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Karina Rodrigues da Silva Banci

História Natural e Ecologia da jararaca-ilhoa, *Bothrops insularis* (Serpentes, Viperidae)

São José do Rio Preto
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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biologia Animal, junto ao Programa de Pós-Graduação em Biodiversidade, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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“*Ipsa scientia potestas est*.”

Francis Bacon (1597)

RESUMO

A jararaca-ilhoa, *Bothrops insularis*, é uma espécie criticamente ameaçada de extinção, endêmica da Ilha da Queimada Grande (IQG), no litoral Sul de São Paulo. Ela faz parte do grupo *Bothrops jararaca*, que também inclui *B. alcatraz, B. germanoi*, *B. jabrensis*, *B. jararaca*, *B. otavioi* e *B. sazimai*. Sua dieta é baseada em presas ectotérmicas, como anuros e lagartos, quando juvenis, e em aves migratórias quando adultas, que visitam a ilha em dois picos migratórios, em março e julho, principalmente. Por se tratar de espécie ameaçada, a manutenção de populações *ex situ* de *B. insularis* é uma recomendação da União Internacional para a Conservação da Natureza. No entanto, em cativeiro, essas serpentes são alimentadas com roedores mensalmente, desde seu nascimento. No primeiro capítulo da presente tese, a partir da comparação morfológica de animais de cativeiro e da IQG (com base em captura e recaptura), avaliamos a influência que esta alimentação diferenciada pode ter sobre o crescimento dos indivíduos. Demonstramos que os indivíduos de cativeiro apresentam maior taxa de crescimento e tamanho, e sua maturidade sexual ocorre mais cedo em comparação aos indivíduos de mesmo sexo da IQG, possivelmente por conta da maior frequência alimentar. Ainda em relação à dieta, desde sua descrição, por se alimentar de aves, a espécie tem sido considerada diurna, ao contrário das espécies do grupo. Entretanto, este aspecto ainda não havia sido devidamente estudado. No segundo capítulo, apresentamos dados comparativos da atividade diária de *B. insularis, B. alcatraz* e *B. jararaca* obtidos a partir de filmagens realizadas durante dez dias consecutivos em cativeiro. Os dados foram então comparados a registros de atividade de animais encontrados na IQG. Este estudo demonstrou que, assim como *B. alcatraz* e *B. jararaca*, *B. insularis* é intrinsecamente noturna, embora sua atividade de caça na natureza ocorra principalmente durante o dia. Demonstramos, ainda, que a variação de temperatura em diferentes estações pode modular a atividade diária de *B. jararaca*. O conhecimento acerca da história natural e ecologia das diferentes espécies, especialmente as ameaçadas de extinção, são essenciais para conhecer quais e como os diferentes fatores ambientais e ecológicos as influenciam. Deste modo, informações sobre história natural e ecologia são cruciais para o delineamento de estratégias de conservação direcionadas a essas espécies. Dado que ilhas podem apresentar condições ecológicas diferenciadas em relação ao continente, ou mesmo em relação a outras ilhas, é importante avaliar a taxa de predação à qual as espécies-alvo do estudo estão sujeitas, analisando, ainda, se este aspecto poderia influenciar seu comportamento defensivo. No terceiro capítulo, a partir de experimentos usando réplicas de massa de modelar, demonstramos que a taxa de predação sobre *B. insularis* na IQG é menor do que aquela sobre

B. alcatraz na Ilha de Alcatrazes e sobre *B. jararaca* em um fragmento urbano de São Paulo, que, por sua vez, são menores do que aquela sobre *B. jararaca* de áreas florestais contínuas. Possivelmente por conta disso, indivíduos de *B. insularis* são menos defensivos do que *B. jararaca*, fugindo mais. O mesmo foi constatado com *B. alcatraz*, porém isso possivelmente está associado ao seu tamanho reduzido e baixa capacidade de se defender contra aves e teiús que as podem predar, o que faz com que sejam mais secretivas do que as outras duas espécies. Finalizando, para estratégias de conservação, é fundamental conhecer os ambientes utilizados pelas espécies, avaliando as variáveis do hábitat e os padrões de deslocamento das mesmas. Utilizando três métodos combinados (radiotelemetria, carretel, e captura e recaptura), foi possível verificar que *B. insularis* aparenta ser uma espécie sedentária, assim como verificado para *B. jararaca*. Verificamos, ainda, que, embora os animais utilizem mais o chão, a probabilidade de encontrar animais sobre a vegetação é maior nos períodos em que as aves visitam a ilha, indicando a ocorrência de uma migração vertical pela jararaca-ilhoa. Existe, ainda, grande influência da alometria na utilização da vegetação. Além disso, com base nos encontros e reencontros ao longo dos anos, foi possível verificar que a jararaca-ilhoa está mais associada a áreas fechadas, sendo encontrada no interior da mata ou em áreas de borda, sempre com grande cobertura do dossel, indicando que a espécie seja mais dependente de áreas fechadas do que *B. alcatraz* e *B. jararaca*. As análises comparativas de *B. insularis* com espécies irmãs também são interessantes para o melhor entendimento da evolução do grupo. Finalmente, esperamos que o conhecimento gerado possa contribuir para a conservação não só da jararaca-ilhoa, mas do grupo *jararaca*.

Palavras-chave: Serpente. Grupo *Bothrops jararaca*. Crescimento. Atividade diária. Predação. Comportamento defensivo. Uso do ambiente.

ABSTRACT

The Golden Lancehead is a critically endangered species, endemic of Queimada Grande Island (QGI), southeastern Brazil. It belongs to the *Bothrops jararaca* species group, that also includes *B. alcatraz*, *B. germanoi*, *B. jabrensis*, *B. jararaca*, *B. otavioi*, and *B. sazimai*. The diet of juveniles of the Golden Lancehead is based on ectothermic prey, as anurans and lizards, and, when adults, on migratory birds which visit the island in two migratory peaks, mainly March and July. For being an endangered species, the International Union for Conservation of Nature encourages the maintenance of *ex situ* populations. However, in captivity these animals are usually monthly fed on mice, since birth. In the first chapter of this thesis, by comparing the morphology of captive and wild individuals of *B. insularis* (using mark-recapture methods), we evaluated the influence of this differential feeding on growth. We demonstrated that captive animals attain larger sizes and growth rate, and anticipated sexual maturity, in comparison to those of the same sex in the wild population, possibly as a result of the higher feeding frequency. Concerning diet, for feeding on birds, the species has been largely considered as diurnal since its description. However, this trait has not been studied in details, yet. In the second chapter, we present comparative data on the daily activity of *B. insularis*, *B. alcatraz*, and *B. jararaca* obtained with video records made during ten consecutive days for each species in captivity. Daily activity of captive *B. insularis* was also compared to those of QGI. This study evinced that as *B. alcatraz* and *B. jararaca*, *B. insularis* is intrinsically nocturnal, even though it hunts during the day in the wild. We also showed that seasonal fluctuations of temperature may shape daily activity of *B. jararaca*. Knowledge on natural history and ecology of different species, especially endangered ones, are essential for recognizing which and how environmental and ecological factors may affect them. Therefore, information on natural history and ecology are crucial for conservation strategies aimed at these species. Considering that islands may present ecological conditions which are different of those found in the mainland, or even in other islands, it is important to evaluate the predation rate under which the target species are, evaluating, also, if such aspect may influence its defensiveness. In the third chapter, after conducting experiments using plastiline snake replicas, we demonstrate that predation rate on *B. insularis* at QGI is lower than that on *B. alcatraz* at Alcatrazes Island and on *B. jararaca* from an urban fragment in São Paulo Municipality, which are, in turn, lower than that on *B. jararaca* from continuous forested areas. Possibly for this reason, individuals of *B. insularis* are less defensive than those of *B. jararaca*, and tend to flee more often. The same was observed for *B. alcatraz*, but this may be related to its reduced size and low ability to defend against birds and tegus which may feed on these snakes, causing them to be more secretive than the other two species. Finally, knowing the habitats used by the species, evaluating environmental variables and displacement patterns, is fundamental for designing conservation plans. Using three combined methods (radiotelemetry, thread bobbins, and mark-recapture), it was possible to verify that *B. insularis* seems to be a sedentary species, as already reported for *B. jararaca*. We also found out that, even though they are observed more frequently on the ground, the probability of finding a Golden Lancehead is higher by the time the birds visit the island, indicating the occurrence of seasonal vertical migration by this species. Usage of vegetation seems to be influenced also by allometry. Additionally, based on mark-recapture methods, it was possible to verify that the Golden Lancehead is more associated with forested areas, with high canopy cover, indicating that this species relies more on such habitats than *B. alcatraz* and *B. jararaca*. Comparative analyses of *B. insularis* with its sister species are also interesting for better understanding the evolution of the group. Finally, we expect that the knowledge acquired may contribute with the conservation, not only of the Golden Lancehead, but of the *jararaca* group.

Keywords: Viperidae. *Bothrops jararaca* group. Growth. Daily activity. Predation. Defensive behavior. Habitat use.

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO GERAL

GROWTH PATTERNS OF THE CRITICALLY ENDANGERED *BOTHROPS INSULARIS*: A COMPARISON BETWEEN WILD AND CAPTIVE POPULATIONS.

LIVING IN THE DARKSIDE: ACTIVITY IN REPRESENTATIVES OF THE *BOTHROPS JARARACA* GROUP (SERPENTES, VIPERIDAE)

DIFFERENTIAL PREDATION RATE IN ISLANDS: CAN PREDATORS SHAPE THE NICHE AND DEFENSIVE BEHAVIOUR OF INSULAR PITVIPERS *BOTHROPS INSULARIS* AND *B. ALCATRAZ*?

HABITAT USE IN THE CRITICALLY ENDANGERED GOLDEN LANCEHEAD, *BOTHROPS INSULARIS* (AMARAL, 1921)

LISTA DE TABELAS

GROWTH PATTERNS OF THE CRITICALLY ENDANGERED *BOTHROPS INSULARIS*: A COMPARISON BETWEEN WILD AND CAPTIVE POPULATIONS.

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SUMÁRIO

INTRODUÇÃO GERAL: A presente introdução foi feita em formato de divulgação científica, e será submetida como artigo à Ciência Hoje

A JARARACA-ILHOA: RAINHA DOS MITOS ENTRE AS SERPENTES

Karina R.S. Banci, Wilian Barbosa Borges, Natália F. Torello-Viera & Otavio A.V. Marques

Você provavelmente já ouviu falar sobre a Ilha da Cobras, a Ilha da Queimada Grande (figura 1). Esta ilha, localizada no Litoral Sul de São Paulo, é o lar da famosa jararaca-ilhoa, *Bothrops insularis* (figura 2). Mas será que as informações que você possui realmente condizem com a realidade? Muitas vezes, as fontes de informação tendem a ser sensacionalistas, trazendo informações que não são de fato verdadeiras. Sabe aquela história de que quem conta um conto aumenta um ponto? Pois é exatamente isso! Além disso, histórias sensacionalistas acabam atraindo mais atenção, resultando em maior audiência, que é o intuito destes meios. Entretanto, em uma era em que percebemos os prejuízos que as *fake news* podem trazer, é fundamental estreitar os laços entre a ciência e os meios de comunicação, para ofertar informações fidedignas e confiáveis às pessoas.

No que diz respeito à Ilha das Cobras, por exemplo, inúmeros são os mitos que a rodeiam. Dizem, por exemplo, que a ilha apresenta a maior abundância de cobras do mundo, e que até 25 mil jararacas-ilhoas vivem por lá. Alegam, ainda, que essa alta abundância é decorrente de esses animais terem sido levados para a ilha, para protegerem tesouros de piratas ou espanhóis. Isso porque seu veneno é tão potente que seria capaz de derreter a carne humana, podendo matar uma pessoa instantaneamente. Isso, inclusive, impediu que pessoas morassem na ilha, uma vez que as serpentes mataram famílias que tentaram, bem como seus animais, e mesmo pescadores que eventualmente aportaram na ilha para pegar banana. Outras inverdades não tão chocantes, dizem respeito à biologia e ecologia de espécie. Muitas vezes, é dito que tal serpente alimenta-se unicamente de aves, e, por este motivo, é exclusivamente diurna e arborícola.

Fig. 1: Imagem de satélite da Ilha da Queimada Grande. Fonte: Google Earth, 2023.

Fig. 2: A jararaca-ilhoa, *Bothrops insularis*, da Ilha da Queimada Grande. Fonte: Karina Banci.

Mas, afinal, o que é verdade?

Para saber o que é verdade, precisamos primeiramente falar sobre a origem da jararaca-ilhoa. Cabe ressaltar que a espécie é irmã da jararaca-comum (*Bothrops jararaca*), bastante comum no continente, nas áreas de Mata Atlântica. Diferente do que dizem os mitos, as serpentes não foram levadas à ilha para proteger tesouro algum. Na verdade, a hipótese aceita atualmente diz que a origem da espécie está associada ao aumento do nível do mar, possivelmente durante a última glaciação. Por conta do degelo, o nível do mar teria subido, inundando áreas que anteriormente faziam parte do continente. Por ser uma área mais elevada, o que hoje é a Ilha da Queimada Grande não foi alagada, mas populações de jararacas ancestrais ficaram isoladas. Em virtude do isolamento e das diferenças ambientais no continente e na ilha, com o tempo, essas populações foram se diferenciando tanto, que deram origem às espécies que conhecemos hoje, a jararaca-comum no continente, e a jararaca-ilhoa, na Ilha da Queimada Grande. Esse mesmo processo teria sido responsável pelo surgimento de outras espécies desse mesmo grupo, como a jararaca-de-Alcatraz, da Ilha de Alcatrazes, por exemplo.

Dentre as diferenças ambientais mencionadas, cabe ressaltar que na ilha não existem mamíferos. Os roedores são os principais alimentos de adultos da jararaca-comum. Uma vez privadas dessas presas, as jararacas-ilhoas acabaram se especializando em predar aves, principal item da dieta dos adultos da espécie. Mais especificamente, duas espécies de aves migratórias constituem a maior parte do cardápio da jararaca-ilhoa. Uma delas é a guaracava-de-crista-branca, que visita a ilha em meados de março, e a outra, é o sabiá-una, que o faz em meados de julho.

Diferente do que costuma ser dito, a jararaca-ilhoa não é exclusivamente arborícola. Entretanto, ela usa mais a vegetação do que a sua irmã continental. Vale ressaltar que a probabilidade de encontrar exemplares sobre a vegetação é maior durante as épocas correspondentes à de migração das aves, o que condiz com o fato de que as aves buscam por alimento (frutas e insetos) nas árvores. Além disso, dado que as aves das quais se alimentam são diurnas, as jararacas-ilhoas também já foram consideradas exclusivamente diurnas. Embora, de fato, só tenham sido observadas se alimentando de aves durante o dia, a maioria dos registros de atividade das jararaca-ilhoas é no período noturno. A atividade noturna parece ser uma característica compartilhada e herdada dos ancestrais no grupo das jararacas, sendo que tanto a jararaca-comum, quanto a jararaca-de-Alcatraz, irmãs da jararaca-ilhoa, também apresentam o mesmo padrão de atividade. Possivelmente, a chegada das aves na ilha cause uma adequação no período de atividade, o que é necessário para a sobrevivência da espécie. Fora dos períodos de migração das aves, porém, as jararacas-ilhoas são encontradas principalmente no chão da mata, onde podem se alimentar de sapos no chão da mata. Além disso, na estação chuvosa, essas serpentes apresentam atividade predominantemente noturna, o que coincide com o horário de atividade desses sapos. As jararacas-ilhoas também podem usar tanto o chão, quanto a vegetação, para se se acasalarem, o que ocorre principalmente do outono até o meio do inverno. É importante ressaltar, ainda, que elas parecem dependentes das áreas florestadas da ilha, e mesmo neste ambiente não costumam se deslocar muito, ficando, geralmente, restritas a um raio de 40 metros.

A densidade de cobras na Ilha da Queimada Grande, diferente do que muitas vezes é noticiado, representa a segunda maior densidade de serpentes do mundo, ficando atrás, apenas, da Ilha de Shedao, na China. As estimativas populacionais mais recentes apontam para a existência de 2.000 a 3.000 cobras na ilha, o que representa algo em torno de 55 indivíduos em uma área equivalente a um campo de futebol (1 hectare)! Essa alta densidade de serpentes é possivelmente decorrente da baixa taxa de predação à qual a jararaca-ilhoa está submetida, doze vezes menor do que aquela à qual a jararaca-comum sofre em certas áreas florestais do continente. Esse aspecto pode explicar, inclusive, o motivo pelo qual esse animal pode ser frequentemente encontrado em campo, e também seu comportamento defensivo restrito quase inteiramente à fuga, diferente de sua irmã continental que possui repertório defensivo bem mais elaborado usado para intimidar os predadores. Apesar disso, cabe ressaltar que se trata de uma espécie peçonhenta, cujo veneno é de fato potente. Porém, ao contrário do que muitas vezes é dito, seu veneno não causa o derretimento da carne, nem é instantaneamente fatal, e, até o presente momento, não há registros de acidentes com esses animais na Ilha da Queimada Grande. Seu veneno tem essencialmente uma ação local agindo na destruição de tecidos e causando hemorragia, não diferindo da jararaca-comum, de modo que o mesmo soro (antibotrópico) pode ser administrado em caso de picadas com essas espécies, assim como com demais representantes das jararacas. O fato de não haver mais humanos residentes na Ilha da Queimada Grande está associado, portanto, não a eventos de acidentes com a jararaca-ilhoa, mas sim à automatização do farol da ilha pela Marinha do Brasil, fazendo com que não haja mais a necessidade de um faroleiro desde 1925. Atualmente, a Ilha da Queimada Grande é uma Unidade de Conservação Federal, fazendo parte da Área de Relevante Interesse Ecológico das Ilhas da Queimada Pequena e Queimada Grande, e desembarque na ilha só é autorizado a pesquisadores, mediante autorização do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO).

Também cabe ao ICMBIO a fiscalização da ilha, mas isso infelizmente não foi capaz de coibir a ação de traficantes de animais, o que resultou em uma redução da população de jararacas-ilhoas nas últimas décadas. Devido a isso, e também por se tratar de uma espécie endêmica, ou seja, que só ocorre na Ilha da Queimada Grande, a jararaca-ilhoa é classificada como criticamente ameaçada de extinção.

Conservação da jararaca-ilhoa

Por se tratar de uma espécie criticamente ameaçada, é fundamental que sejam feitos esforços para a sua conservação. As pesquisas realizadas com a espécie constituem a base para isso, uma vez que a preservação necessita das informações básicas acerca da história natural e ecologia da espécie. Os dados obtidos a partir dos estudos, portanto, devem nortear as estratégias de conservação. Considerando-se as informações disponíveis, fica evidente que a conservação da jararaca-ilhoa depende fundamentalmente, da preservação das florestas da ilha, bem como das áreas de Mata Atlântica do continente utilizadas pelas aves migratórias que servem de alimento para essas serpentes.

Paralelamente, é fundamental que sejam feitos programas de conservação da jararaca-ilhoa *ex-situ*, ou seja, em cativeiro. Da mesma forma, é fundamental que tais programas levem em consideração os requerimentos da espécie na Ilha da Queimada Grande. Indivíduos de cativeiro costumam ser alimentados com roedores, e em frequência maior do que os da ilha, o que pode fazer com que eles cresçam mais e fiquem mais pesados, o que pode comprometer, por exemplo, o uso da vegetação por estes animais, prejudicando o seu deslocamento e também a caça de aves. Além disso, por estarem isolados, os indivíduos de cativeiro podem acabar ficando geneticamente diferentes dos animais da ilha, o que também pode comprometer a sobrevivência da espécie. Deste modo, é fundamental que haja o monitoramento contínuo desses animais.

Para finalizar, a conscientização da população é fundamental para a conservação das espécies como um todo. As serpentes são corriqueiramente tidas como vilãs. Entretanto, elas são fundamentais para a manutenção das cadeias ecológicas. Serpentes que se alimentam de roedores, inclusive, associam-se diretamente ao controle de pragas e vetores de doenças. Além disso, as toxinas presentes no veneno das serpentes podem ter importância farmacológica. A partir das toxinas da jararaca-comum, por exemplo, foi desenvolvido o Captopril, um importante medicamento utilizado para controle da pressão arterial. Estudos recentes demonstram que as toxinas presentes no veneno da jararaca-ilhoa podem ter efeito antitumoral. A conscientização da população, portanto, é fundamental para a preservação do meio-ambiente, e também no combate ao tráfico desses animais, o que ressalta a importância de que os laços entre a ciência e as pessoas sejam estreitados, de modo que informações confiáveis cheguem até elas.

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Original Article

GROWTH PATTERNS OF THE CRITICALLY ENDANGERED *BOTHROPS INSULARIS*: A COMPARISON BETWEEN WILD AND CAPTIVE POPULATIONS

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ABSTRACT

Body size, growth rate, and maturity in snakes are influenced by food intake. The Golden Lancehead, *Bothrops insularis*, is a critically endangered viperid species, endemic to Queimada Grande Island. The diet of adults relies mainly on migratory birds, which peaks in March and July on the island. The aim of the present study was to evaluate the influence of such deprivation of food on the species' growth and maturity in comparison to captive individuals. Our findings reveal that both food intake, temperature, and reproductive requirements might influence body size, growth rate, and sexual maturity in *B. insularis*. More specifically, captive animals attain larger body size and mass, show higher growth rate, and anticipated sexual maturity in comparison to the wild individuals of the same sex, possibly as a result of higher food availability. Females are larger than the males of the same population, which is assumed to be associated with fecundity optimization. Fecundity also depends on energy reserve for vitellogenesis, and, due to the metabolic costs involved, females take a longer time to mature, showing, therefore, delayed maturity when compared to males. Also, because the energy storage depends on the food intake, wild animals show delayed maturity in comparison to captive ones. Food abundance also reflected in greater relative body mass in captive females. Unlike males, which probably convert the energy obtained from feeding in sperm production, besides growth, females possibly store such energy as fat, as a caloric reserve.

Keywords: Viperidae; Golden Lancehead; food intake; sexual maturity; *in situ* conservation; *ex situ* conservation.

1. INTRODUCTION

As with many species of animals, snakes show continuous and undetermined growth throughout their lives (Shine & Charnov, 1992; Saint-Girons, 1994). Growth rate relies on several factors, such as genetic constitution, availability of resources (such as food and water), climatic conditions, and success of capturing and digesting prey (Macartney *et al*., 1990; Saint-Girons, 1994; Lillywhite, 2014). It also varies according to age, so that snakes usually grow early in life, with growth rate decreasing after sexual maturation, closely following an asymptotic pattern (Shine & Charnov, 1992; Lillywhite, 2014).

The reason why growth rate decreases after attaining sexual maturity is that the energy which was previously mobilized for growing starts being directed to the development of the testes and behaviours associated with reproduction (Lillywhite, 2014). For males, it involves the development of the testicles and hypertrophy of the annex glands such as the epididymis and the sexual segment of the kidneys (Saint-Girons, 1994). As for the females, a great energy intake is necessary for the vitellogenesis to occur previously to ovulation, and this process is so bioenergetically expensive that the costs may outweigh those of pregnancy itself (Saint-Girons, 1994; Van Dyke & Beaupre, 2011). For these processes, snakes may allocate either stored reserves (the so-called capital breeders), or energy obtained from recently-ingested food (the income breeders; Van Dyke *et al*., 2012).

For ectotherms, there is also the so-called temperature-size rule, that states that growth is strongly related to temperature, and these species tend to grow slower and mature at a larger body size (Angilletta *et al*., 2004). Individuals of *Thamnophis sirtalis* and *T. elegans* grow faster in warmer temperatures (Arnold & Peterson, 1989; Bronikowski, 2000; Gangloff *et al*., 2015). On the other hand, this pattern was not observed in another species from temperate locality, *Vipera aspis* (Michel & Bonnet, 2010). Nevertheless, data on neotropical species are still lacking.

Bothrops insularis is a viviparous species of the *Bothrops jararaca* complex (Alencar *et al.*, 2016). It is one of the most arboreal species of the genus, and shows some morphological differences when compared to congeneric species (Amaral, 1921; Martins *et al*., 2002; Wüster *et al*., 2005). However, similarly to other *Bothrops* species, females are larger than males (Marques *et al*., 2013). The Golden Lancehead is endemic to Queimada Grande Island (QGI, São Paulo, Brazil), and is classified as a critically endangered species due to its restrict distribution and populational decrease (Silveira *et al*., 2021). For this reason, the National Action Plan for the Conservation of the Threatened Insular Herpetofauna recommends the establishment of captive populations in order to ensure the species' *ex-situ* conservation (Bataus & Reis, 2011). Therefore, the Laboratório de Ecologia e Evolução (LEEV) of Instituto Butantan has housed a captive population of *Bothrops insularis* since 2009. As mentioned above, food availability affects growth rate, and prey can be a limited and ephemeral resource for island snakes (Lillywhite, 2014), such as *B. insularis*. Captive specimens of Golden Lancehead at LEEV receive water and food frequently, being monthly fed on mice when adults, while population at QGI feeds mainly on migratory birds, especially the tyrant *Elaenia chilensis*, that visits the island in mid-March (Marques *et al*., 2012). When in the island, these birds seek for fruits or insects mainly on the vegetation, which may cause the Golden Lanceheads to displace vertically, so that they are seen more often using this substrate in March than in other months (Marques *et al*., 2012). Fed Golden Lanceheads may be found throughout the year, but the frequency of fed animals is rather negligible in comparison to that found in March (Marques *et al*., 2012).

It has been shown that food intake may influence snakes' growth, so that scarcity of prey lead to decreased growth rate (Lindell & Forsman, 2011), and supplementation of prey may cause snakes to be larger (Ford & Seigel, 1994). Likewise, food intake may influence reproductive traits, causing females to show early maturation or even causing the number of reproductive females in "good" years of food availability to outnumber that of when prey was scarce (*e.g.* Ford & Seigel, 1994; Shine & Madsen, 1997). Such influences are even more pronounced when this higher food supply happens in the first years of life, causing a "silver spoon effect" (Ford & Seigel, 1994; Madsen & Shine, 2000). Also, growth patterns differ between sexes in sexually dimorphic species (Madsen, 1983; Plummer, 1985). Given the differences in resources availability to which the captive and the wild populations of *B. insularis* are submitted, the aim of the present work is to test the hypotheses that: (1) captive animals attain larger size and greater mass than the wild ones; (2) sexual maturity (inferred by the animals' SVL, based on Marques *et al*., 2013 and Passos, 2018) of wild animals is delayed in comparison to that of captive ones; and (3) growth rate is higher in captive individuals.

2. MATERIALS AND METHODS

2.1. Data Collection

Captive individuals, as mentioned above, are housed at Laboratório de Ecologia e Evolução, Instituto Butantan. They are kept in boxes individually, in a room with photoperiod of 12:12h, with light phase from 6 a.m. to 6 p.m.. The animals are monthly fed on mice, representing 20% of their body mass, and they are annually measured and weighed. The captive population is now made up of three generations. We only used morphometric data from the two generations which were born in the laboratory, in order to avoid bias arising from the animals having lived a part of their lives in the island, and another part in captivity. In this sense, we used data from 15 females, and 20 males, that were from one to ten years old.

For the wild population, growth data were gathered data from mark and recapture events of animals in the field. The identification of Golden Lanceheads from Queimada Grande Island (QGI) with 12mm passive integrated transponder (PIT) tags is a routine procedure during our expeditions, and made it possible for us to recapture some of the animals (general recapture rate is around 13%; present study). These data were obtained during sampling trips from 2004 to 2023, comprising 35 females and 37 males (which corresponds to a recapture rate of 8%, after filtering for complete records), and included recaptures ranging from 3 months up to almost 8 years after capture.

For body size analyses, we used data of the same captive 15 females and 20 males. As for the wild population, we used data collected for 710 individuals (395 females and 315 males) encountered at QGI from 2004 to 2023. The morphometric data utilized were snout-vent length (SVL) measured from the tip of the nose to the cloaca and tail length (TL), measured from the cloaca to the end of the tail, using a measuring tape with accuracy of 1 mm, total body length (TBL; calculated by SVL + TL), and body mass, weighted using precision dynamometers (in the island; precision of 1 g) or precision scale (in the laboratory, precision of 0.1 g). In case of an individual being recaptured more than once in each field expedition, only the first biometric data were considered.

Daily air temperature and humidity were collected in captivity using thermohygrometer. Unfortunately, we only had meteorological station at QGI during short periods, therefore we considered data measured in the closest meteorological station from Southern littoral of São Paulo state, located in Bertioga (about 110 km away), provided by Instituto Nacional de Meteorologia (INMET, 2022). Previous analyses showed that these data were not normally-distributed, even after being log-transformed. Therefore, they were analysed using Paired Wilcoxon Test, in RStudio 2022.12.0 and The R Stats Package (R Core Team, 2022).

2.2.Growth models and rates

Two different methods were used, one for investigating growth of wild individuals, and another, for captive individuals, due to kind of data gathered for each population. In this sense, once we knew the birth date of all captive individuals, we used the von Bertalanffy Typical model (1; von Bertalanffy, 1938). Data for each sex was also analysed separately. Growth rate for each individual was calculated by the size it attained (SVL) at a certain age (given by time passed between birth and the last measuring event).

For analysing mark-recapture data of the wild population, the von Bertalanffy logistic-by-length model (2) was used (Fabens, 1965). We included literature data from the published literature as a parameter for size at birth (Marques *et al.*, 2013). Data for each sex was analysed separately using non-linear, least-squares regression. Should an individual have more than one recapture event, all of them were considered, taking into account the time interval between them.

(1)

$$L_t = L_\infty * \ (1 - e^{-K*(t-t0)})$$

(2)

$$L_r = L_m + (L_\infty - \ L_m) * (1 - e^{-K*\Delta t})$$

L_t is the average length in age t, L_∞ is the asymptotic average length, K is the growth rate coefficient, t_0 is the age when the average length is zero. L_r is the length at recapture event, L_m is the length at mark event and Δt is the time interval between mark and recapture events.

Growth Rate for the wild population was calculated as the difference in SVL in mark-recapture events divided by the number of days between these events, and each individual was used only once, so that we had 19 observations for females, and 27 for males. For captive animals, instead of mark-recapture, each measurement event was considered as one observation, resulting in 65 observations for females, and 82 for males.

2.3. Statistical Analyses

Differences in SVL and growth rate between sexes and populations were assessed by performing two-way ANOVA, with sex, populations and interactions as fixed factors. ANCOVA was performed for analysing the relative body mass and relative growth rate between groups (captive males and females, and wild males and females), using SVL as a covariate. In case of the interaction being significant, the residuals from the regressions were then extracted and used in an ANOVA, with sex and population as fixed factors. Only data of adult Golden Lancehead were used in the morphological analyses (SVL and body mass), for avoiding biases due to ontogeny. Adulthood in the Golden Lancehead was inferred from SVL, based on data reported in the literature (Marques *et al.*, 2013; Passos, 2018). Tukey analysis was used as a post-hoc test to determine the significance of pairwise comparisons between sexes and populations.

For the morphometric analyses of SVL and body mass, we used samples of all the adult animals captured at QGI for which we had such data (n = 435), and samples of all captive-born adult individuals of LEEV (n = 31). Each animal was only used once, and whenever possible, the most recently measurements were considered.

All analyses were performed in RStudio 4.0.2 (RStudio Team, 2020), using the packages FSA (Ogle *et al*., 2020), FSAdata (Ogle, 2019), nlstools (Baty *et al*., 2015), and plyr (Wickham, 2011).

3. RESULTS

3.1.Environmental data

Mean temperature in captivity ($\bar{x}$ = 23.7; range: 19 – 29.8ºC) was higher than in QGI ($\bar{x}$ = 22.6; range: 12.8 – 38.3ºC; V = 206233; $p < 0.05$). The opposite happened to humidity, that was higher at QGI ($\bar{x}$ = 86.1; range: 32.8 – 100%) than in captivity ($\bar{x}$ = 22.6; range: 28 – 95.5%; V = 6579967; $p < 0.05$).

3.2.Body Size

Some of the animals of the present study attained larger size than the maximum previously reported for the species by Guimarães *et al*. (2010) (females: SVL = 950, TL = 143, TBL = 1093 mm; males: SVL = 775, TL = 137, TBL = 912 mm; Table 1). The largest captive female showed 1250 mm in TBL (SVL = 1050; TL = 155 mm; body mass = 556 g), and the largest captive male was 945 mm in TBL (SVL = 810 mm; TL = 135 mm; body mass = 227 g). The largest female found during our expeditions at QGI was 1130 mm in total length (SVL = 1005 mm; TL = 125 mm; body mass = 240 g). Five captive-born females ranged from 1105 mm (SVL = 965, TL = 140 mm) to 1205 mm (SVL = 1050, TL = 155 mm) in TBL, and four captive-born males ranged between 920 mm (SVL = 780, TL = 140 mm) and 945 mm (SVL = 810, TL = 135 mm) in TBL. The captive females took a minimum of seven years, and the males, a minimum of six years to surpass the previous maximum size reported in literature.

Snout-vent length varied between populations ($F_{(1,737)}$ = 142.874; *P* = 0.000; Figure 1). The Tukey test revealed that captive females attain the largest SVL ($P < 0.001$), while wild males were the smallest amongst all (*P* = 0.000). Captive males were as large as wild females (*P* = 0.999). Linear regression revealed a positive correlation between body mass and SVL (R^2 = 0.69; *df* = 672; *P* = 0.000). Therefore, the residuals of this regression were extracted and used in an ANOVA with sex and population as factors. A significant effect of the interaction sex*population on body mass was observed ($F_{(1,670)}$ = 37.730; *P* = 0.000). The post-hoc Tukey test revealed that captive females showed the highest relative body mass amongst all (*P* = 0.000; Figure 2). Wild females were the relatively heavier than captive males (*P* = 0.00), and wild males were the lightest amongst all ($P < 0.001$).

3.3. Growth rates and curves

Data reported herein comprises 72 growth intervals, 39 of them (ranging from 22 to 4639 days) obtained from the recapture of 35 wild females, and 37 growth intervals (ranging from 32 to 2714 days) from the recapture of 21 wild males of *B. insularis*. Concerning captive Golden Lanceheads, growth rate was inferred from SVL data of 15 females (ranging from 376 to 3612 days of life) and of 20 males (ranging from 8 to 3612 days), considering one interval for each animal.

Even though a slight decrease in growth rate may be observed with SVL increase, such association was not significant (ANCOVA: $F_{(1,186)}$ = 0.750; *P* = 0.387). Therefore, we performed an ANOVA using sex and population as factors, which evinced that population affects growth rate ($F_{(1,221)}$ = 9.415; *P* = 0.002). Post-hoc tests reveal that wild males show the lowest growth rate amongst all (compared to captive females: *P* = 0.000; captive males: *P* = 0.009; and wild females: *P* = 0.020; Figure 3).

Asymptotic values show that females from QGI attain SVL up to about 850 mm, and the males, 600 mm (Figure 4A). Growth curves of the captive animals suggest that females grow up to 1000 mm of SVL, and males, up to about 750 mm (Figure 4B). Data from the literature show that minimum size at sexual maturity is 432 mm for males (Marques *et al.*, 2013) and 555 mm for females from QGI (Kasperoviczus, 2009; Marques *et al.*, 2013), and 403 mm for males and 619 mm for females from captivity (Passos, 2018). Based on such data, the growth curves suggest that captive females reach sexual maturity with about 3 years old, while captive males do so within less than one year. Wild females reach sexual maturity within 3.3 years, and the males, with 3.1 years old.

4. DISCUSSION

Our first hypothesis was partially confirmed: captive animals indeed attain larger body size than wild individuals of the same sex. Nevertheless, wild males are relatively heavier than captive ones, which, in turn, grow larger. The shorter, however relatively stouter body in captive males may represent a trade-off between growth and energy storage as fat. In both conditions (wild and captivity), females were the largest sex. Warmer temperatures may be related to higher growth rate in snakes (Arnold & Peterson, 1989; Gangloff *et al*., 2015). Given that mean temperature in captivity was higher than at QGI, this may explain why captive animals were larger or heavier. Humidity does not seem to have direct effects on growth rate. However, it must be considered that low humidity may be associated with disecdysis, whereas high humidity may cause dermatitis and lesions, especially in captive reptiles (Lillywhite & Gatten Jr., 1995; Oonincx & van Leeuwen, 2017), what may ultimately compromise health conditions and growth in these animals.

We also confirmed herein the marked sexual size dimorphism (SSD) reported for by *B. insularis* (Marques *et al*., 2013), with females being larger than the males, like several other congeneric species (Valdujo *et al*., 2002; Nogueira *et al*., 2003; Hartmann *et al*., 2004; Sasa *et al*., 2009; Nunes *et al*., 2010; Barros *et al*., 2014; Leão *et al*., 2014; Almeida-Santos *et al*., 2017; Stuginski *et al.*, 2017; Silva *et al.*, 2017; Silva *et al*., 2019; Silva *et al*., 2020; Siqueira *et al*., 2022). In reptiles, SSD may be the result of three main selective pressures: (1) sexual selection, (2) fecundity, and (3) reduction of intraspecific competition for prey (Cox *et al*., 2007). Female-biased SSD in the Golden Lancehead seems to be influenced by the two latter. *Bothrops insularis* has an ontogenetic dietary shift, with juveniles feeding on ectothermic prey as anurans, lizards and centipedes, while the adults feed on birds (Marques *et al*., 2002). Perhaps by being smaller, males may rely on such ectothermic prey longer than females. Additionally, as in several other snake species (Shine, 1991), females of the Golden Lancehead have larger head (Wüster *et al*., 2005), which could allow them to feed on larger prey, consequently attaining larger body size. As for the males, some hypotheses may be raised. They could be smaller than the females as a consequence of the reproductive costs associated with mating search, or perhaps being small makes them more agile, and the early maturation may increase their reproductive success (Silva *et al.*, 2020). Additionally, body size in arboreal snakes may influence substrate use by these animals, disfavouring larger and stouter animals (Alencar *et al*., 2017). Males may even be smaller simply because there would be no reproductive advantages or because larger size could be disadvantageous for habitat utilization (Madsen, 1983).

In snakes, fecundity is directly correlated to females' size, and both the evolution of viviparity, and fecundity are associated with the selection for larger females (Fitch, 1981; Shine, 1994; Aubret *et al*., 2002), as already evinced in *B. insularis* (Marques *et al*., 2013). Therefore, larger body size in females may be favoured, resulting in greater litter size, increase in offspring, and females with better body condition after parturition

(Hailey & Davies, 1987, Ford & Seigel, 1994, Shine & Madsen, 1997). Because they have larger head size, females may feed on larger prey, which represents an important input for balancing their great energetic expenditures on reproduction (Shine, 1991). In terms of relative body mass, it is noticeable that this was higher only in captive females. Because captive animals have higher food intake, possibly females are able to store the energy as fat for future reproductive events (Shine, 2003). It must also be considered that wild and captive animals feed on different kind of prey. While in the island the Golden Lancehead feed on birds, captive individuals feed on mice. Possibly the nutritional properties of these prey items are different, and may influence the energetic storage (Kremen *et al.*, 2013).

Mean fecundity of *B. insularis* from QGI was estimated in 8.2 offspring per litter (range: 3-20; Marques *et al.*, 2013). Up to this moment, five litters of the Golden Lancehead were born from the breeding among captive-born individuals. Fecundity (7.4 ± 3.65; range: 3-11; this study) was lower than that which was estimated for wild animals, but the low number of litter samples prevented us from performing statistical analysis to check whether such differences are significant. Nevertheless, these results are intriguing, given that captive females are larger than those from QGI. As previously mentioned, larger maternal body size may be associated with greater litter size or larger offspring. Litter mass, for instance, is influenced by maternal body size when the reproduction is delayed until the females will have stored energy enough to fill the body cavity with embryos (Shine, 2003). As aforementioned, fecundity in *B. insularis* is related to maternal body size (Marques *et al.*, 2013). Apparently, despite the differences in body size, litter size is not different in captive and wild females (this study). It can be hypothesized that the larger body size of captive females associates with increased litter mass.

Unfortunately, we lack morphometrical data of wild neonates, so this could not be tested in the present study.

It is important to consider the effects of this differential growth rate. For instance, increased body size may compromise the use of vegetation, and ultimately, of foraging in *B. insularis* (Banci *et al*., in prep.). Additionally, a negative correlation between fast growth and survival has already been evinced for snakes (Bronikowski & Arnold, 1999; Rose *et al*., 2021). As for the animals at QGI, slower growth and later maturation may impact *B. insularis* of recovering from population declines, as shown for other snake species (Blouin-Demers *et al*., 2006). Apart from the concerns aforementioned, additional care must be taken in order to prevent diseases. Reptiles which are overfed, that is, whose feeding frequency in captivity is not the same as in the wild, may result in rapid growth, obesity and secondary disease (Pellett & Wissink-Argilaga, 2015). Obesity may cause damage and even failure of the liver, ultimately leading a snake to death (Martins *et al*., 2018). These aspects are especially important for conservation *ex situ*.

Our second hypothesis was also confirmed: wild Golden Lancehead do have delayed maturation, in comparison to captive individuals. Also, following the pattern of animals which grow continuously and indefinitely throughout life, *B. insularis* showed a marked decrease in GR after maturation (Shine & Charnov, 1992), reaching even an asymptotic trend in the curves of captive males. Food input may influence maturation age (Ford & Seigel, 1994). The apparent ephemerality of food resources to which the wild population is subjected is clearly reflected by the growth curves.

Both males and females need to mobilize energetic reserves for the development of structures and behaviours associated to reproduction, and such energy comes from food (Saint-Girons, 1994). Males need energy for producing sperm and for searching for sexual partners. However, for viviparous female snakes, the reproduction involves high metabolic costs, especially during vitellogenesis, when mean metabolic costs represent about 30% of the total metabolic demand (Saint-Girons, 1994; Van Dyke & Beaupre, 2011). Therefore, it is expected that the females take a longer period in order to gather such energy, stored in the form of abdominal fat, what, in the case of the delayed maturity may increase litter mass (Shine, 2003). This is evident when we analyse the growth curve of captive animals. Even though both males and females receive proportionally the same amount of food, in the same frequency, females take three times that which males take to reach maturity. By comparing the growth curves of both populations, it is also evident that the delayed sexual maturity in *B. insularis* from QGI arises from a scarcity of resources, because when there is a dietary input, males may mature around one year old. This also reveals that the energetic requirements of males for reproducing are markedly lower than that of the females (Bonnet & Naulleau, 1996). Therefore, because costs of reproduction are lower in males, early maturation may increase their reproductive success (by producing more offspring during life), while delayed reproduction may maximize females' lifetime fitness (Madsen & Shine, 1994).

In our third hypothesis, we predicted that captive Golden Lancehead would show a higher GR when compared to those from QGI. This was confirmed only to males. Because body size is an important constraint for females' fecundity, it is notable that at QGI, GR in females is higher than that of males. Growth rate is influenced by variation in prey abundance, whether temporal or seasonally (Macartney *et al.*, 1990, Lindell & Forsman, 2011). Avian prey, the main food item of adults' diet, is limited and ephemeral at the island, so that this relative scarcity of resources could compromise GR of both males and females. However, because females are larger and have larger head (Wüster *et al*, 2005; Marques *et al.*, 2013) they may feed on larger prey, increasing their GR. It is remarkable that males in the island are the smallest and also show the lowest GR amongst all. It can be hypothesized that this is a consequence of constraints imposed by reproduction. Mate-searching may incur high energetic costs, with increased movement and activity of the males, as observed in many snake species (*e.g.* Shine, 2003; Jellen *et al*., 2007; Glaudas & Rodríguez-Robles, 2011; Bauder *et al*., 2016). At QGI there are two main forested areas, relatively separated by grassland. It has been shown that there is gene flow between *B. insularis* from these two areas (Salles-Oliveira *et al*., 2020), and such genetic similarity may be a consequence of males' movement during the reproductive season.

Body size may not represent an important reproductive constraint for the males, since their metabolic costs are lower than the females'. This is especially true for species in which males do not fight to access a female. In these species, SSD tend to be male-biased, with larger size of males being attained by prolonged growth after maturation (Shine, 1994). Therefore, when there are no advantages arising from size, or even when the larger size may represent a disadvantage in resource partitioning, natural selection may favour smaller males (Madsen, 1983). Because there is no apparent selective pressure for males to be larger, most of the energy obtained from food may be mobilized for reproductive purposes, instead of for growth.

In conclusion, our study suggests that body size, growth rate, and age of maturity in *B. insularis* is greatly influenced by food intake and costs of reproduction. For this reason, the wild population show smaller body size and delayed maturity in comparison to the captive one. Likewise, females show delayed maturity when compared to males of the same population, and wild males show the lowest GR amongst all. Concerning species conservation, the impact of the larger body size in captive animals on other traits, such as habitat use and survival, must be considered, especially if reintroduction of these animals become necessary. In this sense, a period in soft release methods would be crucial for better analysing such matters. As for the Golden Lanceheads in the island, it is mandatory that the conservation strategies encompass the maintenance of the population of the migratory birds.

DATA AVAILABILITY STATEMENT

The data set used or produced from this study and used in this manuscript can be obtained from the corresponding author.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

ETHICAL GUIDELINES

This study was authorized by the Ethic Commission for Animal Usage of Instituto Butantan (certificates CEUAIB #983-12 and # 1543170518), the Brazilian Ministry of the Environment (SISBIO #6295-7 and 16119), and the Brazilian Instituto of Environment and Renewable Natural Resources (IBAMA nº 25.650-1).

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Table 1: Mean ± standard deviation of snout-vent length (SVL), body mass (M) and growth rate (GR) of females and males of the captive and the wild population.

		SVL (mm)	**M (g)**	**GR (mm.day^{-1})**
CAPTIVE	**FEMALE**	921.25 ± 94.1	390.2 ± 157.2	0.21 ± 0.12
	MALE	732.9 ± 60.0	146.0 ± 38.0	0.17 ± 0.16
WILD	**FEMALE**	736.8 ± 74.4	196.2 ± 68.1	0.20 ± 0.16
	MALE	591.8 ± 64.7	85.2 ± 32.2	0.07 ± 0.09

FIGURE LEGENDS

Figure 1: Variation in snout-vent length in *Bothrops insularis* from the captivity (females: F CAPT; males: M CAPT) and from Queimada Grande Island (females: F QGI; males: M QGI).

Figure 2: Relative body mass in *Bothrops insularis* from the captivity (females: F CAPT; males: M CAPT) and from Queimada Grande Island (females: F QGI; males: M QGI).

Figure. 3: Growth rate of wild (A) and captive (B) females and males of *Bothrops insularis*, expressed in millimetres per day.

Figure 4: Growth curves of captive (A) and wild (B) males and females of *Bothrops insularis*. The intersection of the segments with the x-axis correspond to age of sexual maturity, and that with the y-axis corresponds to size of sexual maturity reported in the literature (see text).

FIGURE 1

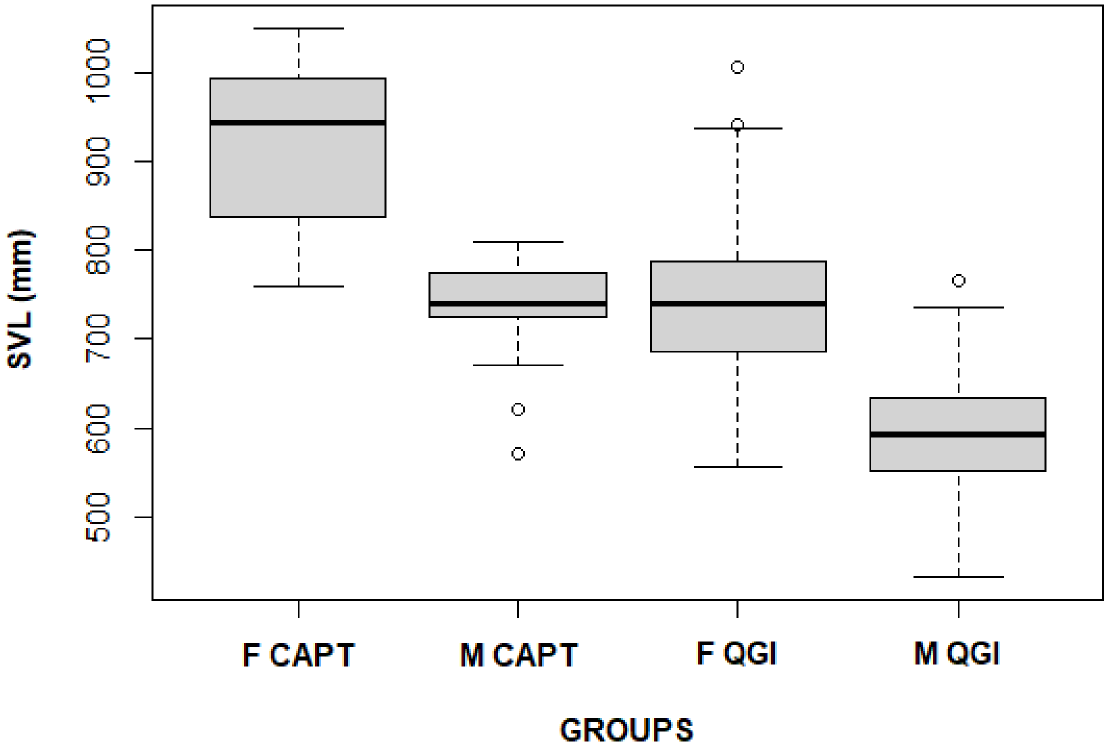

FIGURA 2

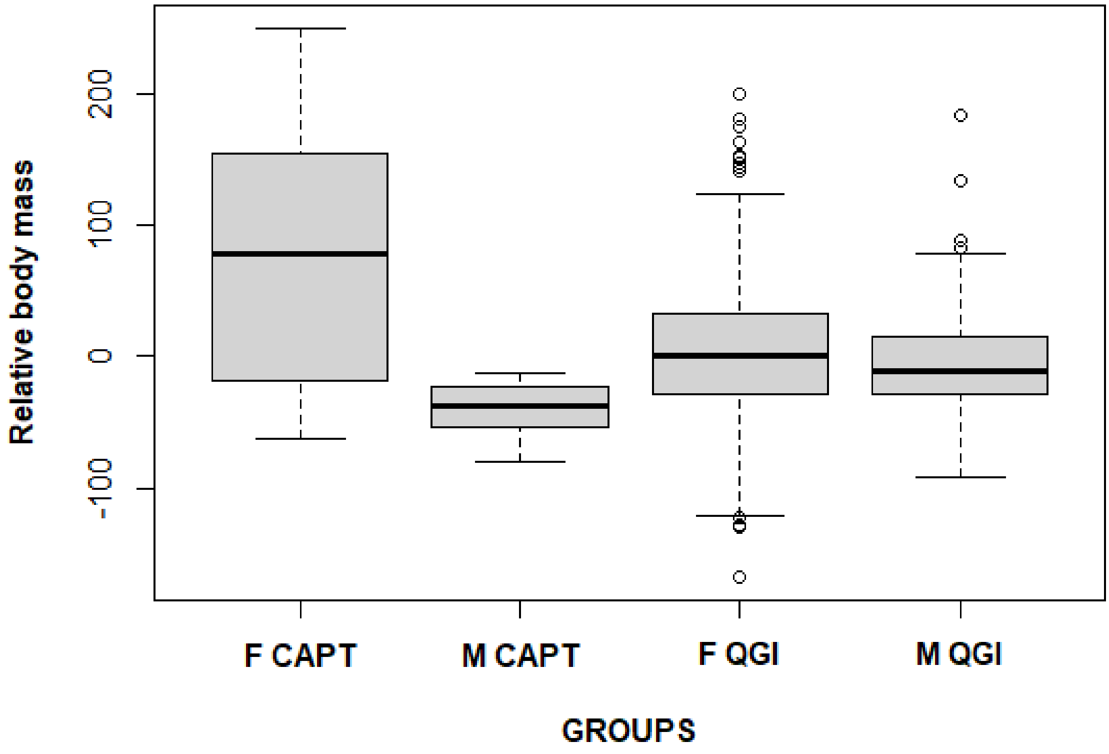
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FIGURE 3

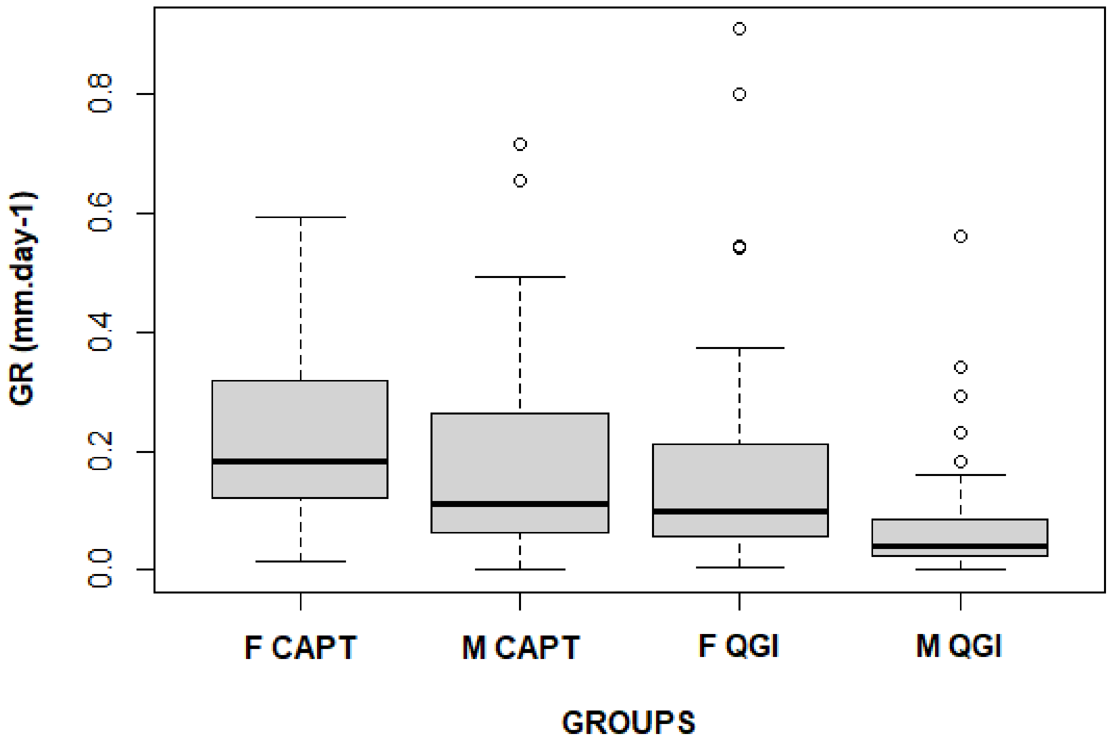
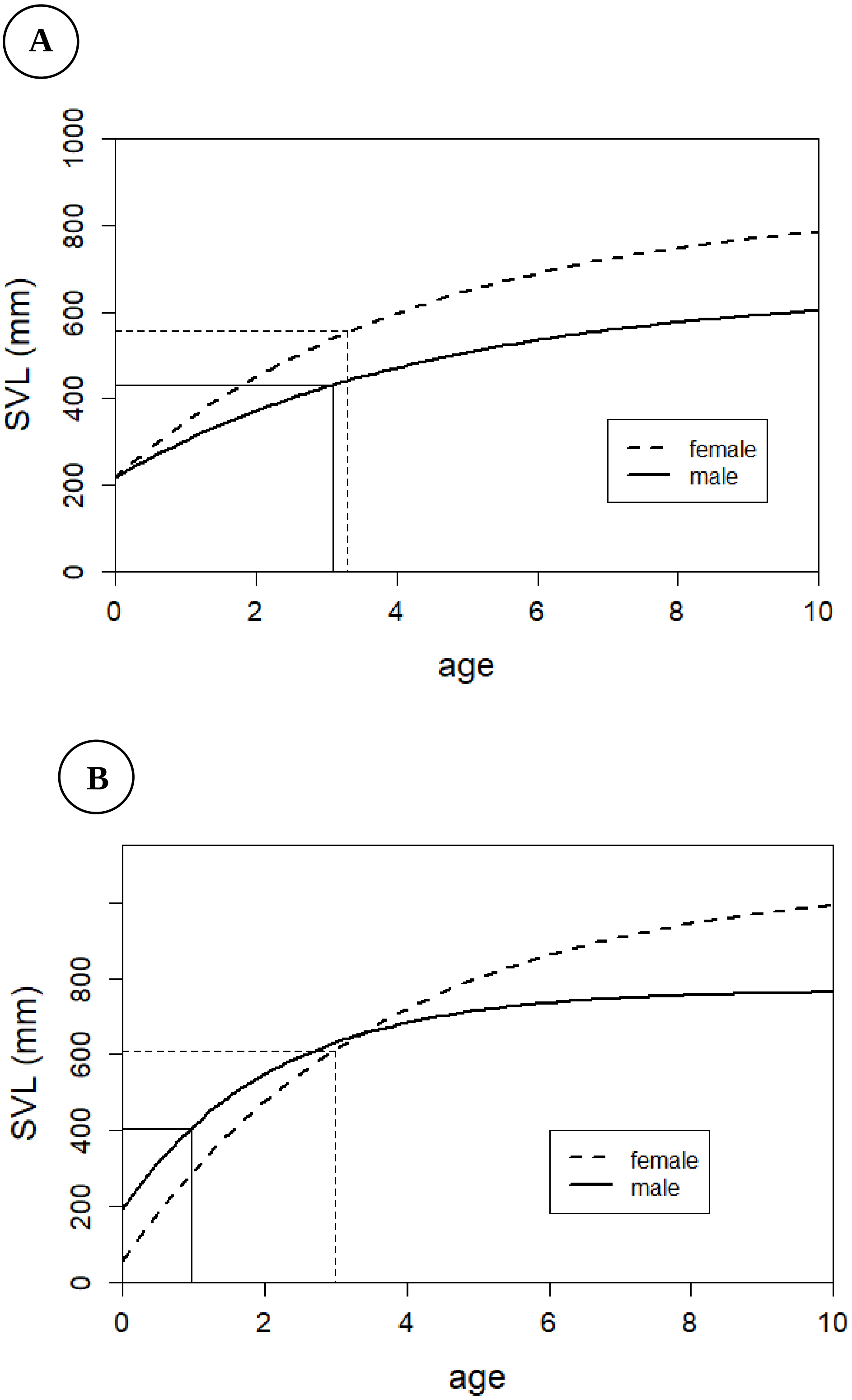


FIGURE 4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Original Article – to be submitted to Amphibia-Reptilia

LIVING IN THE DARKSIDE: ACTIVITY IN REPRESENTATIVES OF THE *BOTHROPS JARARACA* GROUP (SERPENTES, VIPERIDAE)

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Abstract

Species show different movement and activity patterns, due to several environmental and ecological factors, and this synchronicity is crucial for survival. Considering environmental cues, light-dark cycles are the most important *Zeitgeber*, and, as ectotherms, temperature may also be an important *Zeitgeber* for reptiles. The Golden Lancehead, *Bothrops insularis*, is a critically endangered species, endemic to Queimada Grande Island. The adults of the species rely mainly on migratory birds as prey, that visit the island mainly in March and July. For feeding on birds, *B. insularis* was first described as a diurnal species. For being observed active also during the night, it has been considered a diuturnal species. However, this trait had not been studied in detail, yet. Here, we present comparative data on the daily activity of representatives of the *Bothrops jararaca* group, *B. insularis*, *B. alcatraz*, and *B. jararaca*, obtained with video recordings made during ten consecutive days for each species in captivity. The daily activity of captive *B. insularis* was also compared to that observed at QGI. We evince that, as *B. alcatraz* and *B. jararaca*, *B. insularis* is intrinsically nocturnal, even though it hunts during the day in the wild. We also show that seasonal fluctuations of temperature may shape the daily activity of *B. jararaca*, with activity extending throughout the day in colder temperatures. In summary, we show that the light-dark cycle acts as the main

Zeitgeber for these three species, but temperature and prey availability may also shape their daily activity

Keywords: Daily activity; Golden Lancehead; *Bothrops insularis*; *Bothrops alcatraz*; *Bothrops jararaca*.

Introduction

Species show different movement and activity patterns, due to several environmental and ecological factors. If the environment oscillates, species also need to oscillate in order to adapt (Marques *et al*., 1997). Considering environmental cues, light-dark cycles are the most important *Zeitgeber* (Aschoff, 1981). As ectotherms, temperature may also be an important *Zeitgeber* for reptiles (Oishi *et al*., 2010). Circadian organization in reptiles is, therefore, multioscillatory, composed of the retina, the pineal, and the parietal eye, that contain circadian clock and photoreceptors (Oishi *et al*., 2010). However, little is known about activity and chronobiology of snakes. The fortuitous encounter of these animals in the field, allied to their secretive habits make it very difficult to investigate their activity pattern under natural conditions. For this reason, information frequently comes from scattered sources in the literature. For instance, activity of a given species of snake may be inferred from feeding or reproductive events reported, or from the encounter of active individuals in the field during surveys.

Some studies focused on characterizing both daily and seasonal activity of snakes in the field, showing the influence of environmental factors such as temperature, precipitation and humidity on such trait (*e.g.* Diller & Wallace, 1996; Daltry *et al*., 1998; Marques *et al*., 2001; Oliveira & Martins, 2001; Brown & Shine, 2002; Maciel *et al.*, 2003; Turci *et al*., 2009; Eskew & Todd, 2017; Siqueira *et al*., 2021). Moonlight may also affect activity of snakes (*e.g.* Clarke *et al*., 1996; Lillywhite & Brischoux, 2012). Studies using radiotelemetry have also been used for characterizing activity pattern of species (*e.g.* Wasko & Sasa, 2009; DeGregorio *et al*., 2018), and for assessing the influence of both abiotic and biotic factors on it (*e.g.* Slip & Shine, 1988; Sun *et al*., 2001. They have shown, for instance, that even discrete factors, as wind, may affect the activity of snakes (Sun *et al*., 2001). Prey availability (*e.g.* Slip & Shine, 1988; Brown & Shine, 2002; Christian *et al*., 2007; Siqueira *et al*., 2021) and events associated with reproduction also exert great importance on daily and seasonal activity of snakes (*e.g.* Slip & Shine; Glaudas & Rodríguez-Robles, 2011).

Ex situ approaches have also been used for characterizing the daily activity of snakes, utilizing closed-circuit television system (*e.g.* Parpinelli & Marques, 2008; Torello-Viera & Marques, 2017). Influence of prey availability on snakes' activity have also been evaluated using such methods (Torello-Viera *et al*., 2012). Locomotor activity in arenas was also used for inferring circadian rhythm in snakes (Cruz, 2007; Cruz *et al*., 2008; Stuginski *et al*., 2012). Griffiths (1984) evinced that the light-dark cycle acts as a *Zeitgeber* in *Eryx conicus*, whereas temperature exerts a masking effect, altering activity amplitude in this species. Nevertheless, there are still many gaps concerning knowledge on chronobiology of snakes.

Bothops jararaca group currently is associated with the Brazilian Atlantic Forest encompasses seven mainland or island species, namely *B. alcatraz, B. germanoi, B. insularis, B. jabrensis, B. jararaca, B. otavioi*, and *B. sazimai* (Barbo *et al*., 2022a, b). The mainland species *Bothrops jararaca* has the widest geographic distribution of the *jararaca* group (Campbell & Lamar, 2004; Barbo *et al*., 2022a, b). The natural history of this species is relatively well known (Sazima, 1988; Sazima, 1992; Almeida-Santos & Orsi, 2002; Hartmann *et al*., 2003; Janeiro-Cinquini, 2004). Data obtained in the field evince that the species is nocturnal, with activity peak between 6:20 p.m and 8:30 p.m., and found mostly during the wet season, with peak between December and February (Sazima, 1988, 1992). The Golden Lancehead, *Bothrops insularis*, is a critically endangered species, endemic to Queimada Grande Island (QGI), southeastern Brazil (Silveira *et al*., 2021). Its natural history is also relatively well known (Martins *et al*., 2008; Andrade *et al.*, 2010; Bovo *et al.*, 2010; Marques *et al*., 2012; Marques *et al*., 2013; Silva *et al*., 2015). Upon describing the species, Amaral (1921) mentions that, in spite of having pupils for nocturnal habits, the species also show diurnal activity (which are perhaps exclusive), conditioned by their feeding habits on birds, and that he observed active snake between 9 a.m and 11 a.m., and between 3 p.m. and 5 p.m. However, it is very common to observe individuals of this species active at night (pers. obs.). Despite being considered diurnal, more detailed studies on the daily activity of this species are still lacking. As the Golden Lancehead, *B. alcatraz* is also a critically endangered species (Marques *et al*., 2021), endemic to Alcatrazes Island, southeastern Brazil (Marques *et al*., 2002). The natural history of this species is the least well known amongst the three of them, but it has been classified as nocturnal (Marques *et al*., 2002; Marques *et al*., 2019; Marques, 2021).

In this sense, the goal of the present research is to characterize the biological rhythms of these three species in captivity, contrasting them with data obtained in the field.

Material and Methods

For characterizing biological rhythms of species, we used 13 individuals of *B. jararaca* (four females and nine males), five of *B. insularis* (three females and two males), and six of *B. alcatraz* (five females and one male), all housed at Laboratório de Ecologia e Evolução do Instituto Butantan , São Paulo, Brazil. The animals were individually placed in terraria (50 x 35 x 40 cm in length, width and height, respectively), with earth and *sphagnum* as substrate, and water *ad libitum*. In order to avoid bias arising from manipulation, animals were kept untouched in the terraria for three days before the experiments.

Daily activity was assessed by utilizing a closed-circuit television system with cameras provided with infrared light for nocturnal filming. For being ambush species, the identification of activity in viperids may be challenging (Oliveira & Martins, 2001), especially when watching videos with limited visibility. For this reason, locomotor activity was used for evaluating activity herein (Torello-Viera & Marques, 2017). At each hour interval, activity was evaluated, and scored as (0) if the snake was inactive more than half of the time, (0.5) if it showed in intermediate level of activity, and (1) when it was active most of the time. These records were then utilized for constructing the actograms and graphs for characterizing the activity pattern of each species.

We also used data obtained in the field for assessing daily activity of *B. insularis*. During field campaigns at QGI from 2004 to 2023, we gathered 538 records (301 females and 237 males). Activity in these animals was identified based on their posture (Oliveira & Martins, 2001), movement or feeding.

Activity data obtained in captivity were analyzed using binomial logistic regression, having activity as the variable of interest (0 = inactive; 1 = active), and photophase (with light phase between 6 a.m. and 6 p.m.), and mean air temperature and humidity per hour as predictors, and both of them were not artificially controlled. *Bothrops insularis* and *B. alcatraz* were simultaneously filmed during the rainy season, so season was not included as a predictor factor. Because *B. jararaca* was filmed in the beginning (autumn) and in the middle of the dry season (winter), season was included as a predictor for this species. Sex was not included as a predictor due to the low samples. For analyzing data of *B. insularis* from QGI, we also used binomial logistic regression, and activity was the variable of interest. Because *B. insularis* is considered thermoconformers (Bovo, 2009), we used body temperature obtained during the encounter as a predictor. Season was also included as a predictor variable when analyzing activity of snakes from QGI, with rainy season between October and March (Morelatto *et al*., 2000). Unfortunately, it was not possible to measure air humidity in the encounter of most animals, so this variable was not included as a predictor. Binomial logistic regression was also used for comparing levels of activity between species (having activity as variable of interest, and species as predictor).

Analyses were performed using RStudio 2022.12.0 and The R Stats Package (R Core Team, 2022). This study was conducted under authorization of the animal ethics committee of Instituto Butantan (CEUA nº 4920050919, and CEUA nº 1543170518), and of the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO # 63295-12).

Results

In the rainy season, when both *B. alcatraz* and *B. insularis* were filmed, temperature ranged from 19 to 36.8ºC, and humidity ranged from 37.8 to 64%. During the autumn temperature ranged from 14.4ºC to 30.2ºC, in the winter this interval was much narrower, ranging from 14.5ºC to 18.1ºC. Humidity ranged from 34% to 97% in the autumn, and from 35% to 98% in the winter. All species recorded in captivity are nocturnal (see figures from 1 to 8, and coefficients in Table I). However, during the winter, *B. jararaca* is active throughout the day (Figure 4). In comparison to autumn, *B. jararaca* is less active during the winter. *Bothrops alcatraz* showed the lowest levels of activity amongst the three species, followed by *B. insularis*, whereas *B. jararaca* showed the highest levels. Neither temperature nor humidity influence activity of *B. alcatraz* and *B. jararaca* filmed either during the autumn or the winter. In spite of humidity not affecting activity of *B. insularis*, temperature has a negative effect on it.

As mentioned above, we had 536 observations of activity for *B. insularis* at QGI, and 290 (54.1%) out of them were considered as active (75.9% of them in ambush posture, 23.4% moving, and 0.7% feeding; fig. 9). Our observations at QGI showed that there is no difference in the frequency of individuals moving during the day or night under natural conditions. On the other hand, specimens ambushing or preying on prey were found predominantly during the day. In spite of the higher number of observations in the rainy season (58.2%, 45.5% of which were active), animals were mostly found active during the dry season (fig. 10). Activity of *B. insularis* was not influenced neither by sex, nor temperature.

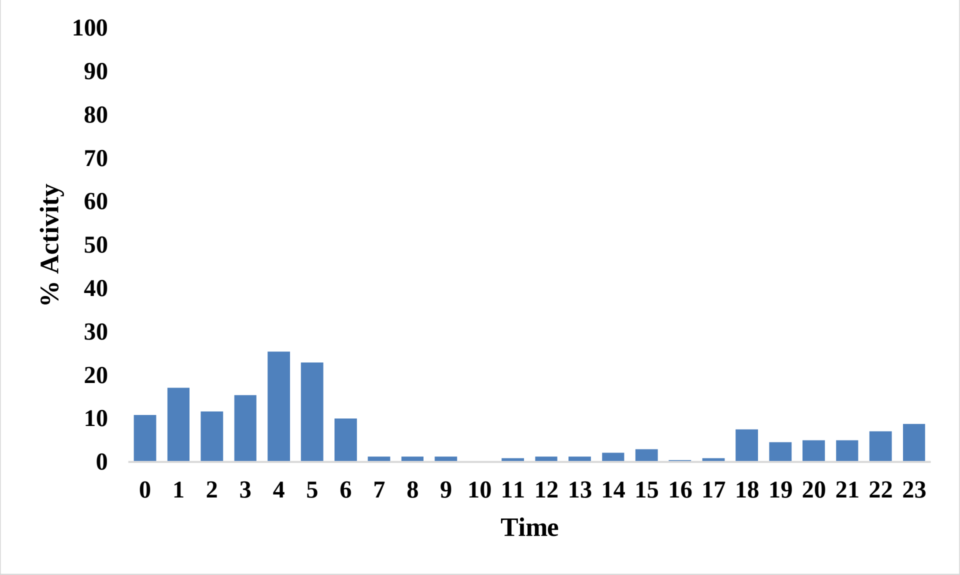

Fig. 1: Daily activity of *Bothrops alcatraz*.

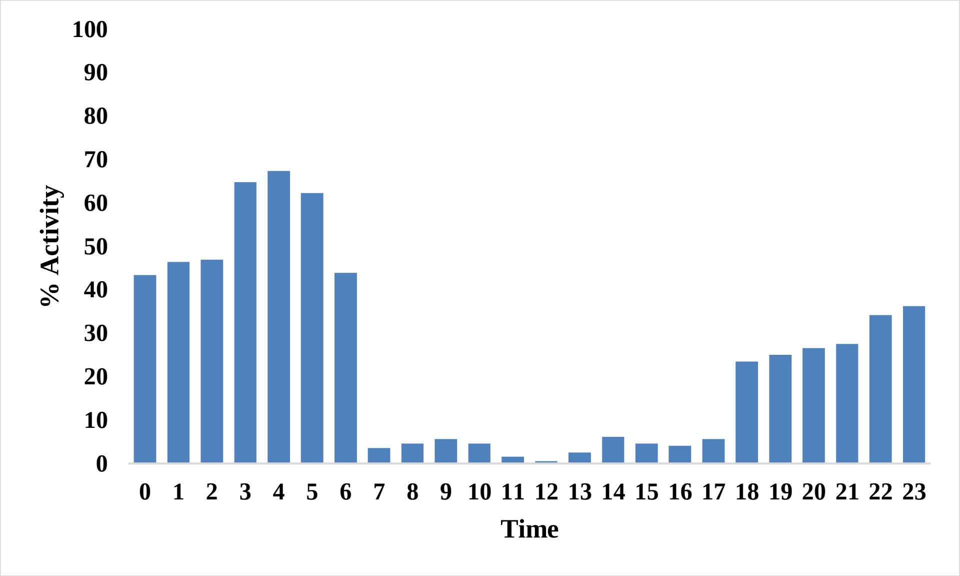

Fig. 2: Daily activity of *B. insularis*.

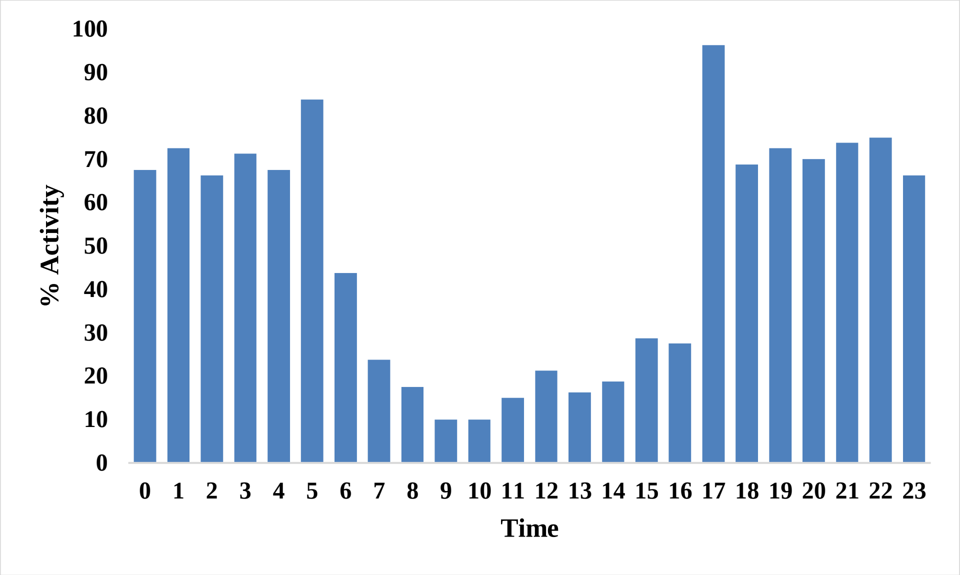

Fig. 3: Daily activity of *B. jararaca* during the autumn.

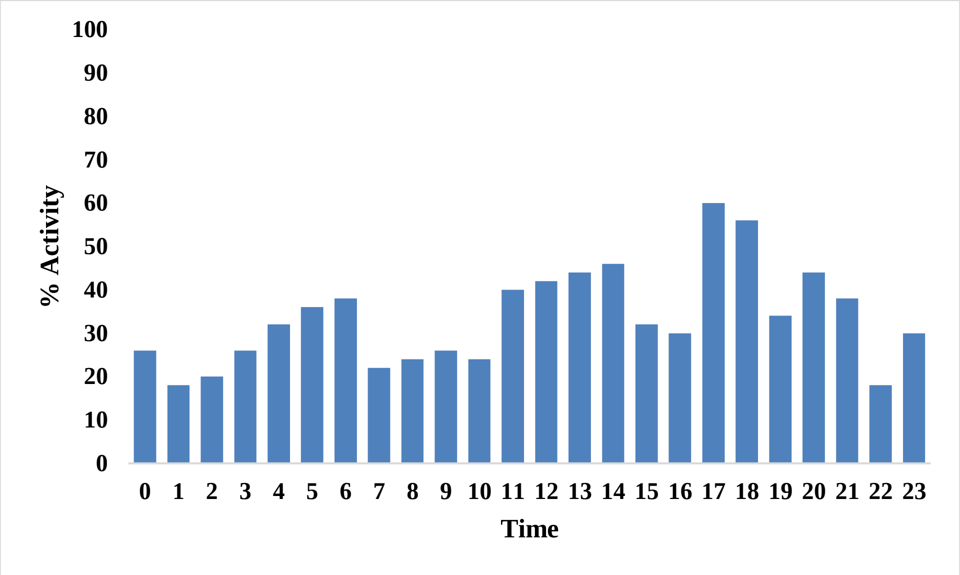

Fig. 4: Daily activity of *B. jararaca* during the winter.

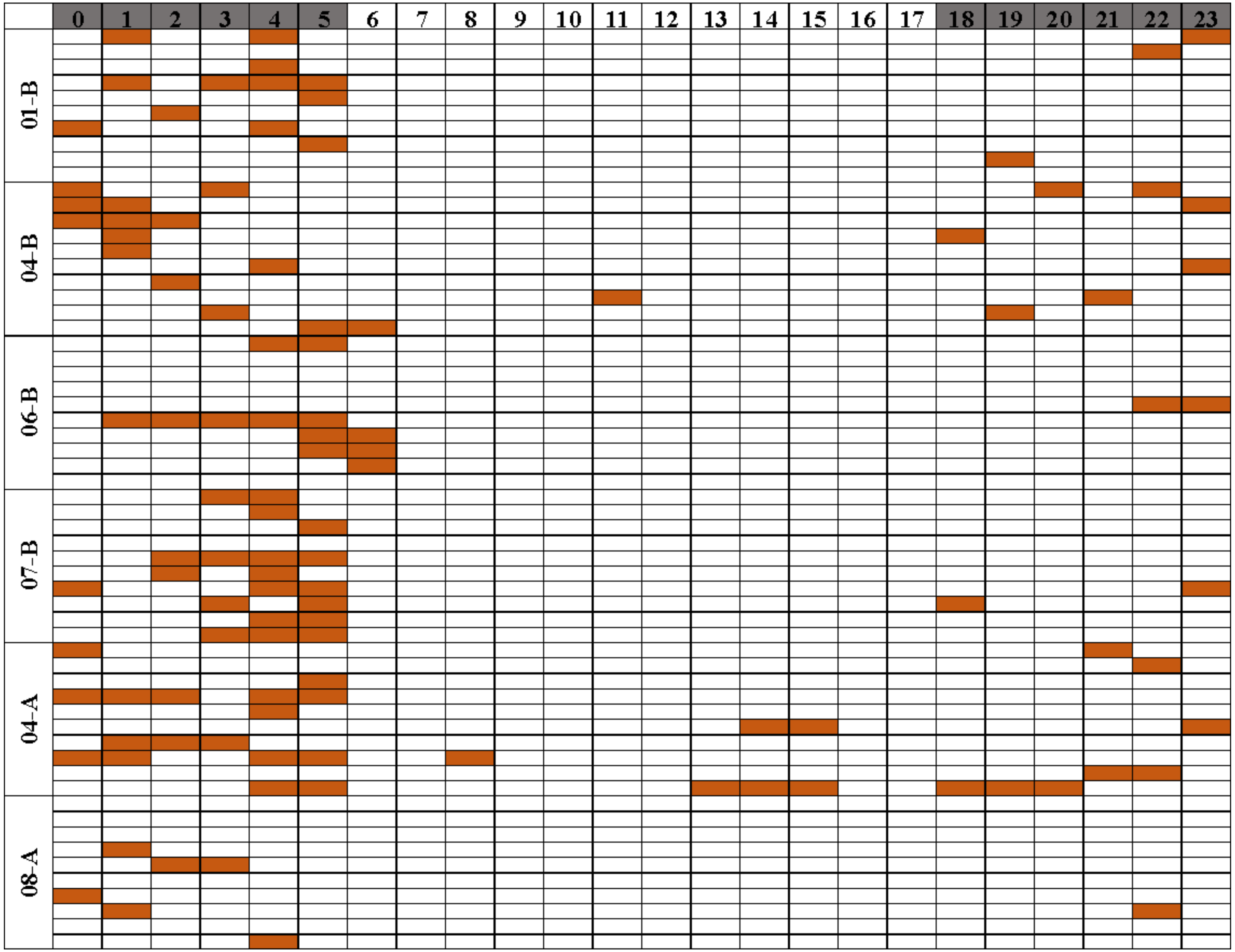

Fig. 5: Actogram representing daily activity of six individuals of *B. alcatraz* (identified in the left column). Hour intervals are indicated in the top row, with fields in grey representing the dark phase. Fields in orange show the periods in which animals were active.

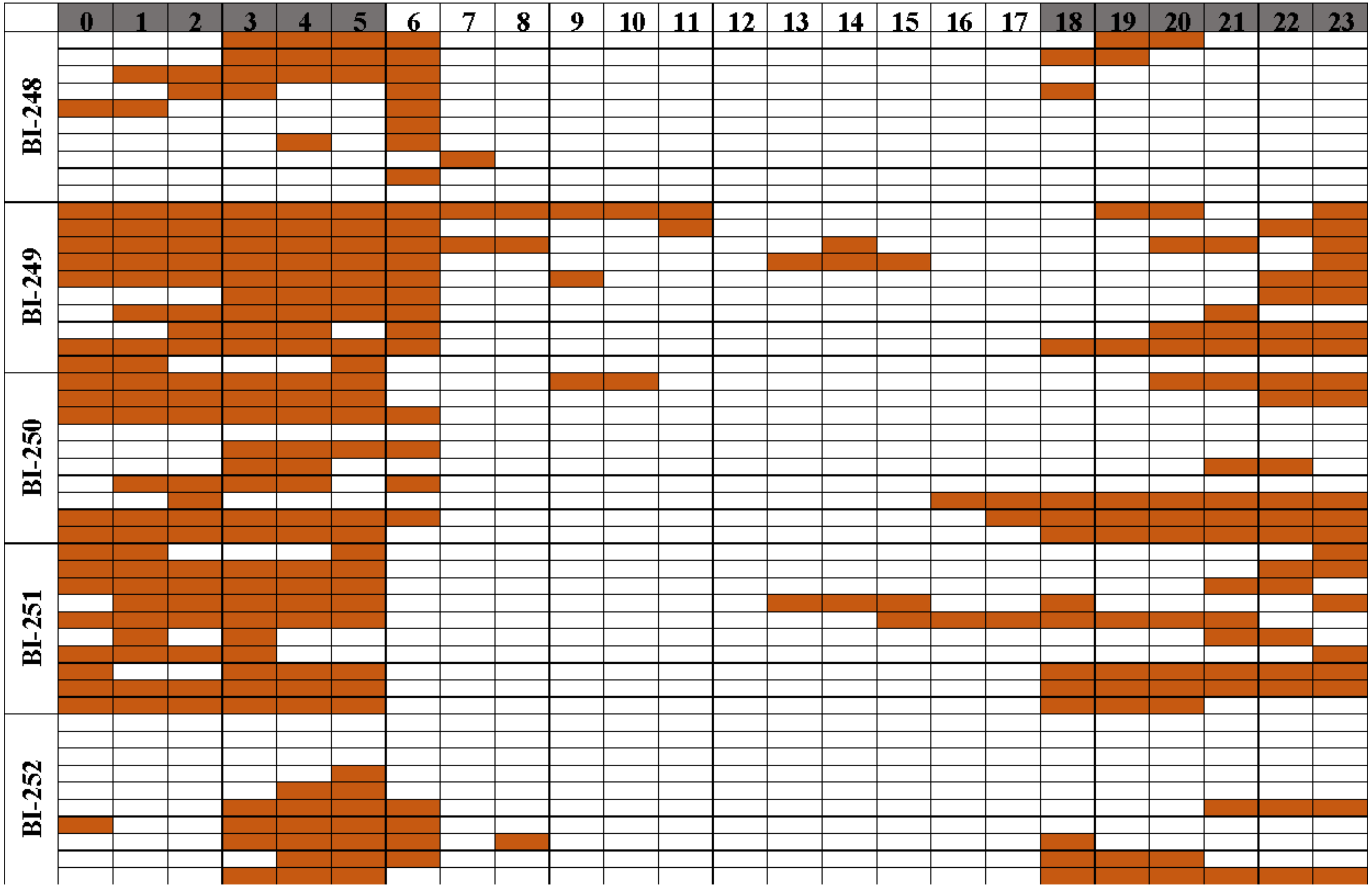

Fig. 6: Actogram representing daily activity of five individuals of *B. insularis* (identified in the left column). Hour intervals are indicated in the top row, with fields in grey representing the dark phase. Fields in Orange show the periods in which animals were active.

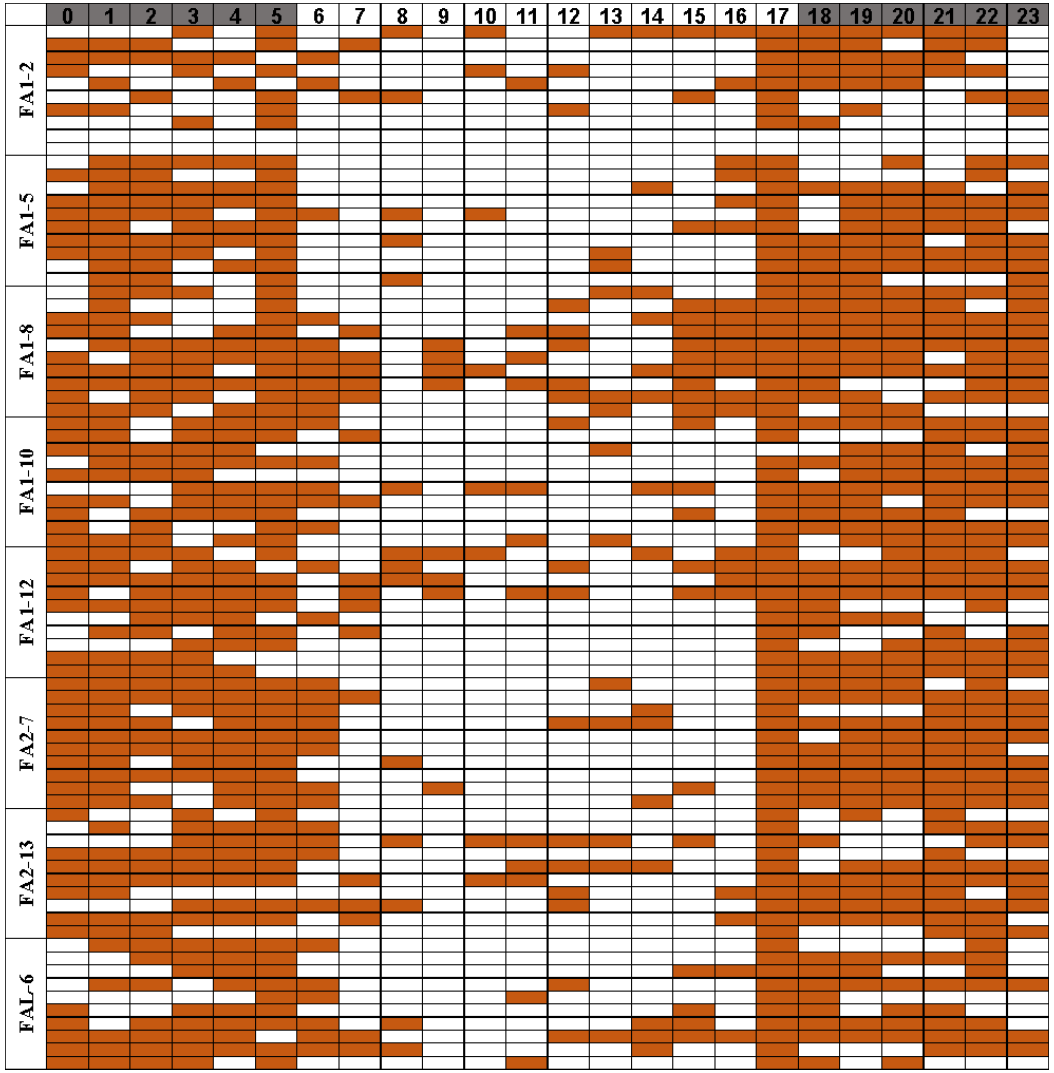

Fig. 7: Actogram representing daily activity of five individuals of *B. jararaca* (identified in the left column) filmed during the autumn. Hour intervals are indicated in the top row, with fields in grey representing the dark phase. Fields in orange show the periods in which animals were active.

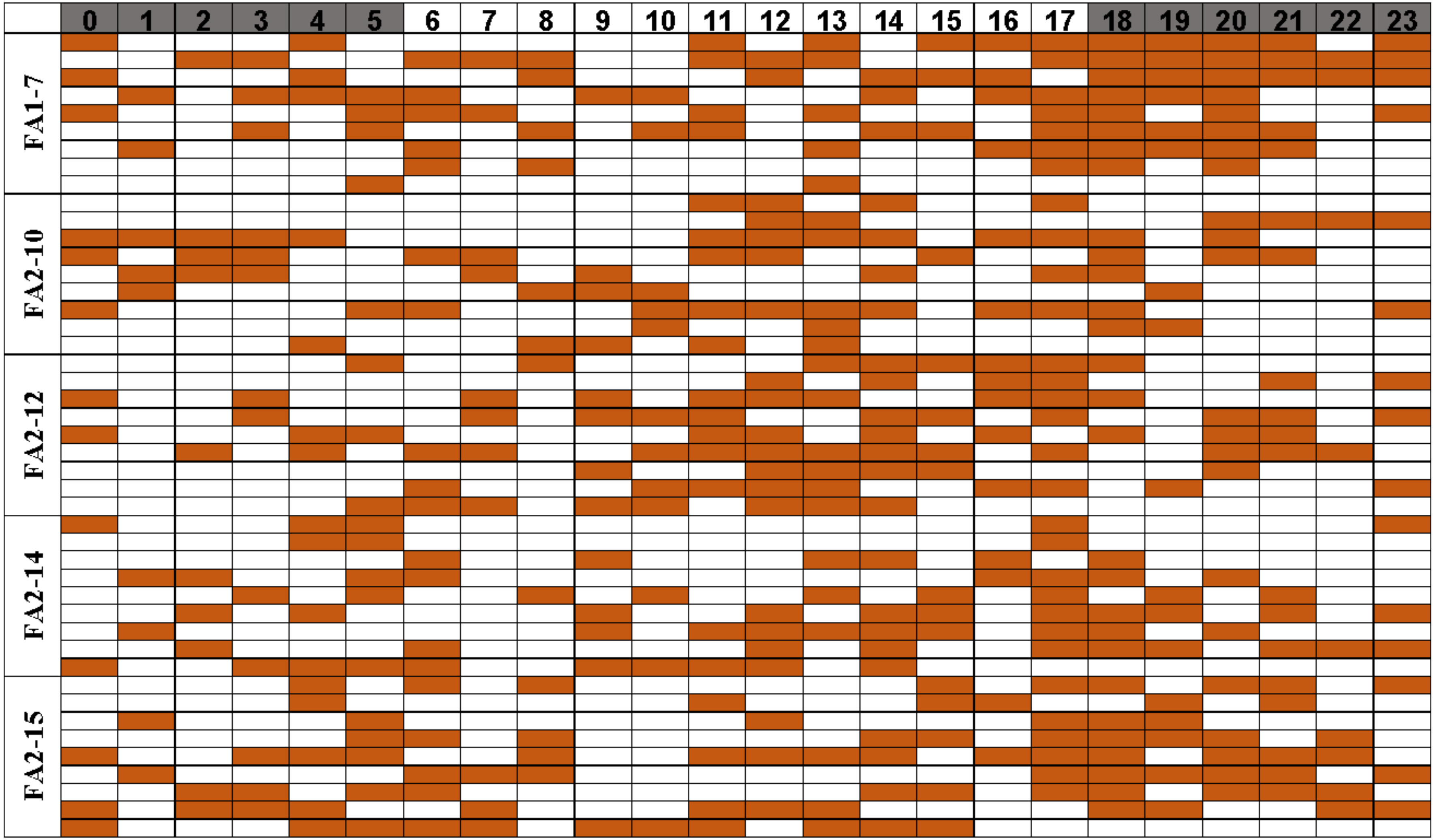

Fig. 8: Actogram representing daily activity of five individuals of *B. jararaca* (identified in the left column) filmed during the winter. Hour intervals are indicated in the top row, with fields in grey representing the dark phase. Fields in orange show the periods in which animals were active.

Table I: Coefficients of the regression models used for analyzing daily activity of *Bothrops jararaca* group in captivity.

Species	Activity level (ref. level: inactive)				Photophase (ref. level: day)				Temperature				Humidity			
	β(SE)	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**
B. alcatraz	-2.4827(0.099)	0.084	0.0688 – 0.101	**< 0.05**	2.308(0.327)	10.055	5.298 – 19.081	**< 0.05**	-0.056(0.037)	0.946	0.880 – 1.016	> 0.05	0.031(0.027)	1.032	0.978 – 1.088	> 0.05
B. insularis	1.4710(0.118)	4.353	3.452 – 5.491	**< 0.05**	2.142(0.175)	8.519	6.045 – 12.007	**< 0.05**	-0.134(0.270)	0.875	0.829 – 0.923	**< 0.05**	0.018(0.020)	1.019	0.980 – 1.059	> 0.05
***B. jararaca* (autumn)**	2.054(0.105)	7.801	6.353 – 9.580	**< 0.05**	1.858(0.124)	6.411	5.030 – 8.172	**< 0.05**	-0.055(0.409)	0.946	0.873 – 1.025	> 0.05	0.006(0.008)	1.006	0.991– 1.021	> 0.05
***B. jararaca* (winter)**	-0.545(0.079)	0.58	0.497 – 0.677	**< 0.05**	0.062(0.129)	1.064	0.827 – 1.368	> 0.05	0.081(0.069)	1.084	0.946 – 1.242	> 0.05	0.006 (0.014)	1.006	0.980 – 1.034	> 0.05

Table II: Coefficients of the regression models used for analyzing daily activity of *Bothrops insularis* at Queimada Grande Island.

B. insularis	Photophase (ref. level: day)				Temperature				Season (ref. level: rainy)				Sex (ref. level: female)			
	β(SE)	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**	**β(SE)**	**OR**	**IC (95%)**	**p**
	1.538(0.238)	4.657	2.923 – 7.420	**< 0.05**	0.040(0.040)	1.041	0.963 – 1.125	> 0.05	1.303(0.271)	3.679	2.164 – 6.255	**< 0.05**	-0.046(0.188)	0.955	0.661 – 1.380	> 0.05

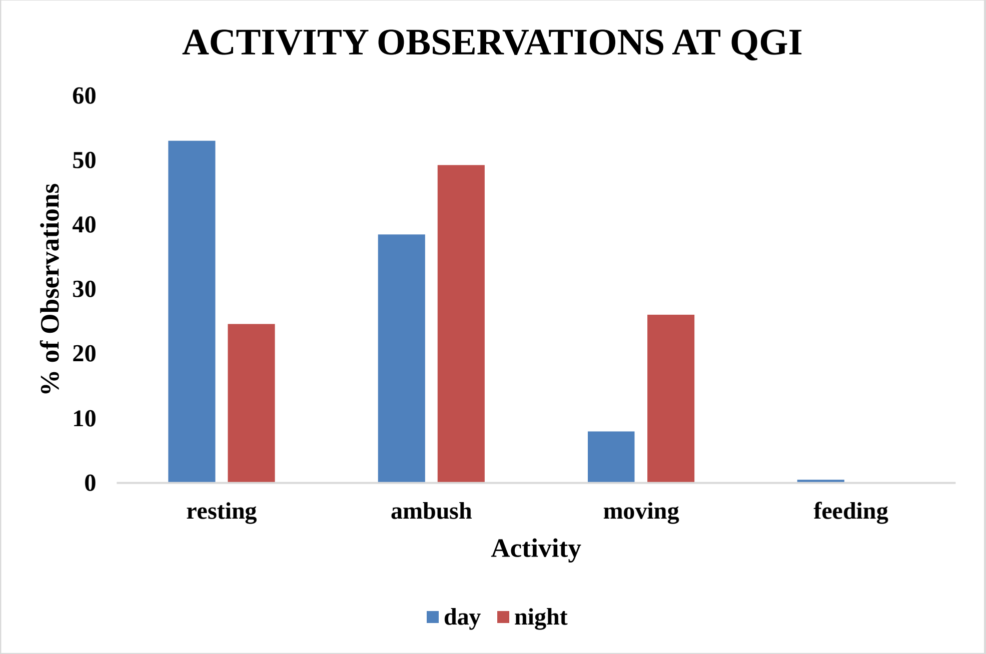

Fig. 9: Activity observations of *B. insularis* at Queimada Grande Island.

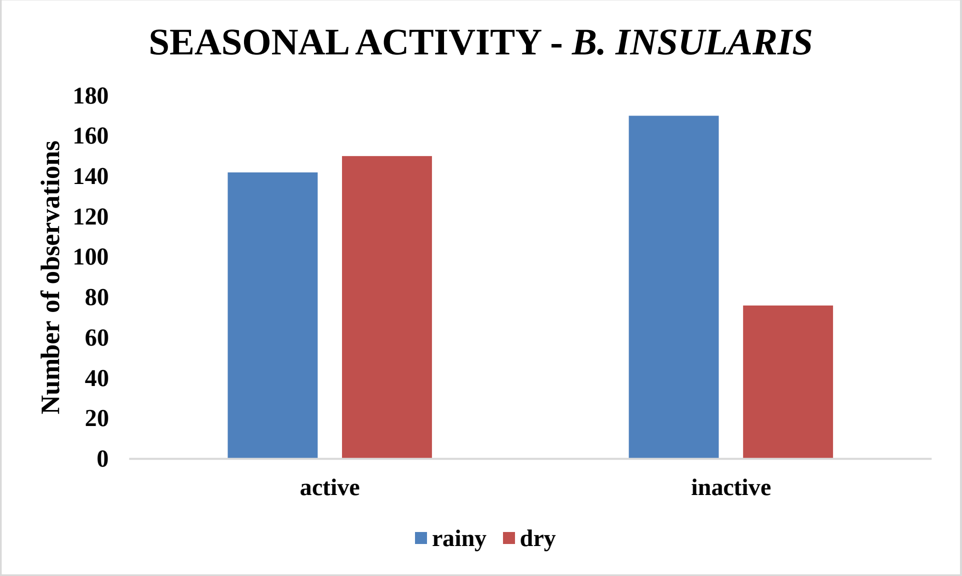

Fig. 10: Seasonal activity of *B. insularis* at Queimada Grande Island.

Discussion

Nocturnal activity is a trait of the genus *Bothrops* and has been reported for at least 17 species occurring in Brazil (Oliveira & Martins, 2001; Marques *et al*., 2005; Cruz, 2007; Cruz *et al*., 2008; Turci *et al*., 2009; Stuginski *et al*., 2012; Rocha *et al*, 2014; Marques *et al*., 2015; Marques *et al*. 2017; Marques *et al*., 2019), *B. leucurus* (Marques *et al*., 2019). Nocturnality has also been reported for other species of the *B. jararaca* group, *B. otavioi* (Barbo *et al*., 2012), and *B. sazimai* (Barbo *et al*., 2016). Concerning the three species studied herein, nocturnal activity had already been reported for *B. jararaca* under natural conditions (Sazima, 1988, 1992), and observation in the field had also pointed out for nocturnal activity in *B. alcatraz* (Marques *et al*., 2002). On the other hand, *B. insularis* have been classified either as a diurnal species (Amaral, 1921; Hoge, 1950) or as showing activity both during the day and the night (Marques *et al*., 2019; Marques, 2021). Our results evince that *B. alcatraz, B. insularis* and *B. insularis* are intrinsically nocturnal, and indicate that photophase may be the Z*eitgeber* for these species, as already evinced for other snake species (Griffiths, 1984). It is noteworthy that, even under natural conditions, *B. insularis* was nocturnal (present study).

Diurnal activity in this species has been attributed to its diet based almost exclusively on birds when adults (Amaral, 1921; Marques *et al*., 2012; Sawaya *et al*., 2023). Hoge (1950) even stated that, despite showing adaptions which are typical in nocturnal Crotalinae, the Golden Lancehead was forced to become diurnal in order to avoid becoming extinct. Our results obtained in captivity show that photophase exert a strong effect on its activity. But observations in the field suggest an increase of activity during the dry season. *Bothrops insularis* feed mainly on migratory birds, and one of this species, *Turdus flavipes*, visit the island in the dry season, with peak of abundance in July (Marques *et al*., 2012). The most important prey of *B. insularis*, *Elaenia chilensis*, visits QGI mainly in March, what corresponds to the rainy season (Marques *et al*., 2012). However, it is possible to observe this species in the island also in the beginning of the dry season (pers. obs.). One of the activity observations included in our analyses is of an individual of *B. insularis* which was feeding on an *E. chilensis* in the end of April. Therefore, high activity during the dry season may be associated with prey availability. Additionally, it must be considered that courtship and mating of *B. insularis* occurs in autumn (Marques *et al*., 2013), during the dry season, what may explain the higher activity during this time of the year.

In captivity, photophase was the only predictor which exerted effect on the activity of the Golden Lancehead. As aforementioned, the diurnality of *B. insularis* have been attributed to its diet based on birds, which are diurnal (Amaral, 1921; Hoge, 1950; Marques *et al*., 2012). Possibly, prey availability exerts a masking effect on the biological rhythm of snakes. Studies carried out in captivity have already suggested that prey availability may influence endogenous rhythm in snakes modulated by photoperiod. Thus, activity phase can change when prey is more available in nature (Torello-Viera & Marques, 2017). For example, diurnal activity has also been reported for *B. jararacussu* (Marques *et al*., 2019). This species was observed active both by day and night in the field, and in some occasions, the individuals were feeding (Hartmann *et al*., 2009, Marques & Araújo, 2011), evincing that prey availability may modulate activity under natural conditions. During our study, captive snakes were not fed, so it was not possible to evaluate this influence. Several representatives of *Bothrops* attract their prey by caudal luring (Martins *et al*., 2002), and we observed that some individual of all three species did this during the experiments. These data were not analyzed, but it is interesting that *B. insularis* was the only species that exhibited this behavior more frequently during the day than during the night. It has already been hypothesized that the coloration of the adults of Golden Lancehead and its tail tip would favor tail luring for attracting birds (Andrade *et al*., 2010), and it is interesting that the individuals filmed displayed such behavior, even though they were born in captivity and have been fed exclusively on mice.

Bothrops jararaca feeds mainly on anuran when juveniles, and on rodents when adults (Sazima, 1992). As *B. jararaca*, several anuran (Wells, 2007) and rodent species are nocturnal (Kilduff & Dube, 1979; Graipel *et al*., 2013; Patton *et al*., 2015). Nocturnal activity in *B. jararaca* may be, thus, favored by prey availability and low exposure to predators, as it has been already evinced by the seasonal activity patterns of this species (Siqueira *et al*., 2021). *Bothrops alcatraz* feeds mainly on nocturnal prey (centipedes *Otostigmus* sp., *Haddadus binotatus, Hemidactylus mabouia*; Marques *et al*., 2022; Guizze *et al*, 2016; Rocha & Anjos, 2007, Sawaya *et al*., 2023, Kasperoviczus, pers. comm.), so, by being nocturnal, this species synchronizes its activity with that of its prey. *Bothrops alcatraz* may be predated by tegus and birds (Banci *et al*., *in prep*.), that are diurnal predators (DuVal *et al*., 2006; Srbek-Araujo *et al*., 2020), so that this species is more secretive and less active during the day (Marques, 1921). Additionally, this species showed the lowest activity scores, what may be a consequence of the low energetic intake that this species ingests by feeding on ectothermic prey (Dierenfeld *et al*., 2002).

Other environmental variables, such as air humidity, may also affect activity of snakes (Daltry *et al*., 1998). In the present study, air humidity did not affect daily activity of none of the three species. Nevertheless, temperature had a negative effect on the activity of *B. insularis*. Differences in the temperature between periods may be also associated with differential daily activity of *Bothrops jararaca verified in the present study* during the autumn and the winter. During the autumn, the activity of this species was nocturnal, extending throughout the day during the winter. It is noteworthy that there was a pronounced difference in temperature amplitude between these two periods. Changes in activity of snakes may be associated with thermal constraints, so that in cooler periods, snakes may extend its activity to the warmer hours, or avoid them in otherwise conditions (Jackson & Franz, 1981; Diller & Wallace, 1996; Torello-Viera & Marques, 2017). The latter may be the case for *B. insularis*, that showed nocturnal activity, with increase in temperature causing decrease in activity. Among the three species, *B. insularis* seems to be the species which is the least resistant to water loss, being more dependent of forested areas (Marques, 2021; Banci *et al*., *in prep*.). This species is rarely encountered under intense insolation in open areas, and its skin seems to be thinner and more elastic than that of *B. alcatraz* and *B. jararaca* (Marques, 2021; pers. obs.). Studies on the physiology and skin histology may be valuable for better elucidating such issues.

In conclusion, the present study strengthens that nocturnality might be a conservative trait among representatives not only of the *jararaca* group, but of the entire genus *Bothrops*. Also, our data support that light-dark cycles may act as a *Zeitgeber* for these species, with temperature and feeding having masking effects on their biological rhythms.

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Original Article – To be submitted to Amphibia-Reptilia

DIFFERENTIAL PREDATION RATE IN ISLANDS: CAN PREDATORS SHAPE THE NICHE AND DEFENSIVE BEHAVIOUR OF INSULAR PITVIPERS *BOTHROPS INSULARIS* AND *B. ALCATRAZ*?

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Abstract

Animals are naturally exposed to several predators, and, given that a single encounter with a predator may cause prey's death, natural selection will strongly act upon its defensiveness, including morphological, physiological, life historical and behavioural aspects. Therefore, defensive behaviour is strongly shaped by predation pressure. Predation rate in islands tend to be relaxed due to lower abundance of predators in such areas. The objective of this study was to test the hypothesis that predation rates upon insular species would be lower than upon *B. jararaca*, their sister species from the mainland, and that, for this reason, *B. insularis* and *B. alcatraz* have different defensive reactions. The encounter of snakes in the field is usually fortuitous, making it very difficult to assess the predation pressure to which they are subjected to. Therefore, in the present study we used plasticine replicas for evaluating predation pressure on the critically endangered insular species *B. alcatraz* and *B. insularis*. Additionally, we tested their defensive behaviour to approach. Both predation rate and defensive behaviour were compared to those previously reported for *B. jararaca*. Our results indicate that predation rate on *B. insularis* at QGI is lower than that on *B. alcatraz* at Alcatrazes Island and on *B. jararaca* from an urban fragment in São Paulo Municipality, which are, in turn, lower than that on *B. jararaca* from continuous forested areas. Possibly for this reason, individuals of *B. insularis* are less defensive than those of *B. jararaca*, and tend to flee more often. The same was observed for *B. alcatraz*, but this may be related to its reduced size and low ability to defend against birds and tegus which may feed on these snakes, causing them to be more secretive than the other two species. Understanding the predation which species are subjected to, and its effect on their defensiveness is fundamental for their conservation, especially in the case of the critically endangered ones.

Keywords: *Bothrops jararaca* group; predation pressure; defense; plasticine replicas; conservation.

Introduction

Animals are naturally exposed to several predators, and, given that a single encounter with a predator may cause prey's death, natural selection will strongly act upon its defensiveness, including morphological, physiological, life historical and behavioural aspects (Kavaliers & Choleris, 2001). Defensive behaviours are both varied and plastic, apart from being context dependent (Cressler *et al*., 2010). There are several studies demonstrating how extensive and plastic the defensive behaviour of snakes may be, as well as how different factors may shape it (Greene, 1988; Allf *et al*., 2016), both intra (*e.g.* Arnold & Bennett, 1984; Brodie III & Russel, 1999) and interspecifically (*e.g.* Araújo & Martins, 2006).

Aspects which influence defensive behaviour encompass extrinsic and intrinsic factors. Studies both in the field and in the laboratory have usually evaluated these factors simultaneously and in different circumstances (Shine *et al*., 2002; Glaudas *et al*., 2005; Aubret *et al*., 2011). Among intrinsic factors, studies focused on a wide variety of aspects, such as heritability (Arnold & Barnett, 1984; Garland Jr., 1988; Torres-López *et al*., 2016), ontogeny (Shine *et* al., 2002; Placyk Jr., 2012; Torres-López *et al*., 2016), body size (Shine *et al*., 2000; Mayer *et al*., 2016), shedding (Glaudas *et al*., 2005), sex (Brodie III & Russel, 1999; Shine *et al*., 2000; Brown & Shine, 2004; Glaudas *et al*., 2005; Aubret *et al*., 2011; Maillet *et al*., 2015), and reproductive stage (Graves, 1989; Brown & Shine, 2004). Concerning extrinsic factors, most studies have focused on the influence of temperature on defensiveness of snakes (Goode & Duvall, 1989; Schieffelin & Queiroz, 1991; Keogh & DeSerto, 1994; Brodie III & Russel, 1999; Shine *et al*., 2000; Aïdam *et al*., 2013; Durso & Mullin, 2013; Gray, 2015). A few studies have also focused on the association between the type of stimulus (Arnold & Bennett, 1984; Burger, 1998) or the substrate utilized by the snakes and the defensive behaviour (Shine *et al*., 2002; Martins *et al*., 2008). And some studies addressed geographic variation in defensive behaviour due to differential predation rates (*e.g.* Aubret *et al*., 2011), including, also, the evaluation of the impacts caused by hunting (Sasaki *et al*., 2009) or levels of human activity in the area on the defensive behaviour of snakes (Atkins *et al*., 2022).

Considering geographic variation on defensive behaviour, it has been shown that *Thamnophis sirtalis* from the mainland may show a broader defensive repertoire in comparison to their insular conspecifics, for being under higher predation pressure (Placyk Jr., 2012). Islands have been of great importance for ecological studies, for representing a sort of natural laboratory due to isolation and particular characteristics. Islands bear higher abundance of specific taxonomic groups than the mainland and their populations have their own traits probably related to their reduced predation (Baeckens & Van Damme, 2020; Terborgh, 2022). It is noteworthy that even isolated forest fragments have functioned as islands in terms of predation pressure to which snakes are subjected to. For instance, *Bothrops jararaca* from smaller fragments are under lower predation rates than those from continuous forested areas (Siqueira & Marques, 2018).

As aforementioned, defensive behaviour is greatly shaped by predation pressure. However, the encounter of snakes in the field is usually fortuitous, making it very difficult to assess the predation pressure to which they are subjected to. Therefore, using snake replicas made of plasticine is a valuable strategy which has been increasingly utilised (Madsen, 1987;

Brodie III, 1993; Kikuchi & Pfennig, 2010; Banci *et al*., 2020). This method was used for assessing predation rate upon *B. jararaca* in forest fragments for example (Siqueira & Marques, 2018).

Bothrops jararaca has a widespread distribution associated with the Atlantic Forest (Campbell & Lamar, 2004). Therefore, considering that this biome is currently restricted to 12% of its original cover (Ribeiro *et al*., 2011), it is reasonable to consider that its populations will be under differential predation pressures throughout the species' distribution, as it has already been demonstrated (see Siqueira & Marques, 2018), and the species has a broad defensive repertoire. *Bothrops jararaca* is closely related to five insular species (Barbo *et al*., 2022), including *B. insularis* and *B. alcatraz*. *Bothrops insularis* is a critically endangered snake species endemic to Queimada Grande Island, Southeastern Brazil (Silveira *et al*., 2021), even though the density of snakes is high (estimated in 55 per hectare; Martins *et al*., 2008). *Bothrops insularis* is commonly known as the Golden Lancehead, because of its distinctive yellowish colour. *Bothrops alcatraz*, is also critically endangered, and endemic to Alcatrazes Island, Southeastern Brazil (Marques *et al*., 2002; Marques *et al*., 2021), and its coloration is very similar to that of *B. jararaca*. Nevertheless, the species does not seem so abundant as is *B. insularis* (pers. obs.). Even though the natural history and ecology of both species have been studied over the past decades, their defensive behaviour and the predation pressure on them are poorly known. Therefore, the objective of this study was to evaluate such aspects, testing the hypothesis that predation rates upon both insular species would be lower than upon *B. jararaca*, their sister species from the mainland, and that, for this reason, *B. insularis* and *B. alcatraz* have different defensive reactions.

Material and Methods

Ethical guidelines

This study was conducted under authorization of the animal ethics committee of Instituto Butantan (CEUA nº 1543170518), and of the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO # 45615-9 and # 63295-12).

Predation rates

In order to assess predation rates on *B. insularis* at Queimada Grande and Alcatrazes Islands we used plasticine replicas. Replicas of *B. insularis* were manufactured by mixing yellow (Acrilex #505) and brown plasticine (Acrilex #531), in order to achieve a colour similar to that of the Golden Lancehead (Figure 1). Replicas of *B. alcatraz* were made mixing brown (Acrilex #531) and black plasticine (Acrilex #520), in the same proportion used to make *B. jararaca* replicas in Siqueira & Marques (2018). Considering that the colouration of *B. insularis* differs from that of *B. jararaca* and *B. alcatraz*, we also used replicas resembling these species at Queimada Grande Island, to control for the effect of the colour on predation pressure.

Replicas were 30 cm long, and were threaded onto iron wires, for being fixed on the substrate, preventing them to be sent away by predators. Replicas were placed in a S-shaped posture, and, because the three species are semi-arboreal (Marques *et al*., 2019), half of the replicas were randomly set on the ground, and the other half on the vegetation. They were set 10-m apart along a 1 km trail which crosses the island. Experiments were made in seven different occasions between 2014 and 2022 at Queimada Grande Island (July and October/2014, January/2015, March/2020, April, May and Juy/2022), utilising 560 replicas of *B. insularis*, and 200 with *B. jararaca* pattern, and in one occasion at Alcatrazes Island (in march/2023), utilising 100 replicas. Replicas were left on the field for 48 hours, and after this period, they were inspected for predation marks made by birds. The recognition of such predation marks is possible because bird pecks leave either a "U" or a "V" shape (Figure 1).

Fig. 1: Replicas used in experiments. "*Bothrops insularis* pattern" (A) resembling *B. insularis* and "*Bothrops jararaca* pattern" (B) resembling *B. jararaca* and *B. alcatraz*. Note the beak marks due to bird attack.

Defensive behaviour

During field experiments from 2013 to 2022, active searches for *B. insularis* and *B. alcatraz* were conducted, both during the day and the night. For each snake encountered on the ground, we registered several biotic factors, such as snakes' body temperature, activity, head and body posture, and activity, keeping the distance. Abiotic variables were also registered, such as season, photophase, weather conditions, substrate temperature and the microhabitat where the animal was. Temperatures were measured using a laser thermometer (precision of 1,5ºC). To assess defensive behaviour, one researcher approached the snake in slow and continuous moves, to a few centimetres (nearly stepping on it), and then molested the snake by moving the sole of the boot in a continuously and repeatedly pushing motion towards the snake's face or anterior body region (*cf*. Duvall *et al*., 1985; Sazima, 1988). The response of the snake to such stimulus was then noted, and the defensive behaviours were classified as: immobility, flight, body retraction and strike. All such experiments were recorded on video, and each snake was tested only once.

After that, the animal was captured, and we measured its snout-vent length (SVL), tail length (TL), total length (TTL) using a measuring tape (precision of 1 mm), and weighted its body mass (BM), using dynamometers (precision of 1g). We also determined the individuals' sex, and checked for the presence of stomach or intestinal content, ticks, and tail mutilation. Each animal also received a microchip tag in the posterior middorsal part of the body.

Statistical Analyses

To assess if there were differences on the predation rate upon brown and yellow replicas at Queimada Grande Island, we performed a chi-square test. Overall predation on replicas of the three species was analysed using binomial logistic regression. Predation (0 = no predation; 1 = predation) was considered as the outcome variable, and substrate (ground *vs.* vegetation) and season (rainy *vs.* dry, being rainy from October to March, and dry, from April to September; Morelatto *et al.*, 2000) were considered as possible explanatory variables. Predation on *B. insularis* and *B. alcatraz* were assessed from our experiments, and we considered predation data on *B. jararaca* previously obtained by Siqueira & Marques (2018) in two localities in mainland: Parque Estadual das Fontes do Ipiranga (PEFI) (23°38'24.6"S, 46°37'03.1"W), a small forest fragment (560 ha) completely isolated and surrounded by an urban matrix; and Parque Estadual da Cantareira (PEC) (23°24'5"S, 46° 35' 24"W) a large and well-connected forest fragment (7,917 ha).

We tested the effect of the predictors on the defensive behaviour of the Golden Lancehead using a Generalized Linear Model for binomial data (logistic regression), where 0 was "flee" and 1 "non-responsive", the only two defensive behaviours displayed by the species (see the results section). Then we compared the full model (all variables included) to a null model to test the hypothesis that defensive behaviour is randomly distributed. The best fit was those with lower score, calculated using the Akaike Information Criteria (AIC).

Analyses were performed using RStudio 2022.12.0 and The R Stats Package (R Core Team, 2022).

Results

Predation rate

Only six out of 560 replicas of *B. insularis* pattern (1.07%), and 2 out of 200 replicas of *B. jararaca* pattern (1%) were attacked. Thus, predation rates at Queimada Grande Island were not significantly different considering these two coloration patterns ($\chi 2 = 0.000222$; $p > 0.988$). Overall predation rate at this locality was 1.05%, and replicas were attacked exclusively by birds. At Alcatrazes, predation rate was 7%, and replicas were attacked by birds (5%) and tegus (2%). At PEC, predation rate was 12.3%, and 5.75 at PEFI, and replicas were significantly more attacked by opossum than by birds (Siqueira & Marques, 2018).

Our results show that attack rate on plasticine replicas was higher at Parque da Cantareira (continuous area; see odds ratio and Confidence Intervals at 95% in Figure 2), followed by Parque do Estado (small forest fragment in urban area) and Alcatrazes Island, and, lastly, Queimada Grande Island.

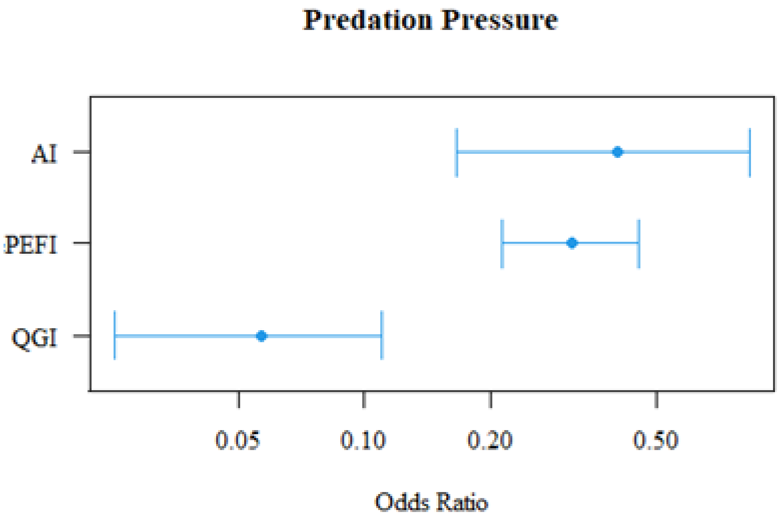

Fig. 2: Odds Ratio and Confidence Intervals (95%) of predation pressure at Alcatrazes Island (AI), the urban fragment (PEFI), and Queimada Grande Island (QGI), in comparison to the continuous forested area in the mainland (PEC).

Defensive behaviour

Defensive behaviour of *B. insularis* reported herein take into account experiments made with 56 individuals (35 females and 21 males). Adult females ranged between 597 and 870 mm in SVL (mean = 681.4 mm), while adult males ranged from 467 to 695 mm in SVL (mean = 602.9). Our sample includes four juveniles, two of each sex. Classification of individuals as either adults or juveniles was made according to data on sexual maturity of the Golden Lancehead provided by the literature (Kasperoviczus, 2009; Marques *et al*., 2013; Passos, 2018). Thirty-two of the trials were made in the dry season, and 24, in the rainy season, and most of them were made during the day (78.6%; n = 44). Most of the individuals were still (83.9%; n = 47), tightly coiled (80.4%; n = 45), and with head placed on the coils (58.9%; n = 33).

Bothrops insularis displayed only two kinds of defensive behaviour during our experiments: immobility and flight. Even so, 55 out of 56 adopted flight as a defence. We found no effect of any variable included in the model (p = 1, AIC = 28) on the defensive behaviour, so that the null model (random effect) produced a better fit to the data (AIC = 11.9).

Defensive trials were made with only five individuals of *B. alcatraz* found during a single expedition (four females and one male), and all of them fled. The male was 508 mm in TTL, and the females ranged from 468 to 535 mm (mean 495.8 mm), suggesting that they were all adults (Marques *et al*., 2002). Because of the low number of animals, no statistical analyses were performed.

Discussion

Predation rate in islands tend to be relaxed due to lower abundance of predators in such areas (Placyk Jr., 2012; Baeckens & Van Damme, 2020; Terborgh, 2022). Because of the restricted area and lower species richness (Wade *et al*., 2003), isolated forest fragments may function as islands, including differences in predation pressure in comparison to continuous areas (Ranta *et al*., 1998). Previous data showed that the population of *B. jararaca* from an urban fragment in the Atlantic Forest (PEFI) is under lower predation pressure than another from a continuous area (PEC) (Siqueira & Marques, 2018). Our data with snake replicas indicate that the two islands, like the mainland forest fragment, also have a reduction in predation pressure on these reptiles. Predation pressure may be the result of both predators' diversity and abundance. Predators of *B. jararaca* include birds (as raptors), mammals (as opossum), other snakes (as *Clelia* sp.), and even large anurans (Sazima, 1992). In two mainland areas (PEC and PEFI), replicas of *B. jararaca* were attacked by birds, but predominantly by mammals (Siqueira & Marques, 2018). The lower predation rate at PEFI is possibly due to the depletion of both birds and mammals, since this forest fragment is isolated in an urban matrix with few conditions to harbour potential snake predators.

At Queimada Grande Island and Alcatrazes there are no mammals (Marques, 2021). Consequently, the absence of these animals may be an important factor to explain the low rate of attacks on replicas recorded on these islands in relation to the continuous area of the Atlantic Forest (PEC). The two islands differ from each other with an extremely low rate of attacks on replicas in Queimada Grande. This island has an area of 43 ha and houses 56 forest bird species, some of which are potential snake predators (Montanhini, 2010; Marques 2021), whereas Alcatrazes has an area of 135 ha and houses 76 bird species, also including potential snake predators (Muscat *et al*., 2014). Besides, Alcatrazes Island includes the large tegu lizard *Salvator merianae* that are abundant in the island (Marques 2021; pers. obs.), and are known to prey on snakes effectively (Kasperoviczus *et al*., 2015). Thus, the spectrum of predators in Alcatrazes is considerably wider than in Queimada Grande, so that predation rate is possibly higher in the former. It should also be considered that the predation pressure on snakes by tegu in Alcatrazes island may be even higher than observed during the experiments for two reasons:

(1) - the replicas consist of essentially visual stimuli to predators, and lizards are more scent oriented, and (2) the experiments were conducted in March, and tegu may have higher activity in other warmer months like October (Srbek-Araujo *et al*., 2020). Another important difference is that in Queimada Grande birds that are potential snake predators are visitors (Montanhini, 2010), whereas in Alcatrazes, half of them are residents (Muscat *et al*., 2014), and they may be more abundant exerting higher predation pressure on snakes.

Bothrops jararaca has a broad defensive repertoire (Sazima, 1988, Araújo & Martins, 2008). Its defensive tactics include immobility, locomotor escape, threat with coiling, and offensive defence with strikes. Under laboratory conditions, the species relies more on striking and tail vibrating (Araújo & Martins, 2008), whereas in the field, immobility followed by flight was the frequent strategy (Sazima, 1988). However, threat and strike preceding the flight were recorded in 20% of the encounters in the field (Sazima, 1988). Threat and strike were not observed in any species on the islands. With the exception of a single individual of *B. insularis* which froze, all specimens tested on both islands (n = 61) fled during confrontation trials. Thus, our initial hypothesis predicted that defensive behaviour of *B. alcatraz* and *B. insularis* differ of *B. jararaca* was confirmed. This hypothesis relied on the premise that predation pressure at both islands would be reduced in relation to a continuous area in the mainland, which happened.

Predation pressure may influence species traits concerning its life history and behaviour. For example, it may reduce their activity due to the trade-off between energy acquisition and predation risk (Cressler *et al*., 2010). *Bothrops alcatraz* replicas were attacked by tegus and birds, that are diurnal predators (DuVal *et al*., 2006; Srbek-Araujo *et al*., 2020). This species feeds mainly on nocturnal prey, (centipedes *Otostigmus* sp., *Haddadus binotatus*, *Hemidactylus mabouia*; Marques *et al*., 2002; Guizze *et al*, 2016; Rocha & Anjos, 2007, Sawaya *et al*., 2023; Kasperoviczus, pers. comm.). It is noteworthy that *B. alcatraz* is the species that attain the smallest body size of the *jararaca* group (Barbo *et al*., 2022, Sawaya *et al*., 2023). Even though it has already been shown that insular crotaline tend to be smaller in comparison do their conspecifics from the mainland (Case, 1983), more recent studies have revealed that body size of insular snakes are mostly related to their diet based on smaller preys (Boback, 2003), which is the case of *B. alcatraz*. In addition to the wide spectrum of predators on Alcatrazes, the reduced body size may cause the species to be even more vulnerable to predation, explaining the fact of *B. alcatraz* being more secretive during the day. Our defensive behaviour data for this species is fragile due to low sample size, but data from captive specimens showed that *B. alcatraz* displayed the highest strike rate relative to B. *insularis* and *B. jararaca* (Alves-Nunes, 2022). Additional field data will be needed to confirm this defensive reaction in *B. alcatraz*. On the other hand, defensive behaviour data of *B. insularis* obtained both in captivity and by us in the field show that this snake has a low tendency to strike, adopting flight as the main defensive tactic. Flight and low defensiveness can be observed in snakes which are not capable of dealing with predators (Sasaki *et al*., 2009). Apparently due to the low predation rate on *B. insularis* these species are not as secretive as *B. alcatraz*, and it can be easily found sitting and waiting for birds during the daylight (pers. obs.). The differences in predator pressure between the two islands may explain the very different niches exploited by each of these island species.

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Original Article – to be submitted to Biotropica

HABITAT USE IN THE CRITICALLY ENDANGERED GOLDEN LANCEHEAD, *B. INSULARIS* (AMARAL, 1922)

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ABSTRACT

The Golden Lancehead, *Bothrops insularis*, is a critically endangered endemic to Queimada Grande Island (QGI), Southeastern Brazil. It belongs to the *Bothrops jararaca* group, and is considered one of the most arboreal species within it. Evidences show a population decrease in last years due to biopiracy. Additionally, the habitat of this species has suffered impacts due to anthropogenic activity, such as fire. Considering that habitat loss is the main cause for reptile extinction in South America, knowledge on habitat use is crucial for species conservation. The aim of this study was to characterize habitat use in *Bothrops insularis*, considering landscape and vegetation structure, and also ecological aspects, such as the migration periods of the birds which they feed on. For characterizing the habitat and vegetation structure, 24 plots were delimited on QGI to obtain indicators to estimate a Biotic Integrity Index (BII) of each plot and the island as a whole. To acquire data of *B. insularis*, three methods were used: radiotracking, thread-bobbins, and mark-recapture. Statical analyses were performed to test whether biotic and abiotic factors may influence habitat use by *B. insularis*. Biotic Integrity Index showed that the overall Atlantic Forest at QGI may be classified as regular. Analysis showed the number of snakes is directly related to BII, maximum and mean height and diameter at breast height, and is negatively related to canopy openness. Specimens were found mainly in forested areas, and animals are found more frequently on the vegetation in the rainy season, and during the bird migratory periods. Sex had no influence on substrate use, but there is a positive correlation between snout-vent length and relative body mass with substrate height used by males. Even though we observed that distance from the initial location is positively correlated with time since the first encounter, it is noteworthy that individuals are rarely found more than 40 meters away from the initial point. Additionally, we observed courtship behaviour in the dry season, especially during the mid-winter. In conclusion, our results evince that *B. insularis* greatly relies on forested areas. Therefore, it is mandatory that conservation strategies aimed at this species take into account also the preservation of this habitat, as well as the connectivity of the forested areas in order to assure the continuity of the species.

Keywords: Substrate use; arboreality; density; displacement; spatial ecology; conservation.

INTRODUCTION

The Neotropical region is very environmentally rich, and includes areas with different levels of conservation. The distribution of snake species may be influenced by environmental characteristics of macro and microhabitats. Some species seem to be less selective regarding habitat use (*e.g. Oxyrhopus guibei*, that occur in anthropized areas; Sazima & Haddad, 1992; Barbo *et al*., 2011), whereas others seem to be highly specialized and dependent on specific habitats (*e.g. Philodryas agassiziii*, restrict to grasslands; Marques *et al*., 2006, 2009). Geographical mapping has been used for inferring habitat use of several species of reptiles, including snakes (*e.g.* Nogueira, 2006; Nogueira *et al*., 2019), what is extremely important for assessing their conservation statuses (Marques *et al*., 2009). However, in order to characterize habitat use precisely, it is crucial to investigate such aspect directly in the field, evaluating environmental characteristics and habitat integrity. The secretive habits of snakes cause their encounter to be very fortuitous, representing a challenge for assessing habitat use by these animals.

In the last decades, radiotracking has greatly contributed for obtaining more accurate information on habitat use by snake species (*e.g.* Fitzgerald *et al*., 2002a; Whitaker & Shine, 2003; Wasko & Sasa, 2009), helping to elucidate, also, which factors may influence their activity. Selection of habitats and microhabitats, as well as home range, may be influenced by several biotic and abiotic factors (Macartney *et al*., 1988).

Males and females of many snake species occupy the same habitat (*e.g.* Shine, 1987; Chandler & Tolson, 1990; Richardson *et al*., 2006), even though intersexual differences may occur. For instance, males of *Morelia spilota* are more arboreal than the females (Shine &

Fitzgerald, 1996), and females of *Acrochordus arafurae* use deeper water bodies than the males (Shine, 1986).

Concerning home ranges, males of many species use larger areas than their conspecific females, mainly because of seeking for mating partner during the reproductive season (Slip & Shine, 1988; Fitzgerald *et al*., 2002b; Roth, 2005; Kapfer *et al*., 2008; Glaudas & Rodríguez-Robles, 2011; Bauder *et al*., 2016). In some species, females may use a different habitat according to their reproductive status (*e.g.* Harvey & Weatherhead, 2006), decreasing activity during pregnancy (Reinert & Kodrich, 1982; Charland & Gregory, 1995), or increasing activity close to oviposition (Slip & Shine, 1988; Brown *et al*., 2005; Roth, 2005; Buchanan *et al*., 2017). However, males and females of some species do not show such difference (*e.g.* Brown *et al*., 2005; Wasko & Sasa, 2009). Habitat use may also be influenced by ontogeny and body size (*e.g.* Whitaker & Shine, 2003; Brown *et al*., 2005). Home range and site fidelity increase with snake size in *Elaphe obsoleta* (Blouin-Demers *et al*., 2007). Adults of *Laticauda colubrina* may frequently visit the mainland, whereas juveniles rarely do so (Shetty & Shine, 2002). Juveniles of *Gloydius shedaoensis* tend to be more arboreal than the adults, what seems to be related to hunting optimization (Shine *et al*., 2002).

Several of the aforementioned studies evince the influence of both biotic and abiotic factor on habitat use by snakes, showing that this trait may vary seasonally according to environmental and ecological local conditions (Slip & Shine, 1988; Wheaterhead & Prior, 1992; Shine & Fitzgerald, 1996; Fitzgerald *et al*., 2002a; Brito, 2003; Shine *et al*., 2003; Vanek & Wasko, 2017).

However, the lack of information for Neotropical snake species is notorious. Studies using radiotelemetry have been conducted with only a few species, such as *Boa constrictor*, *Erythrolamprus zweifeli*, *Oxybelis aeneus*, *Leptophis depressirostris*, *Spilotes pullatus*, *Bothrops asper*, *B. atrox*, *B. jararacussu*, and *Crotalus durissus* (Henderson *et al*., 1976; Nickerson *et al*., 1978; Tozetti & Martins, 2008; Tozetti *et al*., 2009; Wasko & Sasa, 2009; Muscat *et al*., 2021), and some of them, for short periods or low samples. On the other hand, long-term studies, even without using radiotelemetry, have provided important information on habitat use for some species, as *Crotalus durissus*, *Thamnodynastes strigatus*, *Philodryas agassizii*, *P. olfersii*, *P. patagonienis* and a few *Bothrops* species, such as *B. jararaca*, *B. jararacussu* and *B. atrox* (*e.g.* Sazima, 1988; Sazima, 1992; Bernarde *et al*., 2000; Nogueira, 2001; Hartmann *et al*., 2003; Hartmann & Marques, 2005; Marques *et al*., 2006; Sazima & Marques, 2007; Tozetti & Martins, 2007; Sawaya *et al*., 2008; Fraga *et al*., 2013).

The Golden Lancehead, *Bothrops insularis*, is endemic to Queimada Grande Island (QGI), Southeastern Brazil (Amaral, 1921; Vanzolini, 1973; Duarte *et al.*, 1995; Marques *et al.*, 2002a). Several studies have already addressed several aspects of the species' ecology and natural history, encompassing reproduction, diet, thermal ecology and population size (Martins *et al.*, 2008; Andrade *et al.*, 2010; Bovo *et al.*, 2010; Marques *et al.*, 2012, 2013; Silva *et al.*, 2015). Besides differences in morphology and coloration, *B. insularis* show very particular ecological characteristics in comparison to other species of the *Bothrops jararaca* group to which it belongs (Marques *et al.*, 2002a,b; Wüster *et al.*, 2005). This species is the most arboreal in the group, possibly because of its diet based on migratory birds which visit the island in March (*Elaenia chilensis*) and July (*Turdus flavipes*; Marques *et al.*, 2012). Accordingly, it has also been shown that the heart is located more anteriorly in the females, what usually happens in arboreal snakes (Wüster *et al.*, 2005). For this reason, it has been hypothesized that the species may migrate vertically during these periods (Marques *et al.*, 2012). Additionally, previous findings evinced that the snakes seem to be more abundant in more elevated areas, where the forest is higher, what would supposedly favour foraging on birds (Martins *et al.*, 2008). These aspects, however, must be better elucidated.

Bothrops insularis is a critically endangered species (Silveira *et al.*, 2021), and its habitat, covered by Atlantic Forest (Kurtz *et al.*, 2017), has suffered impacts due to anthropogenic activity, such as fires (Duarte *et al.*, 1995; Bataus & Reis., 2011). It is noteworthy that *B. insularis* seems to rely mainly on the forested than on grassy areas from QGI (Martins *et al.*, 2008) Apart from the impact on the original vegetation, excessive captures happened between 1914 and 1924, and from 1946 to 1957 (Duarte *et al.*, 1995), and, up to the present day, there are evidences of illegal capture of the Golden Lancehead for biopiracy (Duarte *et al.*, 1995; Marques *et al.*, 2002a; pers. obs.), what may have contributed to the decrease of the population size (Martins *et al.*, 2008; Guimarães *et al.*, 2014; Abrahão *et al.*, 2021). Although the illegal removal of specimens from the island is an important factor to be considered (Duarte *et al*, 21995; Martins *et al.*, 2008), habitat loss is the main cause for reptile extinction in South America (Rodrigues, 2005; Böhm *et al.*, 2013). Therefore, knowledge on habitat use is crucial for species conservation. In this sense, the main objective of this study was to characterize habitat use in *Bothrops insularis* based on relief, landscape and vegetation structure, taking into account ecological aspects, such as the influence of migration on this trait.

MATERIAL AND METHODS

Ethical Guidelines

This study was conducted under authorization of the animal ethics committee of Instituto Butantan (CEUA nº 1543170518), and of the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO # 45615-9 and # 63295-12).

Habitat Structure

In order to better understand habitat use by *B. insularis*, it is important to evaluate the habitats which are available, by assessing the integrity of the Atlantic Forest at QGI. For that, we used the indicators for the analysis of Biotic Integrity Index (BII) proposed Medeiros and Torezan (2012), Scarascia *et al*. (2020) and Cardoso-Leite *et al*. (2022), with adaptations for the region and the particularities of the island habitats. We delimited 24 plots (10 x 10 m; fig. 1), 50 m apart from each other (except when the relief was not adequate), along the trail which crosses the island. Inside each one of these plots, we sampled the following variables for calculating the integrity index: coverage by leaf litter; number of dead trees standing; coverage by grassy vegetation; quantity and types of vines and lianas, percentage of the plot occupied by clearing; number of epiphytes; number of palm trees. Each variable receives a score from 1 to 5, that represent low and high integrity, respectively (check scores of each variable in Table I), so that the overall index of each plot could range between 7 and 35 (see index classification in table II). We also evaluated maximum and mean canopy height, canopy openness in the centre of the plot, diameter at breast height (DBH) of the trees; number of trees with DBH > 100 mm, and, considering that the snakes frequently use rocks and *Anthurium parasiticum* (Araceae) as shelter, we also evaluated the percentage of the plot covered by them. This evaluation of vegetation and habitat structure was made from January 28th to February 3rd, 2023.

Table I: Indicators used for assessing Biotic Integrity Index at Queimada Grande Island.

VARIABLES	**INTEGRITY INDEX**				
	1	**2**	**3**	**4**	**5**
A -Leaf litter	exposed soil	1 - 25%	26 - 50%	51 - 75%	76 - 100%
B - Dead trees standing	> 3	3	2	1	Absent
C - Coverage by grassy vegetation	> 50%	26 - 50 %	11 - 25%	1 - 10%	Absent
D - Vines and lianas	≥ 3, thin	2 thin	1 thin or absent	thin + thick (≥ 4cm)	only thick (≥ 4)
E - Clearings	50%	26 - 50%	11 - 25%	1 - 10%	Absent
F - Epiphytes	Absent	1 - 5	6 - 10	11 - 15	> 15
G - Palm trees	Absent	1 - 3	4 - 6	7 - 9	≥10

Table II: Classification of the Biotic Integrity Index (BII) obtained at Queimada Grande Island.

BII SCORES	**CLASSIFICATIONS**
7 - 12	Very Low
13 - 18	Low
19 - 24	Regular
25 - 30	Good
31 - 35	Excellent

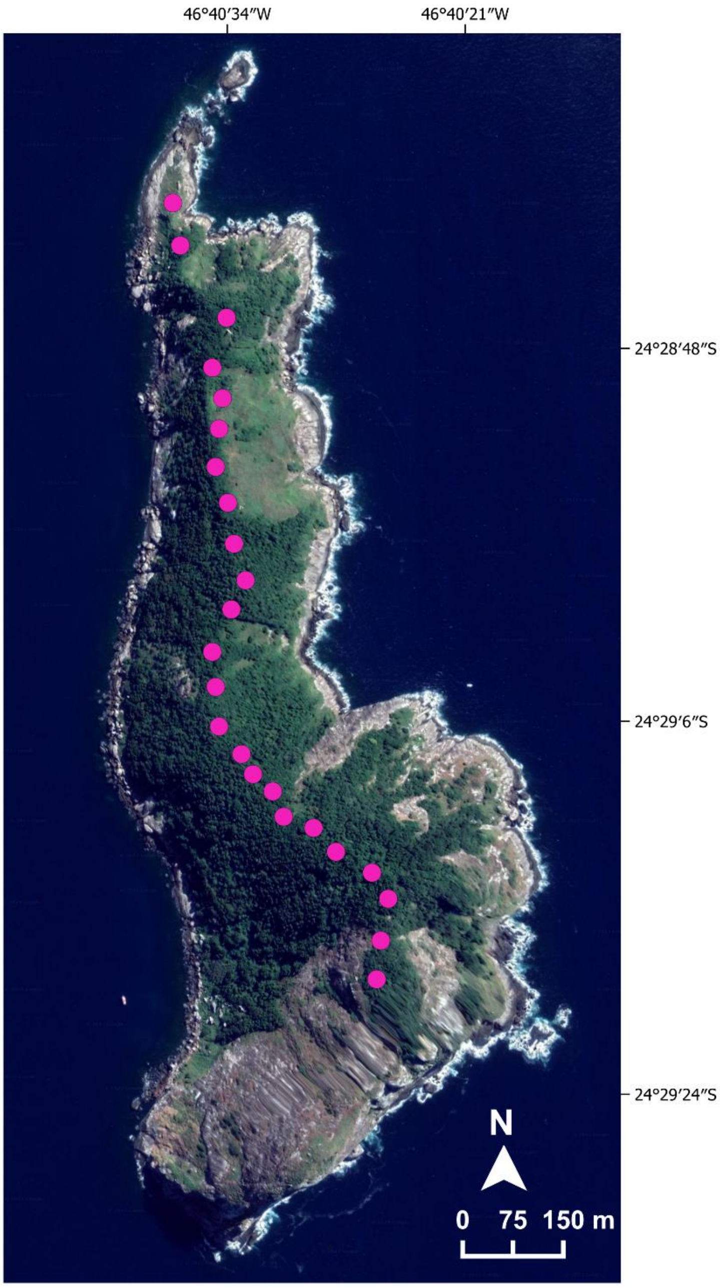

Fig. 1: Distribution of the plots at Queimada Grande Island.

Sampling of the Golden Lancehead

For investigating habitat use by *B. insularis*, we used three techniques combined, namely radiotracking, thread-bobbins, and mark-recapture (Bonnet *et al*., 2002; Tozetti & Martins, 2007). Radio transmitters were implanted in 12 animals (nine females and three males), and these animals were tracked during 2022. Transmitters were surgically implanted in caudal third of the snakes' bodies, with the antenna directed towards the caudal region (Banci *et al*., in prep.). Thread bobbins were fixed with adhesive tape, also in the third caudal region. These studies were carried out from January of 2022 to March of 2023. In both cases, the mass of the devices never surpassed 5% of the snakes' body mass, in order to not compromise its activity (Újvári & Korsós, 2000).

At each encounter, the following data were registered: microhabitat (type of substrate and height) used by the snake, canopy openness, air temperature and humidity, substrate and animal temperature, geographic coordinates, sex, snout-vent length (SVL), tail length (TL), body mass, reproductive status (inferred from the presence of follicles or embryos in the females; Marques *et al*., 2013), stomach or intestinal content (*cf.* Marques *et al*., 2012). Because of the many years of studies at QGI, since 2004 several animals had already been microchipped, and those that were not, received a microchip in the first encounter. This technique provided data on habitat use and displacement obtained from mark-recapture. Geographic coordinates were used for making heatmaps, evincing the distribution and snakes' density throughout QGI. For that, locations of all individuals were used for creating a density map using Kernel Density Estimation (KDE), performed by the heatmap tool of the open-source GIS software QGIS v. 3.30.0 (QGIS Development Team, 2009).

Distance travelled by individuals with thread-bobbins were directly measured in the field using a measure tape, and for those obtained from mark-recapture, we calculated geodesic distance, using the geographic coordinates.

Statistical analyses

In order to check for differences between the plots, according to the variables measured, we performed a Partial Least Square (PLS) analyses. The number of snakes around each plot, considering a radius of 10 meters from the plot centre, was assessed using the "count" function in QGIS v.3.30.0 (QGIS Org.). This number was used as the variable of interest, and BII, canopy openness, maximum and mean height, DBH, number of trees, and percentage of the plot covered by rocks and *Anthurium parasiticum* were used as predictors. As aforementioned, it had already been observed that the abundance of *B. insularis* seems to be higher in forested areas (Martins *et al*., 2008). In order to test this hypothesis, we also analysed canopy openness (variable of interest) in the locations where the animals (96 females and 62 males) were found. For checking whether forest is higher in more elevated areas of the island, linear regression was performed, having either maximum or mean canopy height as variables of interest, and altitude as a predictor.

For assessing whether vertical migration occurs during migratory periods of the birds which the Golden Lancehead feeds on, we used binomial logistic regression. In this case, substrate (0 = ground; 1 = vegetation) was used as variable of interest, and season (dry or rainy), migratory period (0 = non-migratory period; 1 = migratory period) and sex were used as predictors. Migratory periods were the months between February and April (migration of *Elaenia chilensis*), and between June and August (migration of *Turdus flavipes*; Marques *et al*., 2012; pers. obs.). Concerning substrate use, we also investigated whether sex or body size had any influence on the height in which animals were observed. For that, we performed linear regressions using height as variable of interest, and sex and SVL or relative body mass as predictors.

We also tested whether distance from either the initial location (DIL; variable of interest) was a function of sex, SVL and time between encounters (TBE; predictors), using multiple linear regression. After finding no relation between DIL and sex and SVL ($F_{3,\ 84}$ = 2.23; $p > 0.05$), we performed a linear regression having DIL as a function of TBE.

Analyses were performed using RStudio 2022.12.0 and The R Stats Package (R Core Team, 2022), and 'pls' package (Liland *et al*., 2022). Significance was considered when $p >$ 0.05.

RESULTS

From 2022 to 2023, a total of 259 of Golden Lanceheads were encountered during diuturnal visual searches (79.5% captures, and 20.5% recaptures), and 15.8% of them had identifiable alimentary content (19.5% anurans, and 80.5% birds). Radiotracking was not an effective tool during this study, possibly because of the terrain roughness. Therefore, most of the results presented herein arise from thread-bobbins and mark-recapture.

Biotic Integrity Index (BII) showed that the overall Atlantic Forest at QGI may be classified as regular (Appendix 1). Only 51 (19.7%) of the animals were in the surroundings of the plots, and the density of the snakes encountered in a 10 meters ray of each plot is represented in the heatmap (figure 2), with warmer colours corresponding to higher densities. Data collected in each plot are available at Appendix 2. Partial Least Square analysis showed that only the first component explains the number of snakes in the surroundings of the plots. This component explains 50.9% of the model, and shows that the number of snakes is directly related to BII (0.47), maximum (0.45) and mean height (0.44) and DBH (0.42), and is negatively related to canopy openness (-0.37). Canopy openness where the snakes were found ranged from 2.4% to

30.9% ($\bar{x}$ = 9.7±4.9%), and, complementing the previous results, it shows that the species is not found in open areas. A positive correlation was found between maximum ($F_{1,22} = 16.37$; $p < 0.05$; fig. 3) and mean canopy height ($F_{1,22} = 9.55$; $p < 0.05$; fig. 4).

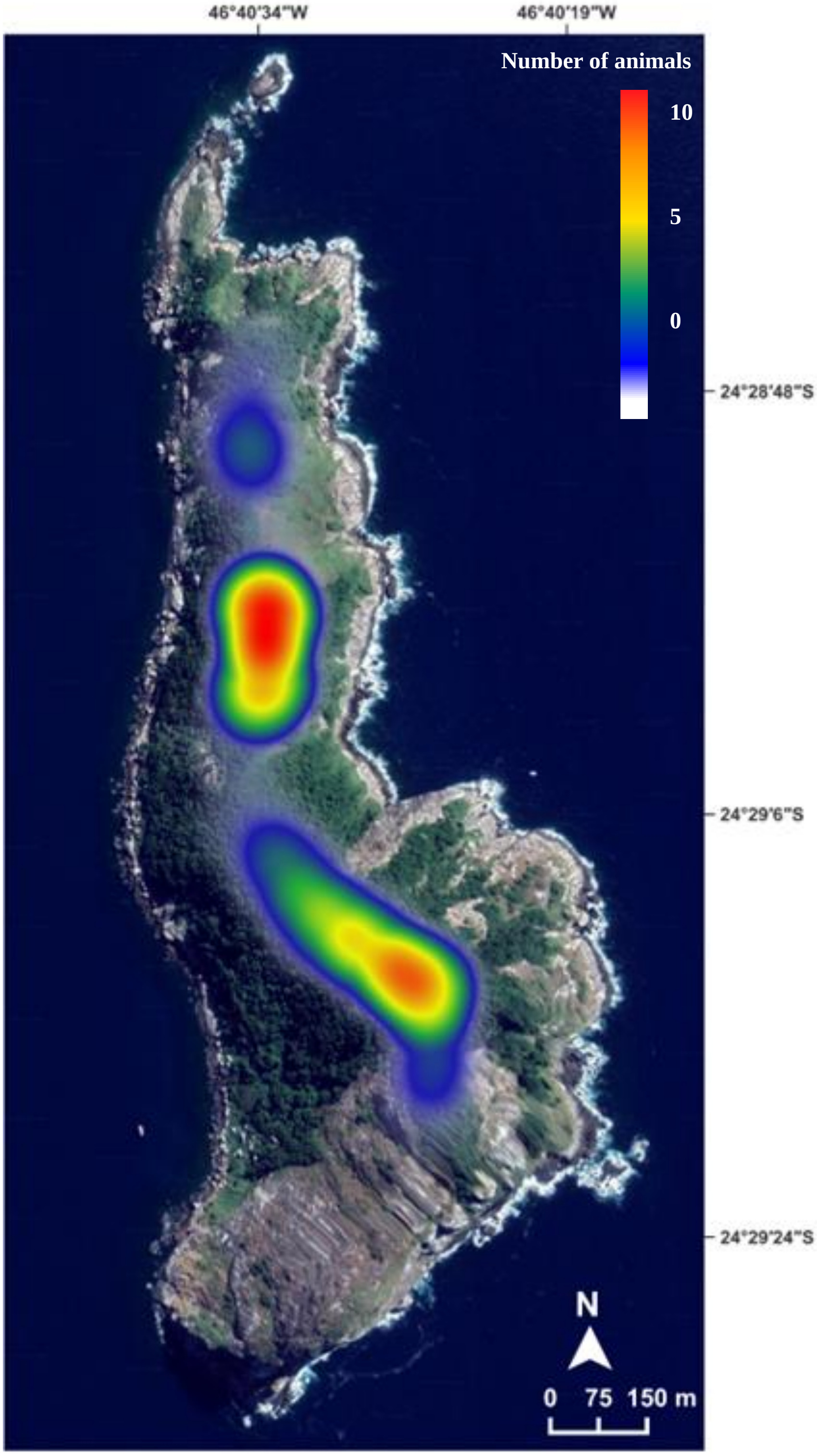

Fig. 2: Density of individuals of *B. insularis* around the plots.

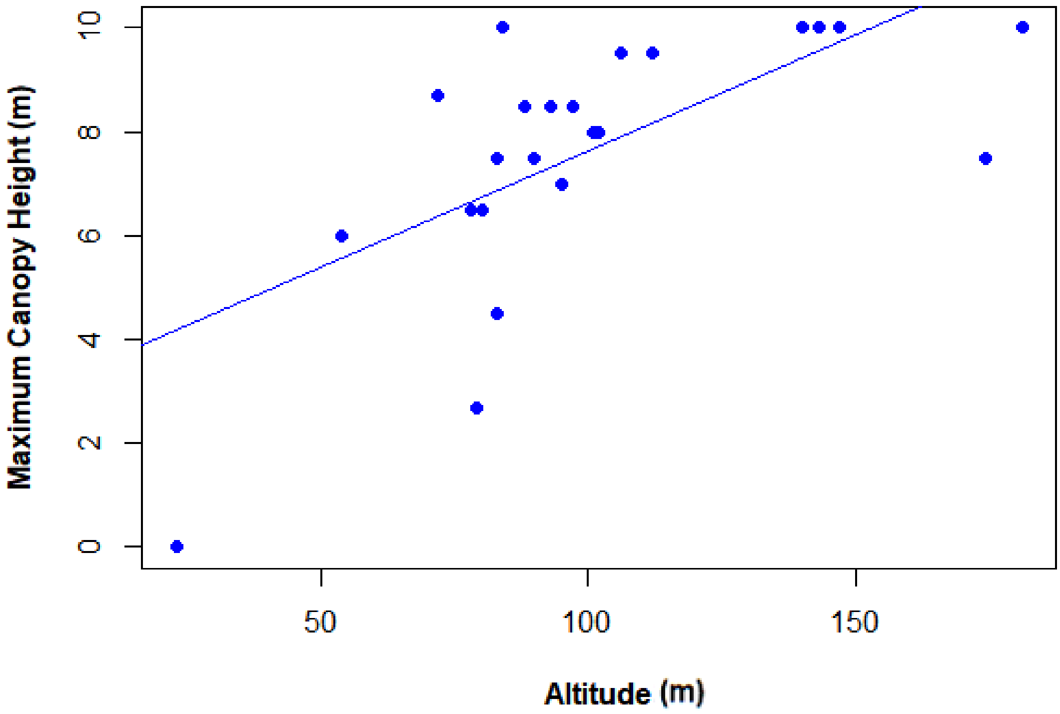

Fig. 3: Correlation between maximum canopy height and altitude at Queimada Grande Island.

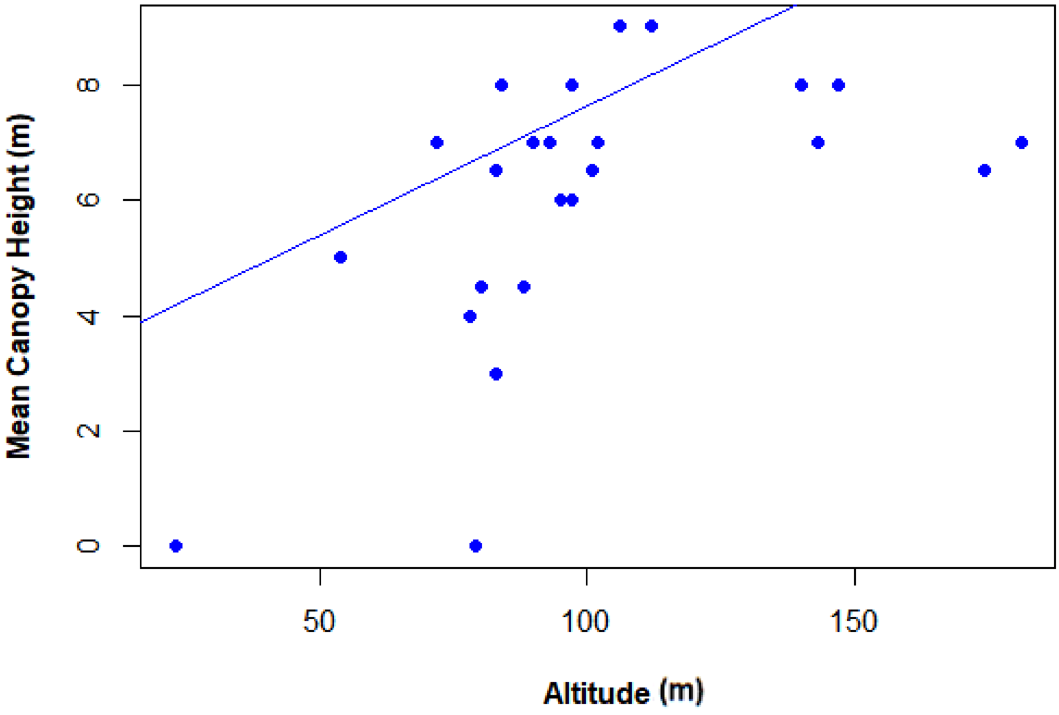

Fig. 4: Correlation between mean canopy height and altitude at Queimada Grande Island.

Animals are found more frequently on the vegetation in the rainy season [β(SE) = 0.59(0.28); OR = 1.52; IC(95%) = 1.07 – 2.14; $p < 0.05$], and during the migratory periods [β(SE) = 0.28(0.17); OR = 1.58; IC(95%) = 1.11 – 2.26; $p < 0.05$]. Sex had no influence on substrate use [sexM: β(SE) = -0.02(0.15); OR = 0.98; IC(95%) = 0.73 – 1.32; $p > 0.05$].

There is a positive correlation between SVL and substrate height used by males ($F_{1,102} = 22.44$; $p < 0.05$; fig. 5). For females, this correlation is marginally significant ($F_{1,139} = 3.67$; $p = 0.06$). The same was observed for relative body mass, with a positive correlation between it and height reached by males ($F_{1,93} = 4.44$; $p < 0.05$, fig. 6), and a marginally significant correlation for females ($F_{1,121} = 3.34$; $p = 0.07$).

Distance from the initial location is positively correlated with time since the first encounter (F1,86 = 4.68; $p < 0.05$; fig. 7). These data were obtained from four animals equipped with radio-transmitter (three females and one male), 46 animals with thread-bobbins (31 females and 15 males), and 36 recaptures (23 females and 13 males).

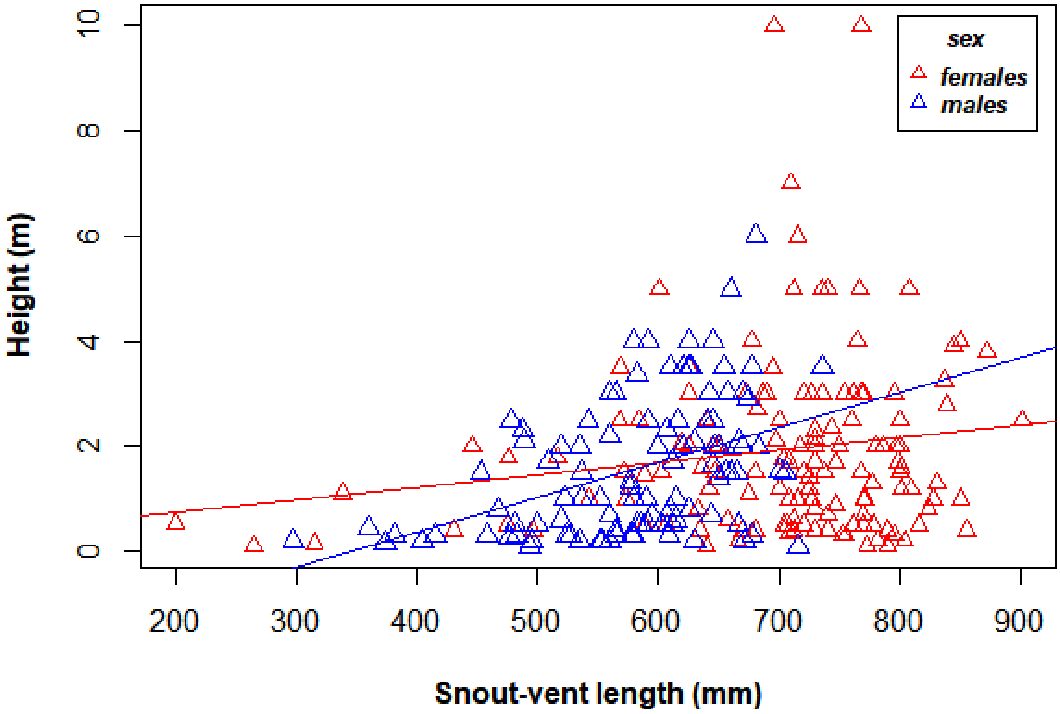

Fig. 5: Correlation between height used by males and females of *B. insularis*, according to their snout-vent length.

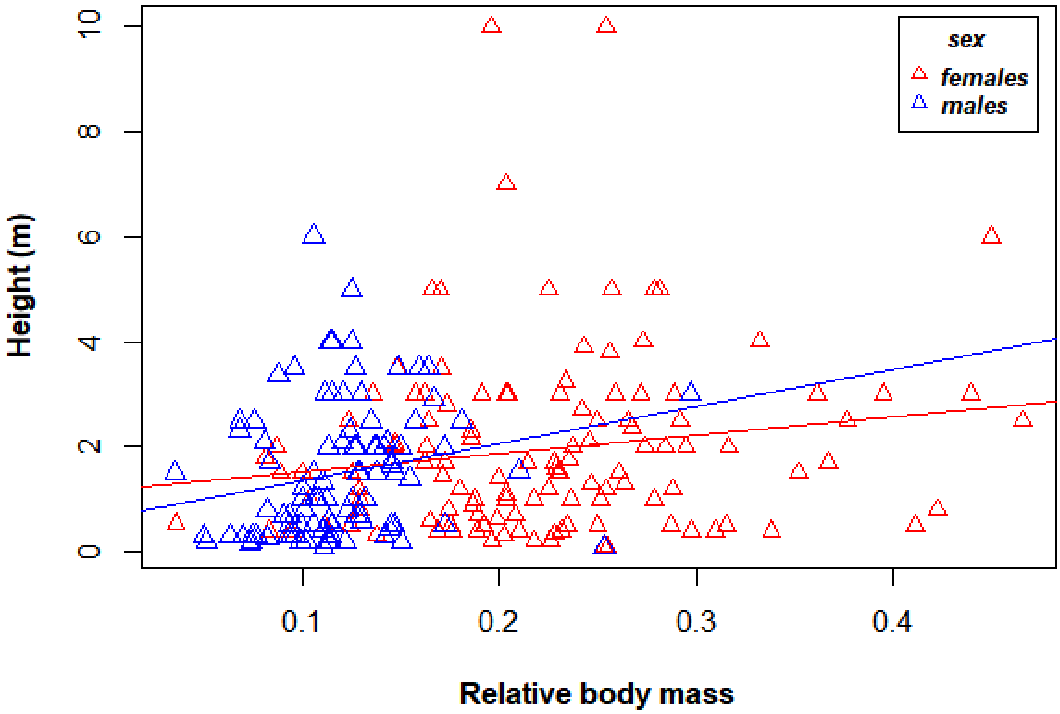

Fig. 6: Correlation between height used by males and females of *B. insularis*, according to their relative body mass.

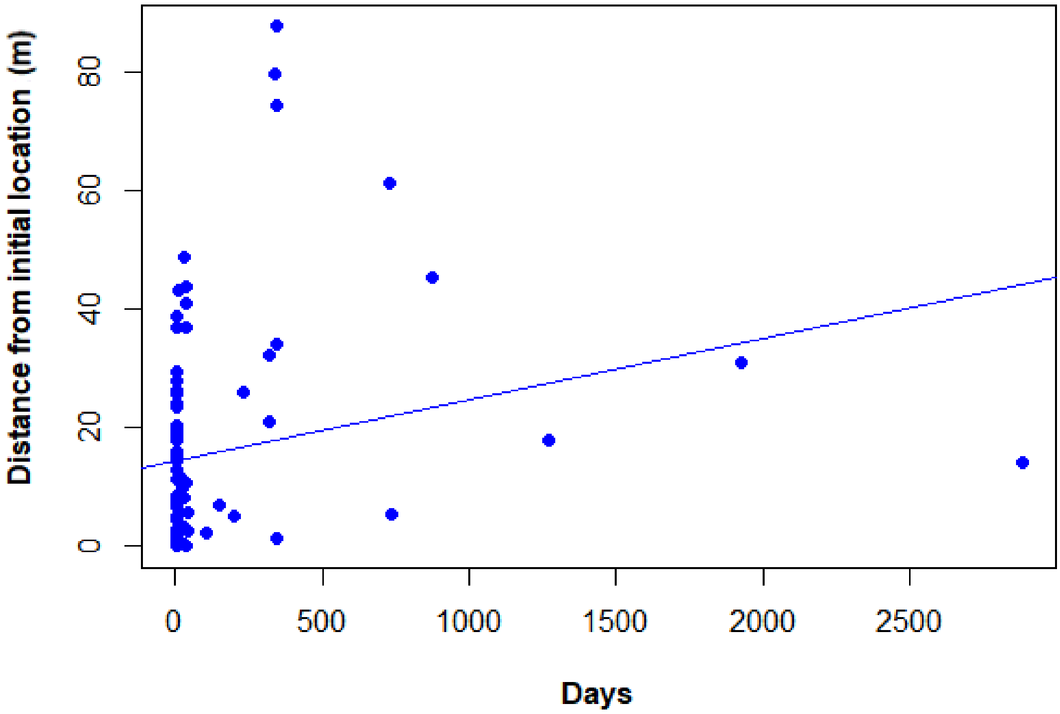

Fig. 7: Correlation between distance from the initial location (in meters) and time since the first encounter.

Interaction between animals

It is noteworthy that, even though we may frequently find snakes relatively close to one another, only during the reproductive season (Marques *et al*., 2013) we could observe seven pairs of males and females in really close proximity (Table III). Most of these encounters happened when tracking animals with thread-bobbins, and it was possible to observe that in five of these occasions, the male was on top of the female (fig. 8), suggesting courtship behaviour. Additionally, most of the courtship and mating activity observed at QGI were in June (Marques *et al*., 2013), whereas our observations were mostly in July.

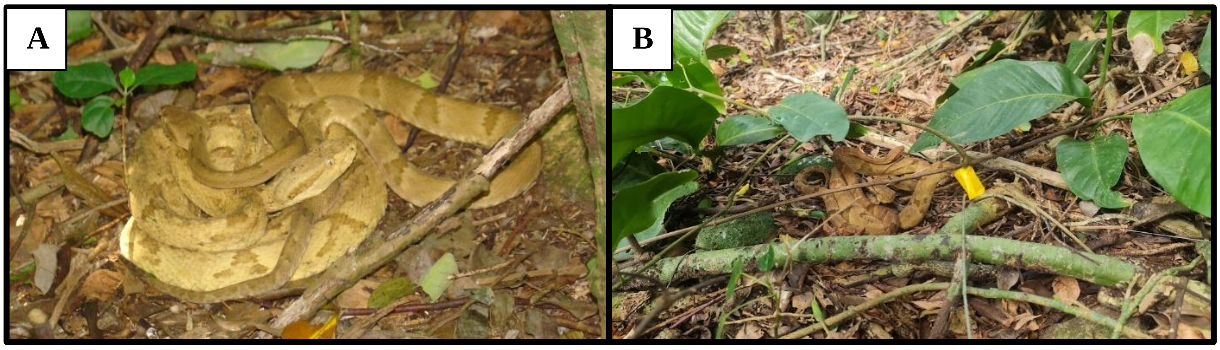

Fig. 8: Males and females found together during the reproductive season, either during visual search (A) or while tracking animal with thread-bobbin.

Table III: Interaction between males and females of *B. insularis* observed at Queimada Grande Island. Individuals not measured are assigned as NM.

Date	Time	SVL + TL (mm)		Observation	Substrate
		Female	Male		
07/31/2014	10:49	886 + 127	645 + 115	Male on top of the female	ground
06/22/2022	15:40	794 + 115	553 + 104	Male on top of the female	ground
06/22/2022	19:33	926 + 131	617 + 111	Male on top of the female	ground
07/13/2022	09:37	761 + 112	711 + 109	Individuals coiled together	ground
07/14/2022	10:50	756 + 106	NM	Male on the entrance of the hollow branch where the female was hidden	vegetation
07/14/2022	11:44	828 + 105	NM	Male close to the female	ground
07/14/2022	12:05	880 + 123	680 + 116	Male on top of the female	ground
08/03/2022	09:43	607 + 102	666 + 116	Male on top of the female	ground

DISCUSSION

The ancestors of *Bothrops* were possibly forest dwellers (Martins *et al*., 2001). Accordingly, several species of the genus inhabit forests, such as those belonging to *B. atrox* and *B. jararaca* groups (Martins *et al*., 2001), despite of some lineages occurring in open and dry areas, such as those belonging to *B. alternatus* and *B. neuwiedi* group (Martins *et al*., 2001; Valdujo *et al*., 2002). The Golden Lancehead belongs to the *B. jararaca* group (Barbo *et al.*, 2022), therefore, it is not surprising finding that the species seems to be dependent of forested areas, as we report herein. The overall integrity of the forest at QGI is classified as regular, what may be associated with the low richness of arboreal species possibly resulting from steep topography and incipient soils, which limits the growth, distribution, and diversity of tree species (Kurtz *et al*., 2017). *Bothrops insularis* may be found in forested areas throughout the island, showing that the species does not necessarily rely on mature or even late succession forests, as observed *Bothrops asper*, another forest dweller species (Ramírez-Arce *et al*., 2021). Even within the *B. jararaca* group, some plasticity on habitat use has been observed. For instance, *B. jararaca* may be found in forest remnants, and more open and even degraded areas (Sazima, 1992; Barbo *et al*., 2011). An association between arboreality and inhabiting forests is intuitive, and, together with *Bothrops insularis*, *B. bilineatus* is considered one of the most arboreal species of *Bothrops* (Martins *et al*., 2001), and it is found exclusively in forested areas (Bernarde *et al*., 2021). Nevertheless, our results point to a preference for areas with higher Biotic Integrity Index, with high canopy, and low canopy openness. Mature and late succession forests are known to harbour a higher diversity of arboreal snakes (Marques & Sazima, 2004). Late succession forests represent better structured habitats, which can offer more perches, favouring snakes' locomotion and hunting sites (Lillywhite, 2014). Also, it must be considered that these habitats may attract more birds. For instance, *E. chilensis*, the main prey of *B. insularis*, usually search for food in higher substrates (Chust *et al*., 2012), and *T. flavipes* may also be associated with late succession forests, considering that the abundance of certain fruits, which this bird feeds on, is higher in such habitats (Souza & Prevedello, 2019).

A previous study had already evinced that *B. insularis* is found mostly in forested areas, with only 10% of the encounters occurring in grassy areas (Martins *et al*., 2008). This preference for forested areas was also evinced herein, and some hypothesis may be raised. First of all, the fact that the species rely on such areas may be related to physiological constraints. Water loss in reptiles is closely associated with habitat preference, and it seems to be more influenced by ecological than phylogenetic determinants (Gans *et al*., 1968). At QGI, hydration may be difficult due to the lack of water bodies in the island, and most of the available water comes from precipitation, causing dehydration avoidance very challenging. Therefore, the use of open areas could be a problem, since direct exposure to solar radiation and higher amount of wind on the island could lead to a fast dehydration (Bovo *et al*., 2012), especially considering that the skin of *B insularis* seems to be thinner compared to that of *B. jararaca* (Marques, 2021; pers. obs.). Physiological and histological studies on *B. insularis* and its skin could help better elucidating such aspect. Another hypothesis that may be raised is that preys of *B. insularis* are more abundant in forested areas, so the preference for this habitat could optimize foraging. Both juveniles and adults of *B. insularis* may feed on frogs, such as *Haddadus binotatus* (Martins *et al*., 2002; Andrade *et al*., 2010; pers. obs.). This species of anuran is found mainly in the leaf litter of forested areas (Heyer *et al*., 1990; Sazima & Haddad, 1992). As adults, the Golden Lancehead feeds mainly on the migratory birds *Elaenia chilensis* and *Turdus flavipes* (Marques *et al*., 2012). Both *T. flavipes* and *E. chilensis* feed on fruits and arthropods in forests (Develey, 2004; Manhães *et al*., 2010; Bravo *et al*., 2017). It can be inferred, therefore, that, because these items are more abundant inside the forest, it reflects on the abundance of birds themselves, and, consequently, of *B. insularis*. A third hypothesis is that predation pressure may be higher in open areas, as mentioned by França (2008).

Upon describing the species, Amaral (1921) stated that *B. insularis* was exclusively arboreal and ornithophagous, being frequently found on frugivorous trees where the birds would feed. It is currently known that, besides feeding on birds, the Golden Lancehead may also feed on ectothermic prey (Martins *et al*., 2002; Andrade *et al*., 2010; pers. obs.). Moreover, *B. insularis* use both the ground and the vegetation, being found more frequently on the latter during migratory periods of the birds (Marques *et al*., 2012). Herein, we confirmed the occurrence of the vertical migration in the periods when the birds visit the island. *Elaenia chilensis* and *T. flavipes* search for food while perched (Manhães *et al*., 2010; Bravo *et al*., 2017). Therefore, the use of vegetation by snakes during ambush could favour foraging for this reason (Marques *et al*., 2012), and also because it may favour the detectability of the bird’s approach and strike precision by the snakes, as already reported for *Gloydius shedaoensis*, another ornithophagous insular snake species (Shine *et al*., 2002). However, *B. insularis* may also capture birds on the ground, as we observed during the present study. Out of the migratory periods, *B. insularis* are commonly encountered on the ground, where it may feed on *Haddadus binotatus* which inhabits the leaf litter (Heyer *et al*., 1990; Sazima & Sazima, 1992). Additionally, when on the ground, *B. insularis* may be sheltered in rock crevices, burrows or even under vegetation, being protected when climate conditions are unfavourable. As aforementioned, we encountered many individuals of the Golden Lancehead hiding underneath *Anthurium parasiticum*.

Arboreal snakes are generally slender, with low relative body mass, and tend to be smaller than their nonarboreal relatives (Lillywhite, 2014). Adaptations to arboreality had already been reported for *B. insularis*, such as anterior heart in females (Wüster *et al*., 2005), slenderness and relative longer tail (Martins *et al*., 2002), and even retinal specialization (Patel *et al*., 2020). We found a positive association between substrate height achieved and SVL and relative body mass only for males, but not for females. This species, as many other representatives of *Bothrops*, show sexual dimorphism, with females attaining larger size than males (Marques *et al*., 2013), and the maximum SVL reported for males is 775mm (Guimarães *et al*., 2010). Our results show that females around this SVL used the highest substrates, up to 10 meters (which is also the maximum canopy height observed at QGI), decreasing the height used as SVL increases from there on. This suggests that, more than reflecting sexual differences on substrate use, it reflects an allometric effect. The same can be said about relative body mass. It is noticeable that relatively lighter animals use the highest substrates, both males and females. Higher arboreality associated with lower relative body mass has been already reported for other species in the Atlantic Forest (Banci *et al*. 2022). Both body size and mass may represent constraints for arboreality, considering the gravitational effects in blood circulation, and that snakes need to be long enough to transpose the gaps between branches, but not too heavy, considering the fragility of the branches (Alencar *et al*., 2017). Therefore, our findings are in accordance with the presumption that arboreality is strongly correlated with an optimal body size, being less heavily bodied and shorter (Harrington *et al*., 2018).

It has been hypothesized that the reproductive pattern may be conservative among *Bothrops* representatives, occurring during the autumn (Almeida-Santos & Salomão, 2002). However, previous observations have already evinced mating and courtship in *B. insularis* also during the winter (Almeida-Santos & Salomão, 2002; Marques *et al*., 2013). In the present study, most of the interactions between males and females were observed in the mid-winter. This extended duration of the reproductive season in *B. insularis* may be a consequence of the climatic conditions, with higher and more stable temperatures at QGI (Duarte *et al*., 1995; Almeida-Santos & Salomão, 2002). Comparative studies involving populations of other representatives of *Bothrops* might better elucidate the influence of climatic conditions on the duration of their reproductive season. Considering data from the literature (Marques *et al*.,

2013) and our observations, mating and courtship are more likely to occur on the ground, even though such events have already been observed on the vegetation (Marques *et al*., 2013; Amorim *et al*., 2019).

Distance from the initial location point was associated with time elapsed since the first encounter. However, it is noteworthy that the animals rarely move more than 50 meters away from the initial location even after almost 8 years, indicating a considerable site fidelity in the species. Most of our data are from animals reencountered after a short period of time (usually a few days after the first encounter), so it is reasonable to consider that such correlation between time interval and distance moved would not exist had more individuals been recaptured after a long period. Site fidelity has already been reported for its sister species, *B. jararaca*, that were found within 1 to 30 meters away from the initial location in over 24 months (Sazima, 1992), but this relative sedentarism does not seem to be a pattern among the genus (*e.g.* Wasko & Sasa, 2009).

In conclusion, our results show that *B. insularis* greatly rely on forested areas. Therefore, it is imperious that conservation strategies aimed at *B. insularis* consider also the preservation of this habitat. As important as this, it is to preserve the Atlantic Forest in the mainland, ensuring the maintenance of populations of birds that visit the island, where they are preyed upon by the Golden Lancehead. The current phytophysiognomies found at QGI does not seem to be concerning for the conservation of the species. It is also important to consider that neither this aspect, nor the low displacement of the species seem to have a negative impact on the gene flow at QGI (Salles-Oliveira *et al*., 2020), but long-term monitoring is crucial for assuring this scenario.

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APPENDIX 1

Plot	A - Leaf litter	B - Dead trees standing	C - Grass	E - Vines and lianas	F - Clearings	G - Epiphytes	I - Palm trees	Total	BII Class	number of snakes
1	2	4	4	4	2	1	1	18	low	
2	3	5	5	4	3	1	1	22	regular	1
3	5	4	5	4	5	1	1	25	good	0
4	5	3	5	4	4	1	2	24	regular	1
5	5	4	5	4	4	1	2	25	good	10
6	5	5	5	4	5	1	2	27	good	1
7	5	5	5	4	4	1	1	25	good	7
8	5	4	5	4	2	1	1	22	regular	1
9	5	5	5	4	2	1	1	23	regular	0
10	5	3	5	4	4	1	1	23	regular	1
11	5	4	5	4	5	2	1	26	good	1
12	5	4	5	4	4	1	1	24	regular	2
13	5	5	5	4	5	1	1	26	good	1
14	5	5	5	4	4	1	1	25	good	3
15	4	4	5	4	5	1	2	25	good	3
16	5	4	5	4	2	1	1	22	regular	4
17	5	5	5	4	2	1	1	23	regular	7
18	4	4	5	4	5	1	1	24	regular	1
19	2	5	5	0	5	1	1	19	regular	2
20	2	5	1	1	5	1	1	16	low	1
21	3	5	4	1	4	1	1	19	regular	2
22	1	5	1	0	5	1	1	14	low	1
23	2	5	4	1	5	1	1	19	regular	0
24	4	5	2	4	3	1	1	20	regular	0
							Mean:	22.3	regular	2.1

APPENDIX 2

Plot	**Area**	**Latitude**	**Longitude**	**Altitude**	**Cannopy**			**DBH**	**number of trees**	**% Rocks**	**% *Anthurium***	**number of snakes**
					% openness	**Max. Height**	**Mean Height**					
1	Forest	-24.48108	-46.676117	78	20.32	6.5	4	37.73	26	70	30	1
2	Forest	-24.48159	-46.67617	80	13.95	6.5	4.5	35.97	16	70	10	0
3	Forest	-24.48207	-46.67599	72	15.25	8.7	7	86	6	15	30	1
4	Forest	-24.48262	-46.67589	84	11.52	10	8	54	10	10	75	10
5	Forest	-24.48311	-46.67573	93	11.06	8.5	7	46.2	14	5	90	1
6	Forest	-24.4835	-46.67594	88	5.62	8.5	4.5	62.67	6	1	50	7
7	Forest	-24.48407	-46.67622	97	8.22	8.5	6	38.67	18	10	25	1
8	Forest	-24.48454	-46.67660	101	11.69	8	6.5	31.29	34	0	50	0
9	Forest	-24.48507	-46.67612	83	12.64	7.5	6.5	44.18	17	5	80	1
10	Forest	-24.48544	-46.67579	97	10.22	8.5	7	61.53	17	0	90	1
11	Forest	-24.48571	-46.67562	106	6.11	9.5	9	53.59	22	0	30	2
12	Forest	-24.48594	-46.67533	102	6.79	8	7	29.46	27	10	40	1
13	Forest	-24.48634	-46.67514	90	4.59	7.5	7	46.56	17	5	30	3
14	Forest	-24.48634	-46.67468	112	7.92	9.5	9	39.7	15	5	30	3
15	Forest	-24.48623	-46.67473	140	8.34	10	8	36	17	25	15	4
16	Forest	-24.48703	-46.67387	143	8.02	10	7	63.21	7	5	0	7
17	Forest	-24.48738	-46.67363	147	8.94	10	8	51.42	18	0	30	1
18	Forest	-24.48794	-46.67374	174	7.32	7.5	6.5	52.48	27	25	25	2
19	Forest	-24.48846	-46.6738	181	14.94	10	7	49.46	32	80	2	1
20	Open	-24.48067	-46.67607	79	95.05	2.7	1	21.62	4	2	0	2
21	Forest	-24.48026	-46.67622	83	15.01	4.5	3	20.35	65	50	10	1
22	Open	-24.47805	-46.676801	23	100	0	0	0	0	0	0	0
23	Forest	-24.47862	-46.67669	54	27.67	6	5	40.15	27	90	0	0
24	Forest	-24.47959	-46.67601	95	13.68	7	6	22.99	36	20	5	1
				Mean:	18.5	7.6	6.0	42.7	19.9	21.0	31.1	2.1