL and corporal temperature differ significantly between both species (SVL, *w-value* = 426, P = 0.03836; Body Temperature, *w-value* = 830.5, P = 0.007278). All raw values collected during the study are concentrated in Appendix 2 and 3 for *A. ocellifera* and *T. cocorobensis,* respectively.

Residuals from ordinary least squares regressions (OLS) used as Body Condition Index can be seen in Appendix 4 for the two species (*A. ocellifera* r^2 = 0.8356, P < 0.0001; *T. cocorobensis* r^2 = 0.9351, P < 0.0001). The means of the corporal condition of the species not differ significantly (*w-value* = 570, P = 0.7127). The field water balance of *A. ocellifera* and *T. cocorobensis* does not have a significant correlation with the respective Body Condition Index (*A. ocellifera* r^2 = -0.0310, P = 0.6690; *T. cocorobensis* r^2 = 0.0309, P = 0.1339) (Figure 2). The Field Water Balance in both species did not show a significant correlation with air temperature (*A. ocellifera* r^2 = -0.0091, P = 0.3923 ; *T. cocorobensis* r^2 = 0.0008, P = 0.3152 , Figure 3A), environmental humidity (*A. ocellifera* r^2 = -0.0358, P = 0.7968 ; *T. cocorobensis* r^2 = -0.0228, P = 0.8013, Figure 3C) and body temperature (*A. ocellifera* r^2 = -0.0342 , P = 0.7456; *T. cocorobensis* r^2 = 0.0162, P = 0.2007, Figure 3D). But it indicated a significant correlation with soil temperature in *T. cocorobensis* but not the same in *A. ocellifera* (*A. ocellifera* r^2 = 0.0462 , P = 0.1408; *T. cocorobensis* r^2 = 0.1118, P = 0.0162 , Figure 3B).

Moisture in Stomach Content Samples

Ameivula ocellifera presented average moisture of 0.57 grams in stomach content samples, corresponding to 75.19% (Table 2). In *T. cocorobensis,* the average moisture in stomach content samples corresponded to 0.81 grams, or 71.96% (Table 2). According to the Wilcoxon test, the difference in weights of stomach contents wet and dry is significant in both species (Table 2). The moisture in stomach content samples differ significantly between species (*w-value* = 372, P < 0.01). The correlation of moisture in stomach content samples and Body Condition Index was not significant (*A. ocellifera* r^2 = 0.012, P = 0.26; *T. cocorobensis* r^2 = -0.02, P = 0.65) (Figure 4).

With regard to moisture in stomach content samples, both species did not show a significant correlation of this measure with environmental temperature (*A. ocellifera* r^2 = 0.08, P = 0.08 ; *T. cocorobensis* r^2 = -0.02, P = 0.75 , Figure 5A) and humidity of the environment (*A. ocellifera* r^2 = -0.03, P = 0.71 ; *T. cocorobensis* r^2 = 0.03, P = 0.0.15, Figure 5C). However, moisture in stomach content samples of *T. cocorobensis* species indicated a significant correlation with soil temperature (r^2 = 0.07, *P* = 0.04 , Figure 5B) and with body temperature (r^2 = 0.09, *P* = 0.03, Figure 5D). Diverging from what was observed in A. *ocellifera* in these parameters (Soil temperature r^2 = 0.03 , *P* = 0.17; Body temperature r^2 = -0.04 , *P* = 0.87, Figure 5B, D).

DISCUSSION

Field Body Condition

Our study explores primary results on the field water balance of Caatinga lizard species in nature. The more water is absorbed, the more dehydrated animals are at the time of capture. Because *A. ocellifera* absorbed comparatively more water during induced hydration, we indicate that, under the assumption of similar body mass, this species experiences greater water imbalance relative to *T. cocorobensis* (Table 1). Body size matters because water loss through breathing and the skin must relate to lung size and skin area, even if tissue thickness may also matter (Mautz, 1980, 1982). In parallel, small animals present less favorable area-volume relationships within similar shapes and tend to lose water at higher relative rates (water loss as a function of water content) (Turk et al., 2010). Then, our conclusions must consider that individuals of *A. ocellifera* have an average snout-vent length somewhat smaller than *T. cocorobensis* counterparts (Table 1). With all other variables being equal, one would expect *A. ocellifera to* lose less total water but to experience relative rates of dehydration higher than *T. cocorobensis*.

The rates of water loss may relate to body condition index, a measure of the energetic state of the animals. The expected relationship is complex because body condition index reflects both an ecological history (the quality of the recent diet of the animal) and current physiological state, admitting that these are not fully independent variables (Lagrue and Poulin, 2015; Warner et al., 2016; Gaston and Vaira, 2020). We did not find field water balance to be a function of body condition index, perhaps given rather homogeneous states. Furthermore, other possible points that may correlate with water balance, for example, it is important to consider too the involvement of temperature of the environment, once thermoregulation and osmoregulation of ectothermic species are interconnected to environmental conditions (Bradshaw, 1975; Seebacher and Franklin, 2005; Vickers et al., 2011; Cooper, 2017).

Environmental conditions are considered predictors of several ecological aspects of lizards, such as thermoregulation, foraging, diet, growth rate, reproduction, and life cycle (Ribeiro et al., 2012; Cooper, 2017; Ferreira et al., 2017; Sannolo et al., 2020). However, the link between the climatic conditions reported at the time of capture may have but tenue link with the current physiological state of animals. In this study, field water balance was not related to climatic variables, and this suggests that immediate climatic data may fail to capture the full range of ecological impacts of climate on the hydric state. Similar observations by Dupoué et al. (2017) for the lizard *Zootoca* *vivipara* (Lichtenstein, 1823) indicated that the environmental humidity did not relate to water balance, regardless of sex. Nevertheless, given the expected link between current and past climate conditions, lizards may maintain water balance via behavioral changes at time scales of a few days, despite climate-induced variation in water loss rates and water availability. Also, the field water balance of the *T. cocorobensis* species was negatively correlated with soil temperature, meaning that the higher the soil temperature, the more hydrated lizards remained. Perhaps the soil temperature is related to the choice of body preferred temperatures, for example, similar to that observed in the laboratory by Sannolo and Carretero (2019), where *Podarcis* lizards in water déficit arranged in a gradient of different substrate temperatures, "chose" lower temperatures.

Herein, we used species with some distinct ecological characteristics, highlighting the foraging mode. Generally, actively foraging lizards have higher energy expenditure metabolic rates than sit-and-wait species (Huey and Pianka, 1981; Nagy et al., 1984). It is reasonable to hypothesize that active foraging lizards also have greater rates of water loss and perhaps an enhanced risk of dehydration. The active lizard *A. ocellifera* (active forager) has higher water unbalance compared to *T. cocorobensis* (sit-and-wait), a finding that corroborates the proposed relationship between foraging mode and water balance, at least in a Caatinga scenario. However, this is a case study with two species, and do not suffice to assume it as a pattern, even more, given contrasting results by Nagy et al. (1984), who compared two species in the same genus (*Eremias*) and with different foraging modes, and the active species had a higher body water content.

After the induced hydration experiment, the two species absorbed an average amount of water. Both *A. ocellifera* and *T. cocorobensis* may experience challenges in keeping water balance during the dry season in their semi-arid environment. We did not address tolerance to dehydration or test impacts of dehydration on performance, so we have no information about how critical the field states here were reported. However, maintaining a water balance is generally important for lizards (Lorenzon et al., 1999; Marquis et al., 2008; Zylstra et al., 2013), and dehydration has known ecological consequences. Wilson et al., for example, concluded that dehydration reduces the running ability of *Uta stansburiana* Baird & Girard, 1852 lizards. Water balance also affects the growth rate in lizards (Lorenzon et al., 1999) and interferes with the oxidative status of pregnant females, decreasing reproductive output (Dupoué et al., 2020b). Completely, the physiological influences involve increasing defense mechanisms with reactivity to stress and plasma concentrations of glucocorticoids, generating a possible suppression of the lizards' immune function (Moeller et al., 2017). Thus, for lizards, investing in strategies that prevent dehydration is a way to maintain a population and offspring's health.

Moisture in Stomach Content Samples

The humidity content of dietary items is an indirect measure of the inclination of lizards to enhance water uptake. In this study, *A. ocellifera* had higher proportional dietary moisture than *T.*

cocorobensis, despite reports about the diet similarity between these two species (Muniz and Freire, 2015; Oliveira et al., in prep). Foraging modes unambiguously differ, and perhaps the more active *A. ocellifera*, despite an overall tendency to consume similar items, is better suited to select given types of food providing more water. Active foraging lizards tend to display higher feeding rates than sit-and-wait counterparts, as they can forage in a larger area and are more likely to find different types of prey (Nagy et al., 1984).

This feature can work as an interesting strategy to get water through the diet. Water consumption in reptiles is made largely from the water available in the environment. However, water may not be available all year round in arid and semi-arid environments (Golightly e Ohmart, 1984; Nagy e Medica, 1986). When we highlighted, for example, the possible influence of a drier season on the reptiles feeding, we observed that lizard species often change the composition of their diet according to the dry and wet season (Znari e Nagy, 1997; Henen et al., 1998; Ribeiro et al., 2011; Ferreira et al., 2017). On the other hand, Wright et al. (2013) observed that the consumption of a meal did not meet the water needs and did not generate a water balance in the Gila monsters *Heloderma suspectum* (Cope, 1869) and also concluded that the consumption of meals entails a long-term water cost. Many vertebrate species with a herbivorous diet depend on dietary water for water balance (Wright et al., 2013), so we might think that the type of diet interferes with the effectiveness of meal consumption on water balance. In addition to the diet composition, it is necessary to understand metabolic rates, how food is processed by the body, what the species' energy needs are, and other physiological parameters. Despite having ingested, proportionally, a more humid diet, we found that *A. ocellifera* continued in a state of greater dehydration than that of *T. cocorobensis*. However, we still cannot say that for this species, the consumption of a meal is not enough for a water balance, mainly because the total dependence on free water in species from xeric environments is not common (Nagy et al., 1984; Nagy and Medica, 1986; Green et al., 1991).

Both species in our study are mainly insectivorous (Muniz and Freire, 2015; Oliveira et al., *in prep*), but *A. ocellifera* (at least in our study area) consumes comparably more termites (This study, Chapter 1). Most termite species in Brazil provide between 66 and 80% water to your predators (Redford and Dorea, 2009). In contrast, *T. cocorobensis* consume mainly ants, and those items provide only 45 to 70% of water (Redford and Dorea, 2009). Naturally, dietary choosing depends on availability, and the Caatinga environment influences social insects' distribution (Ferreira et al., 2017). Lizards may eat what is available, and whether the observed pattern reflects active selection will be formally tested. However, some species of the genus *Tropidurus*, including *T. cocorobensis*, consume some plant matter when in dry environments, such as Caatinga and Restinga (Fonseca et al., 2012; Gomes et al., 2014; Koski et al., 2018), and it has been hypothesized that the inclusion of fruits relates to their the water content (Fialho et al., 2000; Lima and Rocha, 2006). More studies on feeding ecology and metabolic rates of these and other species in semiarid environments would be important to understand the links between food selection and water balance in lizards.

Conclusions

This study brings us pioneering information about the field water balance of *Ameivula ocellifera* and *Tropidurus cocorobensis* in the semiarid Caatinga settings. Despite being syntopic, the field water balance of *A. ocellifera* was lower than that of *T. cocorobensis*, a trait likely integrating physiological, ecological, and evolutionary aspects. The field water balance of *T. cocorobensis* was related to soil temperature, which suggests a strategy to reduce water loss. In parallel, *A. ocellifera* consumes a proportionally more humid diet, which may be part of a behavioral strategy to compensate for water loss. Our results concerning field water balance show that we still cannot say whether the values we found are sufficient to generate some of the known consequences of dehydration, so this point still needs to be explored further through future research. We cannot state whether or not the diet is capable of helping the species' water balance either. We recommend further studies involving the metabolic rates of *A. ocellifera* and *T. cocorobensis* to better interpret the data.

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Table 1. Mean and standard deviation of SVL, body temperature, body weight (before and after induced hydration). Also the difference between these body masses and the data from the Non-parametric Wilcoxon Test. Differences in body mass are expressed both in grams and in percentages. Values are rounded to the second decimal value.

			Body mass (g)				
Species	**SVL (mm)**	**Body temperature (°C)**	**Before hydration**	**After hydration**	**Difference g (%)**	***v-value***	***P***
Ameivula ocellifera	66.43 ± 13.75	36.21 ± 2.51	9.11 ± 6.27	9.38 ± 6.34	0.27 (3.6%)	430	< 0.0001
Tropidurus cocorobensis	71.32 ± 10.06	34.32 ± 2.60	14.00 ± 6.18	13.89 ± 6.01	0.11 (1.6%)	1060	< 0.0001

Table 2. Mean and standard deviation of wet and dry weight of moisture in stomach content samples. Also the difference between these weights and the data from the Non-parametric Wilcoxon Test. Differences in weight are expressed both in grams and in percentages. Values are rounded to the second decimal value.

	Moisture in Stomach Content Samples (g)					
Species	**Wet Weight**	**Dry Weight**	**Difference**	**Difference %**	***v-value***	***P***
Ameivula ocellifera	0.84 ± 0.63	0.27 ± 0.35	0.57	75.19%	406	< 0.0001
Tropidurus cocorobensis	1.14 ± 0.38	0.33 ± 0.32	0.81	71.96%	946	< 0.0001

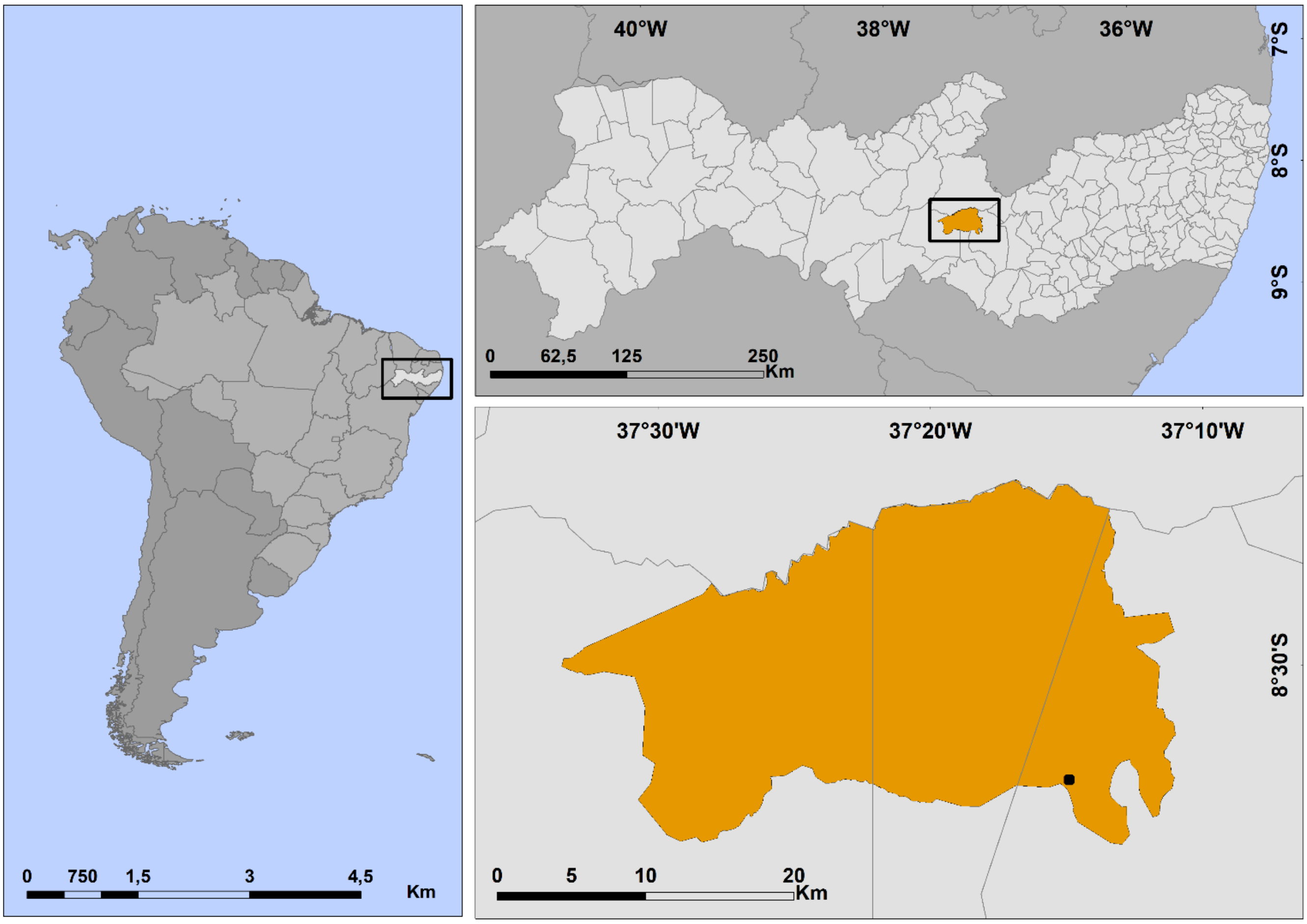

Figure 1. Map with the location of Catimbau National Park. The black dot represents the collection area.

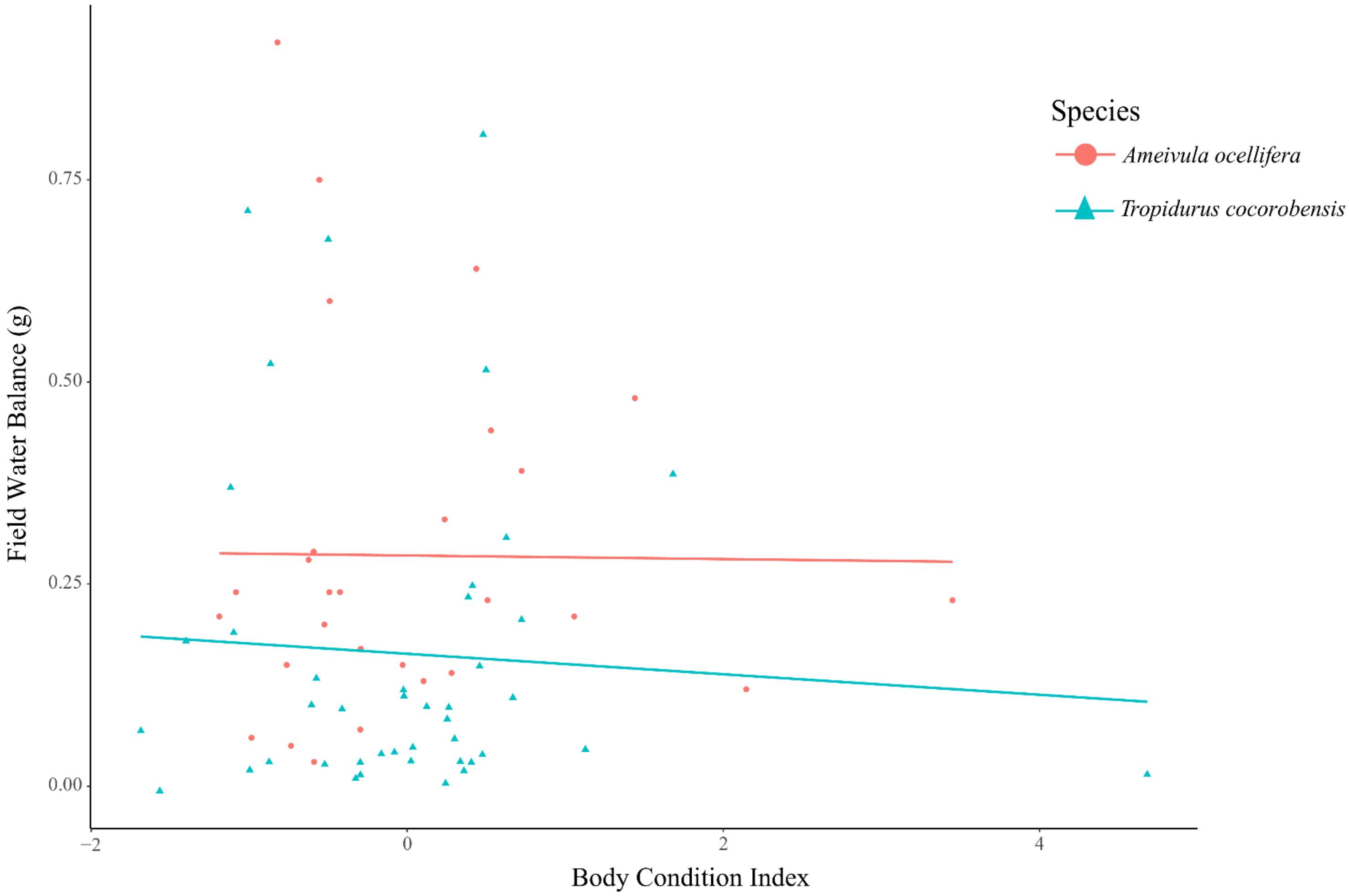

Figure 2. Scatter plot relating the Field water balance (in grams, gross value) with the Body Condition Index of the species *Ameivula ocellifera* and *Tropidurus cocorobensis* from Parque Nacional do Catimbau, PE, Brazil. The pink dots correspond to the species *Ameivula ocellifera*, and the pink line represents the straight line of the simple linear regression of the sample of this species. The blue triangles correspond to the species *Tropidurus cocorobensis*, and the blue line represents the straight line of the simple linear regression of the sample of this species.

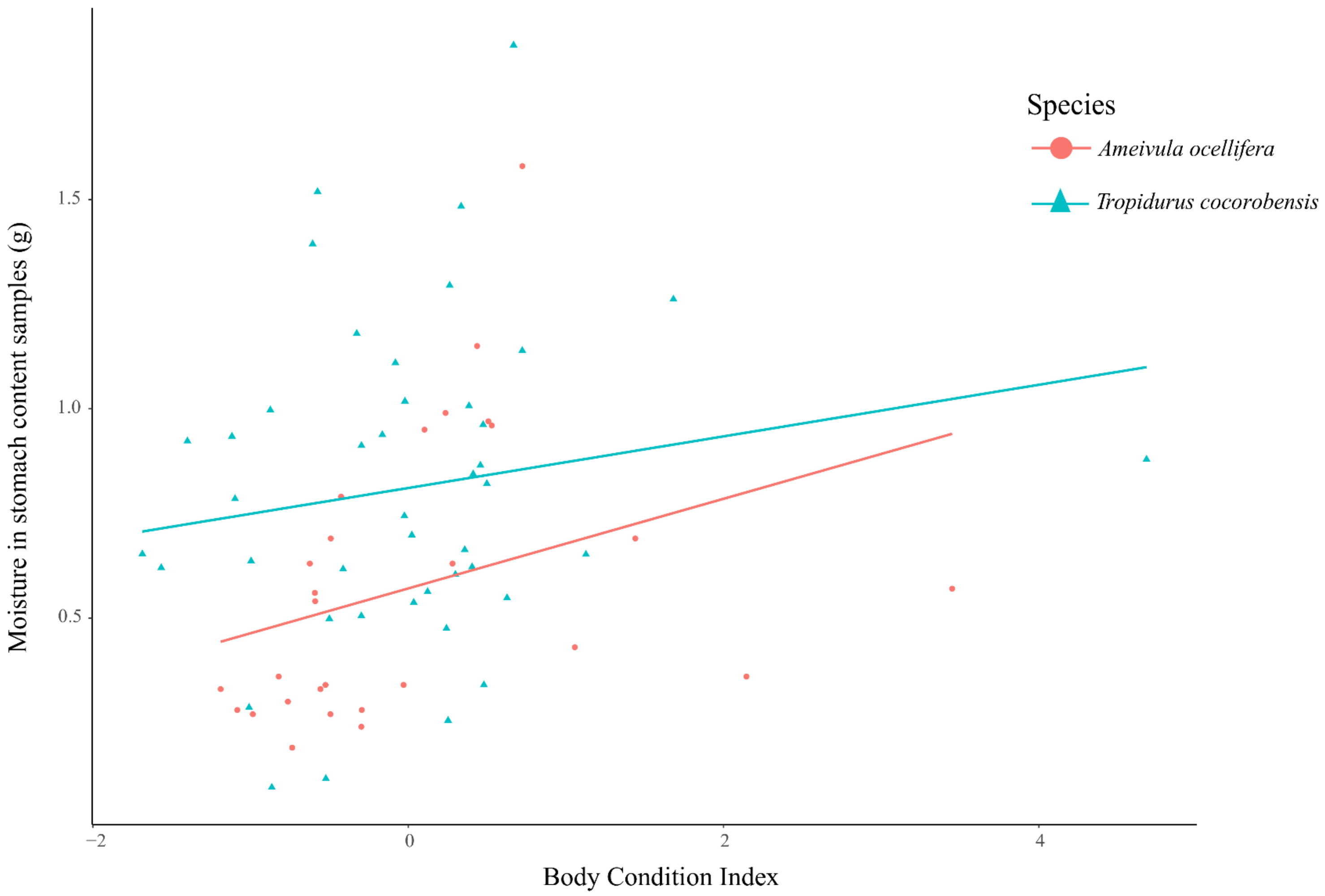

Figure 3. Dispersion graph relating moisture in stomach content samples (in grams, gross value) with the Body Condition Index of the species *Ameivula ocellifera* and *Tropidurus cocorobensis* from Parque Nacional do Catimbau, PE, Brazil. The pink dots correspond to the species *Ameivula ocellifera*, and the pink line represents the straight line of the simple linear regression of the sample of this species. The blue triangles correspond to the species *Tropidurus cocorobensis*, and the blue line represents the straight line of the simple linear regression of the sample of this species.

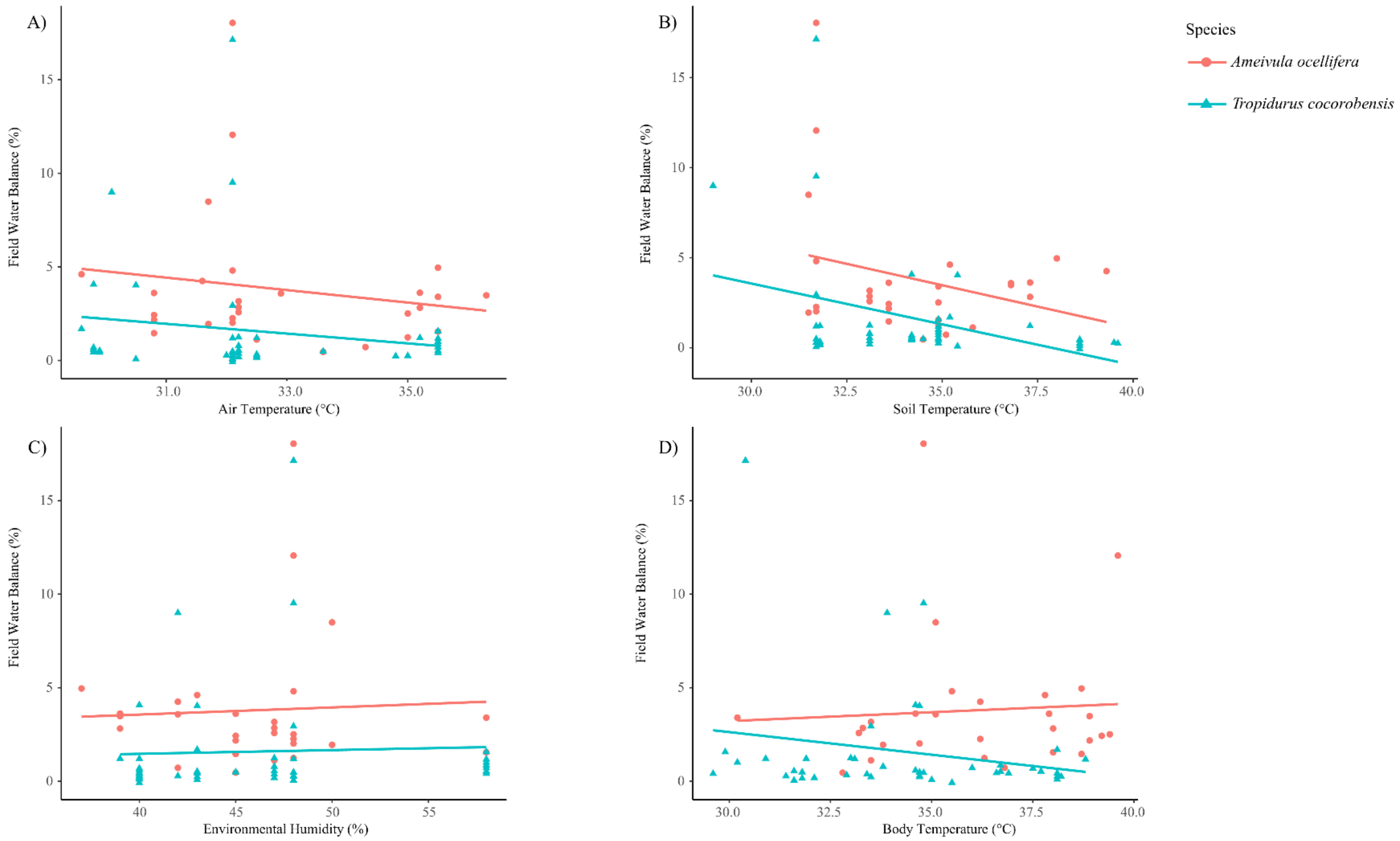

Figure 4. A) Scatter plot relating the Relative Field water balance (in percentage) with the air temperature at the collection site. B) Scatter plot relating the Relative Field water balance (in percentage) with the soil temperature at the collection site. C) Scatter plot relating the Relative Field water balance (in percentage) with the humidity at the collection site. D) Scatter plot relating the Relative Field water balance (in percentage) with the specimens' body temperature. The pink dots correspond to the species *Ameivula ocellifera*, and the pink line represents the straight line of the simple linear regression of the sample of this species. The blue triangles correspond to the species *Tropidurus cocorobensis*, and the blue line represents the straight line of the simple linear regression of the sample of this species.

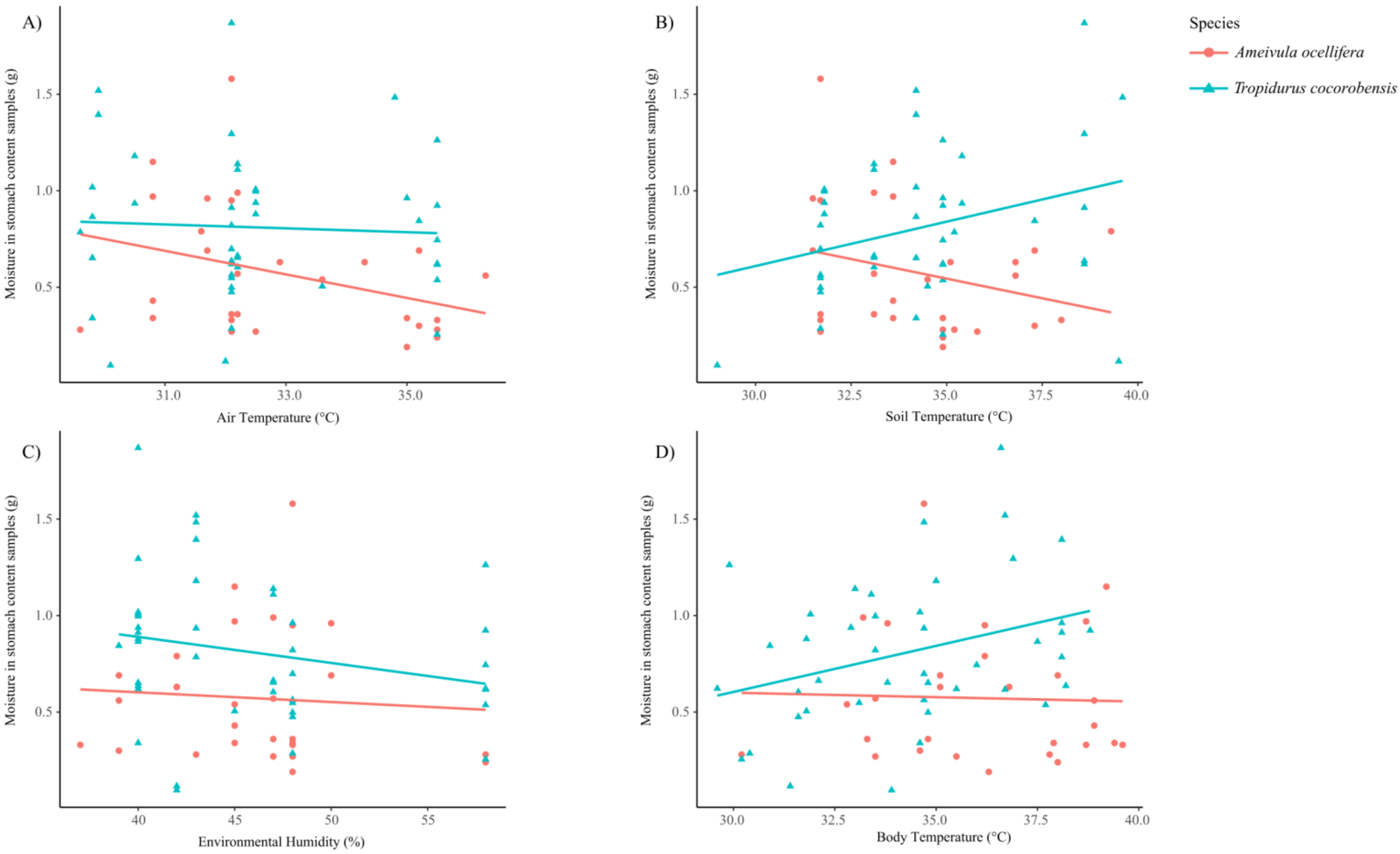

Figure 5. A) Scatter plot relating the moisture in stomach content samples (in grams, gross value) with the air temperature at the collection site. B) Scatter plot relating moisture in stomach content samples (in grams, gross value) with the soil temperature at the collection site. C) Scatter plot relating moisture in stomach content samples (in grams, gross value) with the humidity at the collection site. D) moisture in stomach content samples (in grams, gross value) with the specimens' body temperature. The pink dots correspond to the species *Ameivula ocellifera*, and the pink line represents the straight line of the simple linear regression of the sample of this species. The blue triangles correspond to the species *Tropidurus cocorobensis*, and the blue line represents the straight line of the simple linear regression of the sample of this species.

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4. CONCLUSÃO

Através dos estudos apresentados concluímos que os resultados aqui expostos contribuem para: o entendimento da ecologia trófica de duas espécies de lagartos da Caatinga que dividem o mesmo microhabitat e para o planejamento de medidas mitigatórias acerca das mudanças climáticas. O primeiro capítulo nos trouxe informações importantes sobre a influência do modo de forrageio na composição dos itens a serem predados e mostrou que, apesar de dividirem o mesmo ambiente e os mesmos recursos alimentares, a sobreposição de nicho trófico pode ser baixa, pois a utilização desses itens difere entre as espécies. A caracterização da dieta de lagartos juntamente com a interação e uso do habitat pelas espécies é fundamental para o entendimento das relações tróficas, principalmente no entendimento de como fatores da história natural dos lagartos podem interferir em seus papéis na dinâmica trófica do ecossistema. Também concluímos que nossos resultados acerca do balanço hídrico de campo trazem informações pioneiras sobre a exposição de lagartos da Caatinga à um déficit hídrico. Por estarmos tratando de resultados primários, se faz necessária a exploração do assunto em trabalhos futuros. Acreditamos que a medida do balanço hídrico de campo durante a estação chuvosa da Caatinga pode servir como uma amostra de controle para comparação com os resultados obtidos na estação seca. Além disso, a atenção de futuros estudos com relação às consequências da possível desidratação em funções ecológicas também é interessante. Por fim, recomendamos também uma exploração de pesquisas envolvendo a taxa metabólica dessas espécies, para assim conseguirmos respostas mais concretas quanto à eficiência da ingestão de itens na manutenção de um balanço hídrico das espécies estudadas.

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APPENDIX 1. All specimens examined and their respective vouchers.

Ameivula ocellifera: BRAZIL: Pernambuco: Parque Nacional do Catimbau, Buíque. PMSN 1758, 1759, 1760, 1774, 1806, 1807, 1808, 1809, 1810, 1811, 1813, 1814, 1815, 1816, 1817, 1829, 1832, 1834, 1835, 1840, 1842, 1843, 1846, 1848, 1854, 1857, 1859, 1862, 1865, 1866, 1867.

Tropidurus cocorobensis: BRAZIL: Pernambuco: Parque Nacional do Catimbau, Buíque. PMSN 1761, 1762, 1764, 1765, 1766, 1767, 1768, 1769, 1770, 1771, 1772, 1773, 1775, 1776 1777, 1778, 1779, 1780, 1781, 1782, 1789, 1790, 1791, 1794, 1795, 1805, 1830, 1827, 1828, 1837, 1838, 1839, 1841, 1844, 1845, 1847, 1849, 1850, 1851, 1852, 1855, 1856, 1858, 1860, 1861, 1863, 1864.

APPENDIX 2. Table with raw data collected from the *Ameivula ocellifera* species. The table includes: PMSN acronym voucher corresponding to the specimen field label, body temperature, Snout vent length (SVL), Natural body weight (BM1), Mean body weight 15 and 30 minutes after induced hydration (BM 3/4) and moisture in diet (Wet weight - Dry weight of stomach contents).

Voucher - PMSN	Specie	Body Temp. (°C)	SVL (mm)	BM 1 (g)	BM 3/4 (g)	Hydration State (g)	Moisture in diet (g)
1758	*A. ocellifera*	35.1	69.38	7.80	8.08	0.28	0.63
1759	*A. ocellifera*	36.2	60.77	5.64	5.87	0.24	0.79
1760	*A. ocellifera*	36.8	90.4	19.87	20.01	0.14	0.63
1774	*A. ocellifera*	37.8	61.36	5.16	5.39	0.24	0.28
1806	*A. ocellifera*	36.3	51.89	3.64	3.68	0.05	0.19
1807	*A. ocellifera*	39.4	60.86	6.17	6.32	0.15	0.34
1808	*A. ocellifera*	33.5	61.61	5.27	5.32	0.06	0.27
1809	*A. ocellifera*	38.9	71.27	8.27	8.56	0.29	0.56
1810	*A. ocellifera*	35.1	66.72	7.05	7.65	0.60	0.69
1811	*A. ocellifera*	33.8	92.73	22.48	22.92	0.44	0.96
1813	*A. ocellifera*	38.7	81.34	15.57	15.80	0.23	0.97
1814	*A. ocellifera*	37.9	60.84	5.52	5.72	0.20	0.34
1815	*A. ocellifera*	38.5	86.66	17.79	17.06	-0.73	1.13
1816	*A. ocellifera*	38.9	64.64	9.64	9.85	0.21	0.43
1817	*A. ocellifera*	39.2	100.62	26.40	27.04	0.64	1.15
1829	*A. ocellifera*	34.3	57.32	5.34	5.17	-0.17	0.37
1832	*A. ocellifera*	39.6	63.72	6.21	6.96	0.75	0.33
1834	*A. ocellifera*	34.8	60.82	5.10	6.02	0.92	0.36
1835	*A. ocellifera*	39.6	73.22	9.64	9.52	-0.12	0.55
1840	*A. ocellifera*	34.7	86.84	19.35	19.74	0.39	1.58
1842	*A. ocellifera*	35.5	57.37	4.93	5.16	0.24	0.27
1843	*A. ocellifera*	36.2	57.29	5.60	5.72	0.13	0.95
1846	*A. ocellifera*	30.2	57.17	5.11	5.29	0.17	0.28
1848	*A. ocellifera*	38	54.29	4.25	4.32	0.07	0.24
1854	*A. ocellifera*	33.3	43.8	4.20	4.32	0.12	0.36
1857	*A. ocellifera*	33.2	77.25	12.85	13.19	0.33	0.99
1859	*A. ocellifera*	33.5	48.08	7.26	7.49	0.23	0.57
1862	*A. ocellifera*	32.8	61.25	5.72	5.75	0.03	0.54
1865	*A. ocellifera*	38	78.46	17.10	17.58	0.48	0.69
1866	*A. ocellifera*	34.6	55.3	4.12	4.27	0.15	0.30
1867	*A. ocellifera*	38.7	57.32	4.22	4.43	0.21	0.33

APPENDIX 3. Table with raw data collected from the *Tropidurus cocorobensis* species. The information in the table includes: PMSN acronym voucher corresponding to the specimen field label, body temperature, Snout vent length (SVL), Natural body weight (BM1), Mean body weight 15 and 30 minutes after induced hydration (BM 3/4) and moisture in diet (Wet weight - Dry weight of stomach contents).

Voucher - PMSN	Specie	Body Temp. (°C)	SVL (mm)	BM 1 (g)	BM 3/4 (g)	Hydration State (g)	Moisture in diet (g)
1761	*T. cocorobensis*	31.40	65.61	9.58	9.60	0.03	0.12
1762	*T. cocorobensis*	33.90	56.47	5.81	6.33	0.52	0.10
1764	*T. cocorobensis*	34.60	79.98	19.80	20.60	0.81	0.34
1765	*T. cocorobensis*	34.80	62.62	10.02	10.07	0.05	0.65
1766	*T. cocorobensis*	35.00	67.04	11.21	11.19	-0.02	0.61
1767	*T. cocorobensis*	34.60	80.62	19.16	19.27	0.11	1.02
1768	*T. cocorobensis*	37.50	82.24	21.51	21.66	0.15	0.87
1769	*T. cocorobensis*	31.90	79.71	19.38	19.62	0.23	1.01
1770	*T. cocorobensis*	31.80	53.94	9.34	9.35	0.01	0.88
1771	*T. cocorobensis*	31.20	65.56	8.84	8.81	-0.03	0.64
1772	*T. cocorobensis*	32.90	69.95	12.16	12.20	0.04	0.94
1773	*T. cocorobensis*	33.50	73.16	12.87	12.90	0.03	1.00
1775	*T. cocorobensis*	32.90	82.32	19.26	19.14	-0.11	0.86
1776	*T. cocorobensis*	38.10	70.6	11.23	11.42	0.19	0.79
1777	*T. cocorobensis*	38.20	69.91	11.46	11.42	-0.04	0.81
1778	*T. cocorobensis*	38.10	87.79	23.34	23.44	0.10	1.39
1779	*T. cocorobensis*	36.70	90.31	25.57	25.70	0.13	1.52
1780	*T. cocorobensis*	34.70	66.16	9.17	9.54	0.37	0.93
1781	*T. cocorobensis*	35.00	70.23	12.08	12.09	0.01	1.18
1782	*T. cocorobensis*	34.70	70	12.91	12.94	0.03	1.48
1789	*T. cocorobensis*	38.20	63.24	8.10	8.12	0.02	0.64
1790	*T. cocorobensis*	35.50	64.67	8.12	8.12	-0.01	0.62
1791	*T. cocorobensis*	38.10	72.63	13.45	13.46	0.01	0.91
1794	*T. cocorobensis*	36.90	84.43	22.82	22.91	0.10	1.30
1795	*T. cocorobensis*	36.60	84.97	24.36	24.47	0.11	1.87
1805	*T. cocorobensis*	38.10	74.23	15.72	15.76	0.04	0.96
1830	*T. cocorobensis*	30.40	50.85	4.15	4.86	0.71	0.29
1827	*T. cocorobensis*	34.80	59.51	7.11	7.79	0.68	0.50
1828	*T. cocorobensis*	33.50	76.81	17.52	18.03	0.52	0.82
1837	*T. cocorobensis*	33.10	86.61	25.71	26.02	0.31	0.55
1838	*T. cocorobensis*	31.60	58.67	7.40	7.41	0.00	0.48
1839	*T. cocorobensis*	34.70	81.87	20.42	20.52	0.10	0.56
1841	*T. cocorobensis*	34.70	66.6	10.68	10.71	0.03	0.70
1844	*T. cocorobensis*	29.90	82.08	24.59	24.97	0.39	1.26
1845	*T. cocorobensis*	30.20	60.72	8.24	8.33	0.08	0.26
1847	*T. cocorobensis*	29.60	57.8	7.20	7.23	0.03	0.62
1849	*T. cocorobensis*	37.70	63.27	9.13	9.18	0.05	0.54
1850	*T. cocorobensis*	36.70	68.61	11.13	11.23	0.10	0.62
1851	*T. cocorobensis*	36.00	77.03	16.64	16.76	0.12	0.74
1852	*T. cocorobensis*	38.80	78.82	15.25	15.43	0.18	0.92

1855	*T. cocorobensis*	32.10	64.65	10.13	10.15	0.02	0.66
1856	*T. cocorobensis*	33.40	67.68	11.09	11.13	0.04	1.11
1858	*T. cocorobensis*	31.60	65.94	10.69	10.75	0.06	0.60
1860	*T. cocorobensis*	33.80	66.42	8.69	8.76	0.07	0.65
1861	*T. cocorobensis*	33.00	74.82	16.58	16.79	0.21	1.14
1863	*T. cocorobensis*	31.80	56.08	6.06	6.09	0.03	0.51
1864	*T. cocorobensis*	30.90	81.14	20.53	20.78	0.25	0.84

APPENDIX 4. Body Condition Index values for each specimen of *Ameivula ocellifera* and *Tropidurus cocorobensis* analyzed in the study.

Specie	Body Condition Index	Specie	Body Condition Index
A. ocellifera	-0.623460251	*T. cocorobensis*	-0.521823747
A. ocellifera	-0.425041144	*T. cocorobensis*	-0.86442014
A. ocellifera	0.281116539	*T. cocorobensis*	0.480414263
A. ocellifera	-1.083637852	*T. cocorobensis*	1.127275078
A. ocellifera	-0.735094882	*T. cocorobensis*	-0.020500767
A. ocellifera	-0.029087834	*T. cocorobensis*	0.458012821
A. ocellifera	-0.984781269	*T. cocorobensis*	0.385877644
A. ocellifera	-0.591599178	*T. cocorobensis*	4.681754297
A. ocellifera	-0.490378196	*T. cocorobensis*	-0.163779062
A. ocellifera	0.529943301	*T. cocorobensis*	-0.87379807
A. ocellifera	0.50863441	*T. cocorobensis*	-1.097770925
A. ocellifera	-0.524029471	*T. cocorobensis*	-0.605608653
A. ocellifera	1.056802803	*T. cocorobensis*	-0.574436822
A. ocellifera	0.43721186	*T. cocorobensis*	-1.117243655
A. ocellifera	-0.556061715	*T. cocorobensis*	-0.326008101
A. ocellifera	-0.820994453	*T. cocorobensis*	0.336002612
A. ocellifera	0.723894307	*T. cocorobensis*	-0.99585879
A. ocellifera	-0.492860957	*T. cocorobensis*	-1.565615633
A. ocellifera	0.103567871	*T. cocorobensis*	-0.295948542
A. ocellifera	-0.294051348	*T. cocorobensis*	0.263362265
A. ocellifera	-0.296718157	*T. cocorobensis*	0.668523598
A. ocellifera	2.144968828	*T. cocorobensis*	0.475584335
A. ocellifera	0.236692902	*T. cocorobensis*	-1.008634555
A. ocellifera	3.449477721	*T. cocorobensis*	-0.49955351
A. ocellifera	-0.589354939	*T. cocorobensis*	0.499105293
A. ocellifera	1.44040593	*T. cocorobensis*	0.627129717
A. ocellifera	-0.762483819	*T. cocorobensis*	0.242832417
A. ocellifera	-1.18869459	*T. cocorobensis*	0.123318487
-	-	*T. cocorobensis*	0.023510974
-	-	*T. cocorobensis*	1.681805184
-	-	*T. cocorobensis*	0.253198679
-	-	*T. cocorobensis*	0.405516142
-	-	*T. cocorobensis*	0.035436079
-	-	*T. cocorobensis*	-0.412349276
-	-	*T. cocorobensis*	-0.023771361
-	-	*T. cocorobensis*	-1.399513871
-	-	*T. cocorobensis*	0.358906353
-	-	*T. cocorobensis*	-0.08083813
-	-	*T. cocorobensis*	0.299603443
-	-	*T. cocorobensis*	-1.685374458
-	-	*T. cocorobensis*	0.723518196
-	-	*T. cocorobensis*	-0.296808857
-	-	*T. cocorobensis*	0.412532561

ANEXO 1 - INSTRUCTIONS FOR AUTHORS: SOUTH AMERICA JOURNA OF HERPETOLOGY

General information

The South American Journal of Herpetology (SAJH) is an international journal published by the Brazilian Society of Herpetology that aims to provide an effective medium of communication for the international herpetological community. SAJH publishes peer-reviewed original contributions on all subjects related to the biology of amphibians and reptiles, including descriptive, comparative, inferential, and experimental studies and taxa from anywhere in the world, as well as theoretical studies that explore principles and methods. Beginning with volume 13 (2018), SAJH is published exclusively online through BioOne.

Manuscript submission and evaluation

All manuscripts must be submitted through the SAJH Peer Track System. Manuscripts are considered on the understanding that authors have complied with the SAJH Ethics Policy.

The criteria for acceptance of articles are research excellence, text clarity, figure quality, and compliance with the guidelines for manuscript preparation. Manuscripts that do not comply with these guidelines will be returned to authors without peer review. Submissions are assigned to Associate Editors who seek at least two peer reviews. Associate Editors then submit reviews and recommendations to the Senior Editor for final decision. All communication regarding manuscripts is made through electronic correspondence with the corresponding author only.

Special issues

Recognizing the high demand to publish longer, monographic studies, SAJH will consider manuscripts of approximately 125–350 pages (body of text and references, A4 format paper, double-spaced typescript, with 2.5 cm margins) to be published individually as Special Issues. Only a limited number of Special Issues will be published, so authors must contact the Senior Editors for approval prior to submission. Special Issue manuscripts are subjected to the same standards of peer review as regular manuscripts. Begning with volume 13 (2018), page charges will no longer be applied for Special Issues.

Proofs

Page-proofs will be sent electronically to the corresponding author. Page-proofs must be returned within 72 hours. Authors that are unable to meet this deadline must immediately request an extension, which will be granted at the Editor's discretion depending on production schedule; failure to return the proof within the allotted time will be interpreted as approval with no changes. Only necessary corrections will be permitted. Once page proofs are sent to the author, further alterations and/or significant additions of text are permitted only at the author's expense or in the form of a brief appendix ("Note added in proof").

Cover letter

In addition to any details authors believe the editors should be aware of when processing the ms, the cover letter must include a brief paragraph on the importance of the contribution to the field. In brief, why is it important for this manuscript to be published in SAJH? What problems does it solve? Of what relevance will it be to future studies?

Manuscript preparation

Authors are required to pay close attention to the instructions for manuscript preparation. Manuscripts that do not follow these instructions will be returned without review. Figures must be uploaded separately and not imbedded in the text file, although authors are encouraged to include call-outs in the text to identify preferred locations for figures and tables.

All manuscripts must be written in English using US spelling and grammar conventions. These conventions often differ greatly from Spanish and Portuguese conventions, so we strongly encourage authors to consult appropriate references (e.g., The Chicago Manual of Style, 16th Edition) to ensure proper use and placement of punctuation (especially hyphens, En dashes, Em dashes, commas, semicolons, colons, and periods) and capitalization.

In general, write out single digit numbers (e.g., one, seven, nine) and use numerals for higher numbers, with four exceptions: (1) Write out any number that begins a sentence. (2) Use numerals for calendar years (e.g., 1997). (3) Use numerals for measurements (e.g., 6 mm), mathematical symbols (e.g., < 9 words, 8%), or given as ranges (e.g., 1–20). (4) Use numerals when numbers refer to the same category (e.g., I read 14 papers on Monday and 4 on Tuesday). Use a decimal point (not comma) to separate the integer part of a number from from the fractional part. For numbers of four or more digits (except calendar years), place a comma every three digits from the right (not counting decimals; e.g., 1,045.25 m)

Be consistent when using anatomical and other technical terms for which usage is not entirely standardized (e.g., use "middorsal" or "mid-dorsal," but not both). Although all accepted manuscripts are subjected to thorough English revision prior to publication, submissions that do not meet minimal language requirements to allow evaluation of their scientific content will be returned without peer review. As such, non-native speakers are encouraged to have their manuscripts checked by a native speaker (or equivalent) prior to submission, as this will facilitate review and prevent delays.

All manuscripts must follow the International Code of Zoological Nomenclature and relevant specimens should be properly curated and deposited in recognized natural history collections. Tissue samples should be referred to their voucher specimens. Voucher collection data should be provided in an appendix or occasionally in the text. GenBank or EMBL accession numbers for all DNA sequence data are required for publication.

The scientific name of all species must be given and should be used preferentially in the text, although common names can be given as well. Scientific names must be written according to standard zoological practice (e.g., genus- and species-group names italicized, authorship in parentheses if the species is currently referred to a different genus than the one in which it was described). Authorship and year of publication must be included with all taxon names mentioned in the text (e.g., Colostethus Cope, 1866) and the respective publication must be included in the References section. Unless warranted by special taxonomic considerations, authorship and year should be provided only once in the text, preferably the first time each taxon is mentioned. Alternatively, in articles that address many species and authorship and year would decrease readability, the complete taxonomic references may be provided in an associated table. Authorship of taxa that contain more than two authors must be cited as the first author + et al. (e.g., Adelphobates Grant et al., 2006). Nomenclatural acts must be identified throughout the text, either written in full or using conventional abbreviations, and highlighted using bold (e.g., Genus species sp. nov., Genus species syn. nov.). Common names are not capitalized, although proper names that form part of the common name are (e.g., Cuvier’s dwarf caiman).

The International System of Units (SI) and corresponding symbols should be used to report all measurements (for overview see The International System of Units and the NIST Guide for for the use of the International System of Units), except when this would lead to unnecessary complications. For example, there are standard symbols for minute (min), hour (h), day (d), and year (a, not yr), but not week or month; such non-standard units may be used, but should always be spelled out. Standard geographic coordinates must be written without spaces between numbers and symbols (e.g., 38°57′56.4″N, 95°13′35.9″W) and the corresponding datum should be reported. Standard statistics should be reported as follows: n (sample size), t (t-test statistic), X (sample mean; this will be replaced with the x-bar symbol in production), SD (standard deviation), SE (standard error), r, r2 (Pearson product-moment correlation), R2 (coefficient of determination from regression analysis), P (probability), df (degrees of freedom), and $X2$ (chi-square). Mathematical operators must be separated by a space (e.g., n = 20; 45 ± 1.2). Standard Latin terms and abbreviations, such as ca., cf., e.g., i.e., et al., sp. nov., gen. nov., vs., etc., sensu stricto (s.s.), and sensu lato (s.l.), should not be italicized. All other acronyms and abbreviations must be defined on first mention or in the Materials and Methods section. Dates must be reported as numeric day, full month name, full numeric year (e.g., 18 March 2011) and time of day must use the 24-hour system (e.g., 14:01 h instead of 2:01 p.m.).

Manuscripts must be submitted in Word document format (i.e., doc, docx). All pages must be numbered consecutively. All text (including title page, references, and tables) must be double-spaced and include consecutive line numbers. Text must be left-adjusted and headings must follow specific instructions (see below). Article should be arranged in the following order:

Title page

Abstract

Body of text

Acknowledgments

References

Online supporting information

Tables

Figure captions

The body of text and references should not exceed 40 pages of A4 format paper, double-spaced typescript, with 2.5 cm margins. Authors of longer manuscripts should contact the Editors prior to submission (see also instructions for Special Issues, above).

Title page

This should include the article title and author names and addresses (including email addresses). Article titles should use headline-style capitalization, be concise, and, where appropriate, include names of higher taxa, but they should not include names of new taxa. Names of institutions should be written in full (e.g., Consejo Nacional de Investigaciones Científicas y Técnicas, not CONICET). Multi-author manuscripts must identify the corresponding author and his/her postal and email addresses. For example:

Strengthening Population Inference in Herpetofaunal Studies by Addressing Detection Probability

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3 Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, Estado de México, México.

* Corresponding author. Email: mu.guima@gmail.com

Abstract page

All papers must include an abstract in English of at most 350 words (700 for Special Issues). It should provide a concise summary of the study's objectives, methods, main results, and conclusions. Bibliographic references and new taxon names should not appear in the abstract and abbreviations should be avoided.

Following the abstract, 3–8 keywords must be provided for indexing. Keywords must be arranged in alphabetical order, separated by a semicolon and must not include new taxon names or words already in the title; the first word of every set of keywords must be capitalized.

Authors may also provide a Spanish (Resumen) or Portuguese (Resumo) translation following the keywords. Keywords in Portuguese and Spanish are not permitted.

Body of text

The main body of the text should normally include the following sections: Introduction, Materials and Methods, Results, and Discussion. Primary headings should be in all capital letters, centered, and bold face; the following text should begin on the next line, indented. Secondary headings should use sentence case capitalization and be centered and bold face; the following text should begin on the next line, indented. Tertiary headings should use sentence case capitalization and be flush left and bold face; the following text should begin on the next line, indented. Quaternary headings should use sentence case capitalization, be indented, use bold face italics, and be followed by a period; the following text should be on the same line.

Literature citations in the text must be arranged in chronological order first and alphabetical order second, separated by semi-colon. Citations from the same author(s) and year must be identified by letters, in the text and references, as in the examples below.

Citations in the text should be given as: Silva (1998)…, Silva (1998:14–20)…, Silva (1998: figs. 1, 2)…, Silva (1998a, b)…, Silva and Oliveira (1998)…, (Silva, 1998)…, (Rangel, 1890; Silva and Oliveira, 1998a, b; Adams, 2000)…, (see Silva, 1998, and references therein)…, (H. R. Silva, pers. comm.)…, and Silva et al. (1998) or (Silva et al., 1998) for more than two authors. Adjacent parentheses should be avoided: "… absence of postmalars (present; Fig. 3)," not "… absence of postmalars (present) (Fig. 3)." Exceptions are permissible in special situations, such as when parentheses required by the ICZN: Tropidurus hispidus (Spix, 1825) (Tropiduridae). Field codes generated by citation software (e.g., EndNote) must be stripped prior to submission.

Acknowledgments

Although scientific articles in Portuguese and Spanish title the acknowledgments section "agradecimentos" or "agradecimientos," respectively, the purpose of this section is not to express thanks but to recognize the individuals and institutions who provided critical support for the research (i.e., "reconhecimentos" or "reconocimientos"), and the text should be written accordingly. Specifically, individuals and institutions (other than the authors' home institutions) that provided funding, access to work space, equipment, specimens and tissues, and assistance in carrying out the study or preparing the manuscript must be listed, together with a statement detailing their contribution or involvement. Relevant permits and authorizations must also be listed in the acknowledgments.

References

All literature cited in both the text and online supporting information must be included in the References section. Authors are discouraged from citing dissertations and theses because they usually constitute unfinished works that were either completed and published elsewhere (in which case the published version should be cited) or were not completed and published (in which case the work should not be considered part of the permanent scientific record). However, to allow for the rare, special situations in which dissertations and theses must be cited, the format is included below. Articles that are submitted or in press can be cited as such at the time of submission but must be published or at least publicly available (e.g., via DOI, see below) prior to publication.

The References section is the main source of formatting errors. To help remedy this, we have simplified and streamlined our format. As such, we strongly recommend that authors pay close attention to the following. Important considerations include:

Author names are given as last name followed immediately (i.e., no comma) by initials, each separated by a period and no spaces; suffixes should follow initials, separated by a space (e.g., Brodie E.D. Jr.); authors are separated by a comma without "and" or "&" preceding the last author.

Single- and two-author references must be listed in alphabetical order first, chronological order second. References with three or more authors must always be listed in chronological order. If an article has more than seven authors, list the names of the first six authors followed by "…" and then the last author's name in the reference entry.

Multiple references of same authorship (e.g., Silva, 1998a, b) should be listed in the same order as they are cited in the text (i.e., Silva, 1998a, must precede Silva, 1998b), with the corresponding identifying letter following year of publication.

To facilitate indexing and cross-referencing, articles available from permanent online repositories must include their respective handle. To ensure that handles are truly permanent, SAJH accepts Digital Object Identifiers (DOIs) exclusively. Stable URLs are no longer accepted. Increasingly, ancient (and not so ancient) literature can be accessed online through the Biodiversity Heritage Library, and many works available there have been assigned a DOI. Please note the formatting of DOIs in the examples below:

Article. Authors. Year. Article title. Journal Name volume:page–page. doi:doi.number

Abdala C.S., Quinteros A.S. 2014. Los últimos 30 años de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. Cuadernos de Herpetología. In press.

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Frost D.R., Grant T., Faivovich J., Bain R.H., Haas A., Haddad C.F.B., … Wheeler, W.C. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History 297:1–370. doi:10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2

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Book. Authors. Year. Book Title. Publisher, City.

Martins M., Sano P.T. 2009. Biodiversidade Tropical. Editora UNESP, São Paulo.

Noble G.K. 1931. The Biology of the Amphibia. McGraw-Hill, New York. doi:10.5962/bhl.title.82448.

Linnaeus C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differential, synonymis, locis, Tomus I. Editio decima, reformata. Laurentiis Salvii, Holmiae. doi:10.5962/bhl.title.542

Book chapter. Authors. Year. Chapter title. Pp. chapter pages, in Editor Names (Eds.), Book Title. Publisher, City.

Martins M., Marques O.A.V., Sazima I. 2002. Ecological and phylogenetic correlates of feeding habits in Neotropical pitvipers (genus Bothrops). Pp. 307–328, in Schuett G.W., Höggren M., Douglas M.E., Greene H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Eagle Mountain.

Dissertation or thesis. Author. Year. Title. Degree Requirement, Institution, Country.

Angulo A. 2004. The Evolution of the Acoustic Communication System in Members of the Genus Adenomera (Anura: Leptodactylidae): A Comparative Approach. Ph.D. Dissertation, University of Toronto, Canada.

Website content. Authors. Year. Title. Version. Accessible at website. Accessed: access date [if version not available]

Frost D.R. 2013. Amphibian species of the world: an online reference. Version 5.6 (9 January 2013). Accessible at http://research.amnh.org/herpetology/amphibia/

Uetz P. (Ed.). 2012. The reptile database. Accessible at http://www.reptile-database.org/. Accessed: 07 February 2013.

We also encourage (but do not require) authors to use WebCite® (http://www.webcitation.org) to archive the website. In this case, provide the regular citation followed by the archival site URL provided by the service.

Uetz P. (Ed.). 2012. The reptile database. Accessible at http://www.reptile-database.org/. Accessed: 07 February 2013. Archived by WebCite at http://www.webcitation.org/6EGLJNIh0

Software. Authors. Year. Software name, Version. Available from: website or company name and address.

Maddison W.P., Maddison D.R. 2009. Mesquite: a modular system for evolutionary analysis, Version 2.7.1. Available from: http://mesquiteproject.org

Software packages. Authors. Year. Package title, Software name. Available from: website or company name.

Harmon L.J., Weir J., Brock C., Glor R., Challenger W., Hunt G. 2009. Geiger: analysis of evolutionary diversification, R package. Available from: http://CRAN.R-project.org/package=geiger

Online supporting information

All online supporting information must be cited in the text as Figure S1, (Fig. S2), Appendix S1, Table S1, Audio S1, Video S1, etc. and be listed in the Online Supporting Information section. This section must begin with the opening statement: "The following Supporting Information is available for this article online:" followed by the list of supplementary information, as cited in the text, and a brief caption for each file.

Appendix

In addition to providing information that is not essential to the text (e.g., specimens examined, GenBank accession numbers) as online supporting information, it may be provided in an appendix following the References section.

Specimens examined should, preferably, be reported in the following format:

Species name (n = number of specimens): COUNTRY: State: County: Municipality: Specific locality, COLLECTION ACRONYM number(s); …

Amphisbaena anaemariae (n = 8): BRAZIL: São Paulo: Teodoro Sampaio: Parque Estadual do Morro do Diabo, MZUSP 96810; Goiás: Campinaçu: MZUSP 103743; Luiziania: MTR 11453, 115454; São Salvador do Tocantins: UHE São Salvador, MZUSP 99394; UHE Cana Brava, MZUSP 97217; UHE Serra da Mesa, MZUSP 97047, 97171.

Tables

Tables should be on separate pages in either the main document file or separate files and be accompanied by a legend at the top. Tables must be numbered in the same sequence in which they appear in the text. Authors are encouraged to indicate where the tables should be placed in the text. Tables should be comprehensible without reference to the text and not report the same data presented in figures or listed in the text. Tables should be formatted exclusively with horizontal lines. In the text, tables should be referred to as Table 1, Tables 2 and 3, Tables 2–6. Tables provided as supporting information must not be included here (see below).

Figure captions

A brief caption must be provided for each figure cited in the text, including enough information for the figure to be understood without reference to the text. Figures provided as supplementary information must not be included here.

Figure preparation

Figures must visually compress information in order to complement, not repeat, the information provided in the text. Important but non-essential figures should be submitted as Supporting Information (see below). SAJH publishes a limited number of color figures at no cost to authors. When color reproduction is not essential, authors should submit gray scale graphics. Previously published figures will not be accepted. All figures must be cited in text as "(Fig.)" and "Figure." Use lower case "fig." and "figure" when referring to figures in other papers. Authors are encouraged to indicate where figures should be placed in the text. Each part of a composite figure should be identified by capital letters and referred in the text as Fig. 1A, Fig. 1B, Fig. 2C–D, etc. Where possible, letters should be placed in the upper left corner of each illustration of a composite figure. Font style and final size (i.e., after reduction to page or column width; see below) should be standardized among figures. A scale bar should be marked on each figure so that absolute sizes are clearly apparent. If a scale bar is not provided, then the caption should provide some size reference (e.g., snout–vent length in photographs of whole specimens). On no account should magnification factors (e.g., x7000) be stated in the captions.

High quality graphics files should be submitted through PeerTrack in common electronic formats (e.g., JPEG, TIFF, PNG). Figures should be submitted at final size, maximum length = 23.0 cm; page width 17.5 cm, column width 8.7 cm) at resolution of at least 300 dpi. Vector art (e.g., AI, EPS, SVG) can also be submitted. We recommend that authors use Allen Press's Allen veriFig™ service to check figure quality and format prior to submission, as this can prevent production delays. To log in, authors must provide a valid email and enter the password "figcheck."

We encourage authors to submit through PeerTrack a high quality, original photograph that has not been published or submitted elsewhere as a candidate cover image. We ask that this image be mentioned in the cover letter and that a legend be provided following other figure captions.

Online supporting information preparation

SAJH permits online supporting information to accompany articles, including appendices, figures, and supplementary text—preferably as PDF files—as well as audio and video files. Supplementary files are associated with the corresponding article on the BioOne website and special links are included in the online Table of Contents to highlight that the article has supplemental information available. All online supporting information must be cited in the text as Figure S1, Appendix S1, Table S1, Audio S1, Video S1, etc. and also be listed in the Online Supporting Information section (see above). Literature cited in online supporting information must be included in the References section of the main article; this ensures proper tracking for indexing. Although online supporting information will be sent for peer review, supplementary files usually will not be sent for English revision.

All Supporting Information must be submitted online with the main manuscript files. Please name your online supporting files as Supporting Files and upload them with the main document. This allows the submission web site to combine all the relevant files together for review but keep them separate for publication.

ANEXO 2 - INSTRUCTIONS FOR AUTHORS: JOURNAL OF EXPERIMENTAL BIOLOGY

1. General information

JEB requires authors to submit their manuscripts online using the Bench>Press manuscript processing system. Authors are required to read our journal policies before preparing their manuscripts, and all manuscripts should adhere to the journal's terms of submission.

All pre-submission or general editorial queries should be directed to the Editorial Office.

1.1. New submissions – format free

To make manuscript submission as easy as possible for authors, JEB allows format-free submission.

At first submission, authors may submit their manuscript in any format; however, we do encourage authors to read the manuscript preparation guidelines below and to **use 1.5 line spacing and line numbers to facilitate viewing by editors and reviewers**.

All manuscripts must adhere to our guidelines regarding manuscript length.

1.2. Revised submissions

On JEB, >95% of revised submissions are accepted for publication.

All revised manuscripts should adhere to the guidelines below on preparing text and tables, figures, movies and supplementary information.

Authors should complete and submit a submission checklist with their manuscripts. This form asks authors to confirm that they have followed best practice guidelines regarding experimental subjects, data reporting and statistics. The checklist is based on the NIH Principles and Guidelines for Reporting Preclinical Research and is intended to help ensure high standards for reporting and to aid reproducibility.

2. Manuscript length

The following table shows the maximum word count of the main text (including the main text and figure legends, but not the title page, abstract, materials and methods section or reference list) and maximum number of display items (figures and tables) for different article types.

Articles exceeding the limits specified above will be returned to authors at submission. Note that final word limits will depend on the paper submitted and are at the discretion of the Editors.

3. Preparing the text and tables

The information below relates to a standard Research Article. For all other article types, please refer to the style and layout guidelines provided on our article types page.

3.1 File formats

For manuscript text and tables, our preferred **file format** is **Microsoft Word .docx** (or .**doc**). We also accept Pages (rtf format) and LaTeX.

Please include tables as part of the manuscript file. Tables must be editable and not embedded as an image.

Authors working in LaTeX can download and use our template. Please upload a single PDF at first submission and include any component files, such as .st (style file), .cls (class file) and .bib (bibliography file) at revision submission. Please note that LaTeX files will be converted to Microsoft Word files during the production process and that authors will be required to check the conversion of symbols and special characters carefully at the proofing stage.

For mathematical equations, our preferred file format is MathType. We also accept Equation Editor (Microsoft Word) and LaTex.

3.2. Article sections

3.2.1. Title page

This section should include a **title** of 120 characters or less that clearly and concisely summarises your specific findings and avoids specialist abbreviations, a **running title** of 40 characters or less, the full **names** (including middle initials) **and affiliations of all authors** (including present addresses for authors who have moved), and the **corresponding author's email address**. Please note any cases where authors contributed equally to the work. Please also include **3-6 key words** for indexing purposes (select key words that will make your manuscript easily searchable).

3.2.2. Summary statement

Provide a brief Summary Statement for use in emailed and online tables of content alerts. The text should be between 15 and 30 words, and should explain, without overstatement, why someone should read the article. Please do not simply repeat the title, and avoid unfamiliar terms and abbreviations, as the text should be comprehensible to non-experts. We reserve the right to edit the text.

3.2.3. Abstract

Provide a brief abstract of no more than 250 words for Research Articles (150 words for Short Communications and Methods & Techniques). This should succinctly and clearly introduce the topic of the paper, summarise the main findings and highlight the significance of the data and main conclusions. The abstract is used by abstracting services without modification and is often read more frequently than the full paper and therefore needs to be comprehensible in its own right. Do not include subheadings or references, and avoid any non-standard abbreviations.

3.2.4. Introduction

This section should succinctly provide the background information that is required to set the results into their proper biological context. It should not contain subheadings.

3.2.5. Materials and methods

This section should include sufficient detail to understand and to replicate the experiments performed, in conjunction with cited references. To facilitate detailed description of materials and methods (allowing the reader to fully understand and replicate the experimental protocols), this section does not count towards the word limit for article length. The materials and methods should be divided into sections, and should include subsections detailing reagents, animal models and statistical analysis. Provide names and locations (town, state, country) for ALL equipment and reagent suppliers. Give Latin names and taxonomic authority (e.g. Linnaeus) for all experimental species. Reporting standards should follow those recommended in our journal policies and submission checklist.

3.2.6. Results

This section should describe the results of the experiments performed and should be broken up by subheadings to organise the findings presented and walk the reader through the results. Reproducibility of results must be included – see our submission checklist for further information. Please ensure that the distinction between new results and published findings/established facts is clear.

3.2.7. Discussion

This section should explain the significance of the results and should place them into the broader context of the current literature. The Discussion may contain subheadings to highlight important areas that are expanded on in the text.

3.2.8. Acknowledgements

This section should mention any individuals or groups that are not named as authors but have contributed to the research presented (e.g. in terms of reagents, time, expertise) or writing of the manuscript. Please also include details of support from core facilities.

3.2.9. Competing interests

Include a statement to identify any potential influences that readers may need to know about when thinking about the implications of the presented research. For more specific information regarding the affiliations and associations that must be disclosed, please see our journal policies page. Authors without financial or competing interests should explicitly assert this and include the statement ‘No competing interests declared’.

3.2.10. Funding

Details of all funding sources must be provided. It is the responsibility of the corresponding author to provide the relevant funding information from ALL authors. Please provide the official funding agency name as listed on the Crossref Funder Registry, i.e. 'National Institutes of Health', not 'NIH', and all relevant grant numbers. If your Funder is not listed in the Registry, please provide the name in full.

Where individuals need to be specified for certain sources of funding, please add initials after the relevant agency or grant number. Please use the following format: This work was supported by the National Institutes of Health [AA123456 to C.S., BB765432 to M.H.]; and the Alcohol & Education Research Council [hfygr667789]. Where no specific funding has been provided for the research, please state ‘This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors’.

3.2.11. Data availability

All publicly available datasets supporting your work should be included in the Data availability section. Details should include repository name, identifier such as accession number or doi and, where possible, include a hyperlink to the URL of the dataset. Datasets should be made publicly available at the time of publication. For more information on our data deposition requirements, see our Journal Policies .

Please note that JEB endorses the Force 11 Data Citation Principles and recommends that references to datasets should also be included in the reference list with DOIs/accession numbers and hyperlinks, where available.

3.2.12. References

All references cited in the text, tables and figure legends should be included in a single reference list at the end of the article. We strongly encourage the citation of the primary literature over review articles wherever possible, and for this reason do not have a limit on the number of references that can be included. For specific information about reference formatting, please see the references section below.

3.2.13. Figure legends

Figure legends should be listed at the end of the manuscript. The first sentence of the legend should summarise the figure and be in bold. Each figure legend should stand alone and should contain enough information to ensure that the figure is understandable without having to refer to the main text. Figure panels should be labelled with uppercase letters (A, B, C, etc.), and each panel should be described in the legend. Any abbreviations not given in the main text should be defined. For further details on what should be included in figure legends, please refer to our submission checklist.

3.2.14. Appendices

This optional section can be used for information that is critical to the manuscript but would interrupt the flow of the article and is not suitable for inclusion as supplementary information. It should be formatted according to normal journal style. All figures, tables and equations should be numbered separately from the main text as Fig. A1, Table A1, Eqn A1, etc. Please note that the text, figures and tables in an Appendix count towards the overall manuscript length.

3.3. Preparing the text

3.3.1. General information

- Prepare manuscripts in English (either US or UK spelling is acceptable but be consistent within the manuscript). Your writing should be comprehensible to editors and reviewers, and your writing style should be concise and accessible. If English is not your first language, please consider using a language editing service prior to submission.

- Ensure that the language in your manuscript is original and does not contain previously published passages of text (including those from your own publications) – see our journal policies for more details. All accepted manuscripts are routinely screened using plagiarism-detection software.
- Use 1.5 line spacing and continuous line numbering throughout the paper in order to facilitate online reviewing.
- Do not embed figures in the text.
- Cite each figure, table and movie in the text in numerical order. Figure or table parts should be labelled with uppercase letters (A, B, C, etc.). Use the following format for citations: Fig. 1A,B or Figs 1, 2 or Table 1 or Movie 1.
- If necessary, display equations should be cited using the following format: Eqn 1.
- For supplementary figures, tables and equations, cite as Fig. S1, Table S1, Eqn S1.
- Define abbreviations at first mention and include a List of Symbols and Abbreviations used.
- For special characters not available on a standard keyboard (e.g. Greek characters, mathematical symbols), use the Symbol font or the 'Insert Symbol' function in Microsoft Word, where possible. For special characters that are not available via this route, please use MathType characters; do not use embedded images (e.g. GIF).

3.3.2. Units and nomenclature

- Units of measurement should follow the SI system, e.g. ml s^{-1} rather than ml/s. Guidance on using the SI convention can be found here. Type a space between a digit and a unit, e.g. 1 mm (except 1%, 1^{o}C).
- Use s.e.m. and s.d. for standard errors, etc.
- Taxonomic nomenclature: the Latin names and taxonomic authority (e.g. Linnaeus) should be provided for all experimental species. All species names should be italicized.
- Genetic nomenclature: gene names should be in italic type, but the protein product of a gene should be in Roman type. Genetic nomenclature should be in accordance with established conventions and should be approved by the relevant nomenclature curator if applicable. See below for some relevant links.
    - HGNC list of genome databases: https://www.genenames.org/useful/all-links#ovgdb
    - *Caenorhabditis elegans*: https://www.wormbase.org
    - *Dictyostelium*: https://dictycr.org/
    - Chicken: http://birdgenenames.org/cgnc/guidelines
    - *Drosophila:* https://flybase.org/wiki/FlyBase:Nomenclature
    - Human: https://www.genenames.org/about/guidelines
    - Maize: https://www.maizegdb.org/maize_nomenclature.php
    - Mouse: http://www.informatics.jax.org/mgihome/lists/lists.shtml
    - *Saccharomyces cerevisiae*: https://www.yeastgenome.org/

- *Schizosaccharomyces pombe*: https://www.pombase.org/submit-data/gene-naming-guidelines
- *Xenopus*: https://www.xenbase.org/gene/static/geneNomenclature.jsp
- Zebrafish: https://zfin.atlassian.net/wiki/spaces/general/pages/1818394635/ZFIN+Zebrafish+Nomenclature+Conventions

3.3.3. References

3.3.3.1. References in text

References in the text should be cited using the Harvard (name, date) referencing system.

Each reference cited in the text must be listed in the Reference list and vice versa: please check these carefully. Where references are cited only in supplementary information, please provide a separate supplementary reference list and do not include these in the main reference list.

Literature citations in text are as follows.

- One author – (Jones, 1995) or (Jones, 1995; Smith, 1996).
- Two authors – (Jones and Kane, 1994) or (Jones and Kane, 1994; Smith, 1996).
- More than two authors – (Jones et al., 1995) or (Jones et al., 1995a,b; Smith et al., 1994, 1995).
- Manuscripts accepted for publication but not yet published: include in Reference list and cite as (Jones et al., in press).
- Manuscripts posted on preprint servers but not yet published: include in Reference list and cite as (Smith et al., 2016 preprint).
- Citation of unpublished data: we strongly discourage the citation of unpublished data or data not shown. Where it is necessary, use the format (S.P. Jones, unpublished observations/data not shown); note that the editor or journal office may request that these data should be included prior to publication. Personal communications (the unpublished observations of scientists other than the authors) can only be cited with written permission from the scientist in question, and should be cited in the text using the format (full name, institution, personal communication). Unpublished work should not be included in the Reference list.
- PhD theses: include in Reference list and cite as (Smith, 2016).
- Website URLs: cite in the text but do not include in the Reference list; provide the URL and, if the website is frequently updated, the date that the site was accessed.
- Dataset: we recommend that all publicly available datasets are fully referenced in the reference list with an accession number or unique identifier such as a DOI. Cite as (Jones and Jane, 1994).

- Authors should avoid citing articles from journals that are suspected to be predatory in nature (see https://thinkchecksubmit.org/ for an online resource designed to help researchers identify trusted journals).
- Citation of retracted articles is strongly discouraged. If it is necessary to cite a retracted paper, the notice of retraction must also be cited and it must be obvious to the reader that the article has been retracted. Editors may question why a retracted publication has been cited.

3.3.3.2. Reference List

References are listed in alphabetical order according to surname and initials of first author.

- Use the following style:

Journal

Rivera, A. R. V., Wyneken, J. and Blob, R. W. (2011). Forelimb kinematics and motor patterns of swimming loggerhead sea turtles (*Caretta caretta*): are motor patterns conserved in the evolution of new locomotor strategies? *J. Exp. Biol.* **214**, 3314-3323.

Book

Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution.* Oxford, UK: Oxford University Press.

Book chapter

Feller, G. (2008). Enzyme function at low temperatures in psychrophiles. In *Protein Adaptation in Extremophiles* (ed. K. S. Siddiqui and T. Thomas), pp. 35-69. New York: Nova Science Publishers, Inc.

Preprint server

Baillie-Johnson, P., van den Brink, S. C., Balayo, T., Turner, D. A. and Martinez Arias, A. (2014). Generation of aggregates of mouse ES cells that show symmetry breaking, polarisation and emergent collective behaviour in vitro. *bioRxiv* doi:10.1101/005215.

PhD thesis

Jones, A. R. (2016). Title of thesis. *PhD thesis,* University of Washington, Seattle, WA.

Dataset with persistent identifier

Zheng, L.-Y., Guo, X.-S., He, B., Sun, L.-J., Peng, Y. and Dong, S.-S. (2011). Genome data from sweet and grain sorghum (Sorghum bicolor). *GigaScience Database*. https://dx.doi.org/10.5524/100012.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P. and Beerli, P. (2001). Data from: The strength of phenotypic selection in natural populations. *Dryad Digital Repository*. https://dx.doi.org/10.5061/dryad.166.

- If there are more than 10 authors, use 'et al.' after the 10th author.
- Within a group of papers with the same first author, list single author papers first, then papers with two authors, then et al. papers. If more than one reference exists for each type, arrange in date order. Use a and b for papers published in the same year.
- 'In press' citations must have been accepted for publication and the name of the journal or publisher included.

3.4. Preparing tables

Prepare tables in 'cell' format and include in the same file as the main text. Tables must be editable and not embedded as an image.

The title of the table should be a single sentence and should summarise the contents of the table. Details referring to one or more isolated item(s) in the table are best given in a table footnote. Units should be given in parentheses at the top of each column (do not repeat in the table)

3.5. Preparing display equations

Our preferred file format for equations is MathType. We also accept Equation Editor (Microsoft Word) or LaTex.

Please number all display equations, consecutively. They should take the form:

$$Q = \frac{-\kappa A_p [p]}{\mu T_p}, \quad (1).$$

Units should be defined in the text rather than included in the equation.

4. Preparing figures

4.1. General information

Figures should be numbered in a single series that reflects the order in which they are referred to in the text.

Figures should be prepared at the smallest size that will convey the essential scientific information; final figure size is at the discretion of the journal. For further information on how to arrange your figures to optimise viewing by reviewers and readers, download our figure layout guidelines.

At initial submission, you may submit a single PDF file containing all text and figures. Once an article has been accepted for publication, you are required to submit separate files for each figure (see below for file formats).

Figure legends should be included in the main text file and not in the figure file.

There are no charges for the use of colour in figures, although gratuitous use of colour in graphs and diagrams should be avoided and colour should only be used to improve scientific clarity.

We strongly encourage the use of colours that are suitable for colour-blind readers, particularly in the preparation of fluorescent microscopy images. Most notably, we discourage the use of red/green for the display of 2-channel images; authors should consider an alternative colour combination (e.g. magenta/green).

4.2. Preparing graphs and diagrams (line art)

4.2.1. General information

- The maximum figure size, including lettering and labels, is 180 mm × 210 mm.
- Line thicknesses and symbols should be of sufficient size to ensure clarity if the figure is reduced in size.
- For graphs, our preferred symbols are filled and open circles, triangles, squares, or diamonds; where possible, the same symbol should be used for the same entity in different figures.
- Colour: supply line art in RGB (not CMYK) mode, as this maximizes colour quality and is how the figures will be displayed online; do NOT use Spot, Pantone or Hex colours and do NOT assign a colour profile.
- Text labelling: use 12 pt bold uppercase letters (A, B, C, etc.) to distinguish figure panels; other labelling should be 8 pt Arial font (sentence case) (headings should be bold); for gene sequences, use Courier font to ensure that each letter is the same width; use Symbol font for Greek characters.

4.2.2. File formats

Authors should submit their source figures in an editable format (vector graphic) that retains font, line and shape information. This format ensures that we can edit where necessary and produce high-quality print and online PDFs.

We accept the following file formats for graphs/line art: EPS, PDF, and WMF.

- Applications such as Adobe Illustrator, Canvas, DeltaGraph, Corel Draw, Freehand, MatLab and SigmaPlot provide these formats.
- Please ensure that you 'export' or 'save' with (text/font) information included
- Save text/font information as ‘text’ not ‘curves’ or ‘outlines’.
- If combining images, always 'embed' images; do NOT simply 'link' them. In Adobe Illustrator, copying and pasting or dragging an image directly from Adobe Photoshop will embed the image. Alternatively, if you use the 'Place' command, uncheck 'Link' in the dialogue box. For other software applications, please refer to the documentation (often there will be a 'link', 'proxy', 'OLE' or 'OPI' option, which must NOT be used with EPS files).
- *Note that submission of JPEG or TIFF format for graphs/line art may delay production of your article.*

4.3. Preparing photographic images

4.3.1. General information

Photographic images (also known as bitmap images) are made up of pixels (e.g. light, fluorescence and electron microscopy, gels, and traditional photography)

- The maximum figure size, including lettering and labels, is 180 mm x 210 mm.
- Images should be saved at a resolution of 300 pixels per inch. Any image quality option should be set to maximum.
- For micrographs, use a scale bar to show the magnification and give the length of this in the figure legend.
- Colour: supply images in RGB (not CMYK) mode, as this maximizes colour quality and is how the figures will be displayed online; do NOT use Spot, Pantone or Hex colours and do NOT assign a colour profile.
- Text labelling: use 12 pt bold uppercase letters (A, B, C, etc.) to distinguish figure panels; other labelling should be 8 pt Arial font (sentence case) (headings should be bold); for gene sequences, use Courier font to ensure that each letter is the same width; use Symbol font for Greek characters.

4.3.2. File formats

Accepted file formats are: **EPS/PDF** (vector based, such as Adobe Illustrator).

- EPS / PDF format for figures with mixed data, such as line drawn vector-graphs and Photographic Images.
- TIFF format with text-layers enabled, for Photographic Images only.
- ARIAL or HELVETICA must be the font choice used throughout figure preparation.

PowerPoint images: we do NOT accept PowerPoint files. Instead, please save as PDF using the instructions below.

- Go to 'print' and then choose 'Save as PDF' in the print dialogue box
- You can download free software which will enable you to print EPS/PDF files to disk:Download software for Windows
Download software for Mac

4.4. Image manipulation

Any alterations made to figures using computer software must be consistent with our image manipulation policy. The images presented in the manuscript must remain representative of the original data, and the corresponding author will be asked to confirm this at submission. Please read our requirements for preparing your figures (download PDF) to avoid a potential delay in the publication process or rejection on the basis of non-compliance with these guidelines.

All accepted manuscripts are routinely screened by our production department for any indication of image manipulation. If evidence of inappropriate manipulation is detected, the journal's Editors might ask for the original data to be supplied and, if necessary, may revoke the acceptance of the article.

4.5. Figure permissions

It is the responsibility of the author to obtain permission to use figures from another publication in any article submitted to JEB and to ensure that any such use is credited to the source. Any fees associated with use of the figure are the responsibility of the author. Written permission from the author and/or publisher of the original material, as appropriate, should be provided at the time of submission, otherwise publication may be delayed. If a figure has been modified from a previously published figure, please check with the copyright owners to see whether permission is required and include a complete citation/reference for the original article.

5. Preparing movies

Our preferred **file format** for movies is **.mp4**, but we also accept .mov. Movies should be prepared at the smallest file-size that will convey the essential scientific information. We have a limit of 500 MB for all movie files. If your movies exceed this limit, please contact the Editorial Office for advice before submission.

Please include the titles and captions of all movies in your supplementary information PDF (see section on preparing supplementary information). Please keep captions as short as possible and ensure that they explain what is being shown in the movie and any necessary details of how the movie was made.

Movies should be numbered in a single series that reflects the order in which they are cited in the text, e.g. see Movie 1. Movie 2, etc. Please do not use alphabetical labelling, e.g. Movies 1A-C should be relabelled as Movies 1-3.

All movies will play in place in the full-text online version of the article and a link to each movie will be included in the supplementary information PDF.

When preparing your movies, please note the recommendations below :

- Use a resolution of no greater than 1280x720 (720p), as most readers will view on a desktop or mobile device
- Use a well-characterized video compression codec such as H.264 and use multi-pass encoding if available
- Do not exceed a bitrate of 2500mbps for 720p H.264-encoded video
- Keep duration to the minimum required to illustrate your point
- Do not include an audio track unless it is essential
- If including audio, use a well-characterized audio compression codec such as AAC
- Do not exceed a bitrate of 128kbps, sample rate of 44.1kHz, or channel count of 2 for encoded audio

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Data that are essential for interpretation of the results of the main paper should be included in the main paper. Supplementary information provides access to supporting data that do not appear in the printed article or PDF but that accompany the final version of a paper online.

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