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Snakes and ladders: A review of ball python production in West Africa for the global pet market

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Abstract

The ball python (*Python regius*) is the most traded, CITES listed, live animal exported from Africa. Recent studies have raised concerns as to whether production methods in Africa are sustainable, humane and compliant with legislation. To aid future management we explored export patterns, using the CITES and U.S. LEMIS database, for live ball pythons from across their range in West Africa to identify the main exporters and the main markets supplied, and to assess associated trends, and compliance with nationally-established export quotas. We found that export to supply the global pet trade remains almost exclusively carried out by three range countries – Benin, Ghana, and Togo. The USA was the largest importer from all three countries, although Ghana appeared to be less dependent on the USA market than either Togo or Benin, exporting to a more diverse range of countries, particularly in Asia. Between 2003 and 2017 there was a decline in annual importer-reported exports from Benin and from Ghana, but not from Togo. Ghana appears to operate as a regional trade hub, re-exporting ball pythons imported from Benin and Togo, and exports more ball pythons reported as captive-bred. Trade records from all three countries exhibited a switch from predominantly wild-sourced to predominantly ranched individuals. However, at a range-wide level, differences in the use of source codes among exporting range states, and inconsistencies in reporting of trade among range states, as well as inconsistencies in the use of source codes between exporting and importing countries, represent areas of potential concern. We recommend a regional-level policy approach for this highly sought-after species, to safeguard ball pythons and local livelihoods.

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Keywords
CITES, *Python regius*, ranching, reptile, wildlife trade, Benin, Ghana, Togo

Introduction

International wildlife trade can only be sustainable if harvest (or offtake) at the national level is sustainable (Leader-Williams 2002) and thus, for species that are distributed across multiple countries, sustainability must depend not only on the level of use within one range state but collective use within all range states across the species' natural distribution. Accordingly, for wide-ranging species subject to use and trade, effective conservation management requires an understanding of where (across the species' range) exploitation and fragmentation of populations is greatest. This information provides the basis for appropriate regulation, and the necessary adaptive management that underpins legal trade. Harvest quotas, for example, are set and implemented at a national level, but are complicated by the fact that (in the absence of physical barriers) both wildlife and people move across borders (e.g., Bräutigam et al. 1994; Eniang et al. 2008; Bassey et al. 2010; Ayilu et al. 2016). In particular, people hunt, sell and trade wild animals (legally and illegally) across borders in accordance with variation in local abundance (of the species), hunting and trade regulations, enforcement, and markets (e.g., Shepherd and Nijman 2007; Selier et al. 2014; Krishnasamy et al. 2018; Nowak 2019). At an international level, identification and understanding of trade chains is complicated by additional trade links among source countries, disparities in reporting among them, and differences in destination (market) countries (which might influence trends in demand) (cf. Carpenter et al. 2004; Blundell and Mascia 2005; Robinson et al. 2015).

In this study we explore export patterns for live ball pythons (*Python regius*) from across their West African range, focusing specifically on captive-production systems. Ball pythons are an internationally renowned and popular pet, particularly in the USA, where they are sourced from both domestic captive breeding and international export from range countries (cf. McCurley 2005; Prestridge et al. 2011; Herrel and van der Meijden 2014; D'Cruze et al. 2020a). Among all African species that are listed under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and traded alive, ball pythons (listed on Appendix II of CITES, i.e. species for which trade is permitted, but regulated, to avoid utilization incompatible with their survival) represent the most common in terms of quantity (CITES Secretariat 2012). In an attempt to sustainably meet international consumer demand for this species, some range countries have adopted the use of ranching to produce ball pythons for export (e.g., Ineich 2006) where "ranching" is defined by CITES as "*rearing, in a controlled environment of animals taken as eggs or juveniles from the wild where they would otherwise have had a very low probability of surviving to adulthood*" (Lyons et al. 2017). Between 1996 and 2012 there was an almost 50-fold increase in global imports of ranched reptiles, dominated by ball pythons from Africa (Robinson et al. 2015), which were exported predominantly from Benin, Ghana and Togo (Toudonou 2015).

Native to western Africa and northern parts of central and eastern Africa, ball pythons occur in a wide range of savannah habitats, including open woodlands, rainforest margins, forest/plantation mosaics, and agricultural land, from Senegal to South Sudan and Uganda (Auliya and Schmitz 2010; see also Luiselli and Angelici 1998; Fig. 1). Ball pythons are predominantly nocturnal and are hunted during the day when they can be found resting in abandoned rodent burrows, termite mounds, or under dead oil palm trees and piles of grass and leaves (Harris 2002; D'Cruze et al. 2020b). To supply juvenile pythons for export, hunters collect neonates and gravid females (predominantly between January and March), the latter kept (by the hunters themselves or supplied to holding facilities) until the eggs are laid (D'Cruze et al. 2020b). According to hunters, females are typically later released back into their capture area (D'Cruze et al. 2020b), although it is not clear how long they are kept, when they are released, or what the specific release protocol is. Hunters also collect clutches of eggs (usually in February and March) that are sold directly to the farms (D'Cruze et al. 2020b) where they hatch approximately two months after laying (Aubret et al. 2003). The majority of juvenile snakes are exported post-hatching at 15–30 days of age (Sambo, pers. comm.; 15 April 2019) (under CITES Appendix II permit) and a proportion (in Togo approximately 20%) are released (Ineich 2006), although it is unclear on what basis the proportion to be released is determined.

Locally, in range countries, ball pythons are also taken from the wild for bushmeat, leather and use in traditional medicine (Auliya and Schmitz 2010; Segniagbeto et al. 2013; D'Cruze et al. in 2020c). Here we are concerned only with live exports for the international pet trade, which is considered the biggest threat to the species' conservation status (Auliya and Schmitz 2010). Our aim was to gain an objective understanding of the relative role played by each of the three main range states – Benin, Ghana and Togo – in the export of ball pythons. Specifically, we used CITES trade data, with additional data from the U.S. Fish and Wildlife Service Law Enforcement Management Information System (LEMIS), to assess trade trends (in numbers of animals involved, animal source, and compliance with export quotas reported to CITES), identify the markets (importing countries) supplied (and apparent trends in those markets), and the number of source-country exporters involved. A key aspect of the analysis was to quantify (as far as possible) formal and informal trade links between the three main source countries. Our trade analysis provides general lessons for many regionally-occurring species traded by multiple countries, particularly those for which there are clear links between in-situ (wild) and ex-situ (captive) sources that may cross national borders.

Ghana, Togo and Benin

The centre of the ball pythons' distributional range is Ghana (238,535 km^2) and Benin (112,622 km^2) that frame Togo (56,790 km^2) to the west and east, and in the south all three countries have a coastline to the Gulf of Guinea (Fig. 1). These three countries are recognised as the main reptile exporters of sub-Saharan western Africa with several species being harvested at significant levels for the international "exotic" pet trade (Affre et al. 2005), and Lomé, the capital of Togo, is a major hub for international live reptile export,

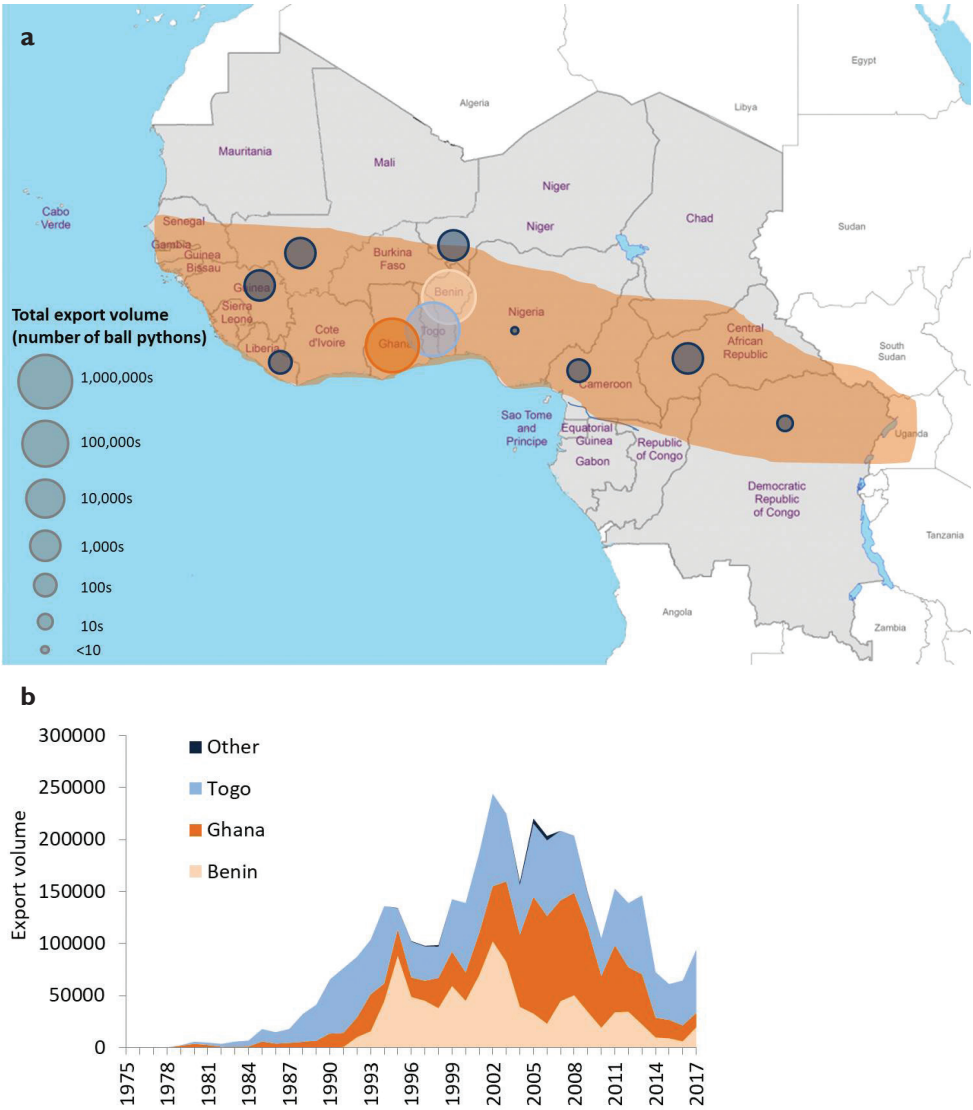

Figure 1. a Approximate distributional range of ball pythons in west and central Africa (shaded orange), showing relative export volume (number of individuals) of ball pythons exported from range states (depicted as proportionally-sized circles), 1978–2017. **b** Trends in annual exports for the three main exporting countries and all others combined. Species range from Auliya and Schmitz (2010) and Barker and Barker (2006). Trade data based on importer-reported quantities from the CITES Trade Database (http://trade.cites.org/).

particularly snakes (Jensen et al. 2019). International trade of ball pythons is regulated through CITES, an international agreement, that opened for signing in 1973 and entered into force in 1975. Ghana joined CITES in November 1975 (entering into force in February 1976), followed by Togo in October 1978 (entering into force in January 1979)

while Benin did not join CITES until February 1984, almost 10 years later (entering into force in May of 1984). According to CITES records, export quotas [see CITES Res. Conf. 14.7 (Rev. CoP15)] for ranched ball pythons from Benin, Ghana and Togo, and wild-caught ball pythons from Ghana and Togo, were all established by 1995 (CITES Notification No. 874). In Benin, the commercial export of wild-caught ball pythons was suspended in 1993 (CITES Notification No. 839), and quotas for the export of wild-caught individuals re-established in 1999 (CITES Notification No. 1999/21). Most recently, in 2008, Ghana also established quotas for captive-bred specimens (UNEP 2019).

Methods

All trade records pertaining to ball pythons exported alive from range states (as listed on the IUCN Red List) were downloaded from the CITES Trade Database (https://trade.cites.org/) in September 2019. We included all importing countries and territories (as listed by CITES), and all source codes (as outlined in Lyons et al. 2017), and purposes of trade (as defined in UNEP-WCMC 2013) (note that source codes have only been recorded since 1990). Data were downloaded as a comparative tabulation. Export quotas for Benin, Ghana and Togo, communicated with CITES, for ranched, wild, and captive-bred specimens (available since 1997), were obtained from the Species+ website (UNEP 2019). First, we described total native range exports and quantified the relative role of Benin, Ghana, and Togo, alongside other range countries actively exporting ball pythons. Second, for each of Benin, Ghana and Togo, we quantified total ball python trade (number of individuals exported, purpose, and source) between 1978 and 2017 (2018 data were not available at the time of the study), used time series analysis to assess trends, identified those countries providing the largest share of the market, and compared numbers reported traded with annual country export quotas. For analysis, both exporter- and importer-reported annual quantities in trade records were used, and compared; where numbers from only one dataset are presented, the dataset was selected based on completeness, and the dataset used stated in the text. Each "live animal" reported was presumed to represent an individual animal. For an earlier analysis of a subset of these data on ball python trade from Togo see D'Cruze et al. (2020e).

Additional trade data, for the period 2000–2017, detailing individual export and import companies, were obtained from the U.S. Fish and Wildlife Service Law Enforcement Management Information System (LEMIS) via a Freedom of Information Act (FOIA) request submitted to the Fish and Wildlife Service, Office of Law Enforcement (received on 8 May 2018, control number FWS-2018-00788). This level of information was only available for ball python trade to the USA.

Finally, to provide further insight on global market trends, for those importing countries (or regions) identified as important for range country exports, we repeated the CITES data collation (in November 2019) using all live ball python trade records from all exporting countries. For all major importing countries (or regions) identified, we assessed overall import trends over time, and, for the USA, known to be a major

captive producer of ball pythons (McCurley 2005), we also assessed exports (trends over time, markets supplied, and animal source) (see Suppl. material 1).

Trade data were described using graphs and summary statistics. To test for trends over time in CITES trade records, we summarised the records by year and used the tslm function in the "forecast" package (Hyndman 2017) in R to fit linear models to the resulting annual time series data, and to quantify and test the significance of trends. The entire time series was plotted for visualisation, but trends were assessed quantitatively over the most recent 15 years (2003–2017) rather than the entire time series available, because this represents the time during which ranching has been used extensively to produce animals for export. Importing country trends were assessed graphically only. Analysis of variance (ANOVA) and post-hoc Tukey's tests were used to test for absolute differences in trade parameters among countries (including year as a factor in the model where appropriate). Chi-squared tests were applied to test for country-level differences in summary statistics (with post-hoc pairwise comparisons carried out using the pairwiseNominalIndependence function in the "rcompanion" package, Mangiafico 2019). For all tests, statistical significance was accepted at $p < 0.05$. All statistical analyses were carried out in R (version 3.5.1, R Core Team 2018).

Results

Range state exports, 1975–2017

The CITES Trade Database holds a total of 2,129 trade records of ball pythons exported from range states, documenting total exports of between three and four million individual ball pythons (exporter-reported exports: 3,121,022; importer-reported exports: 3,979,680) since the first recorded export of the species in 1975. Since that time, 11 of 18 range states exported ball pythons but only six reached total exports over the period of 5,000 or more: Benin, Ghana, Guinea, Mali, Niger and Togo. The following numbers are based on importer-reported data but the relative ranking of range states as ball python exporters is the same regardless of the specific dataset used. Exports from Mali peaked in 1995 at 5,750, and comprised 14.2% of annual ball python exports that year, but <3% the following year, and <1% in all other years. Exports from Guinea and Niger comprised a small proportion of annual exports in all years, peaking at 3.6 and 2.1% (in 1997 and 2005, respectively). In total, through the 1980s, 1990s and 2000s, exports from Benin, Ghana and Togo collectively comprised 98.8% of all ball python exports from range states (28.6%, 44.0% and 26.2%, respectively; Fig. 1). Exports of ball pythons from Guinea, Mali and Niger were last recorded in 2008, 2010 and 2009 (respectively), and, since 2011, Benin, Ghana and Togo were the only range states to have reported ball python exports. Guinea and Liberia have been under a CITES trade suspension for all commercial trade since 2013 (CITES Notification No. 2019/075), and 2016 (CITES Notification No. 2018/012), respectively, as was Guinea-Bissau between 2016 and 2018 (CITES Notification No. 2018/011).

Benin, Ghana and Togo exports and production, 1978–2017

Export volume and trends

In total, CITES trade records document the export of approximately one million live ball pythons from each of Benin, Ghana and Togo, up to 2017, over 99% of which were intended for commercial use. Annual country-level exports varied between 100 and over 100,000 individuals with similar patterns among the three countries: peaking in the mid-1990s (but not in Ghana) and in the early- to mid-2000s (Fig. 1b, Fig. 2). However, over the most recent 15 years (2003 - 2017), there was a statistically significant decline in annual importer-reported exports in Benin (from 82,303 to 19,577 per year; trend=-3246, $F_{1, 13} = 16.54$, $p = 0.001$; Fig. 2a) and in Ghana (from 77,510 to 13,887 per year; trend=-6625, $F_{1, 14} = 38.75$, $p < 0.001$; Fig. 2b), but not in Togo (trend=-1,021; $F_{1, 13} = 1.48$, $p = 0.246$) where annual exports averaged 54,800 per year but showed considerable fluctuation (SD = 14,296; Fig. 2c) (exporter-reported data were not used to assess recent trends due to missing data). Considering only exports in the most recent five years in which there were no missing data (2011–2015), and taking account of year effects, exports from Togo were statistically significantly higher (annual mean between 2011 and 2015 = 54,163) than from Benin (annual mean = 21,876; Tukey post-hoc test, $p = 0.001$) (but not Ghana: annual mean = 38,415; $p = 0.078$).

Countries differed in their tendency to under- or over-report exports (exporter-reported quantities < importer-reported quantities and vice versa, respectively; $\chi^2 = 9.22$, df = 2, $p = 0.010$, based on the frequency of cases) and in the absolute value of discrepancies between exporter- and importer-reported quantities (ANOVA: $F_{2, 83} = 7.46$, $p = 0.001$). Exports from Togo were statistically significantly more likely to be under-reported (exports under-reported in 75% of 32 years) than from Ghana (where exports were over-reported in 62% of 34 years; post-hoc pairwise comparison $p = 0.006$; Fig. 2a–c) and discrepancies in reported quantities from Togo were statistically significantly greater than from either Ghana (Tukey's post-hoc test, $p = 0.002$) or Benin ($p = 0.012$). Exports from Benin showed no particular tendency to be under- or over-reported (50% of 20 years reported in both cases), and did not differ from Ghana in terms of the absolute value of reporting discrepancies ($p = 0.989$).

Source

Trade records from all three countries exhibited a switch from predominantly wild-sourced to predominantly ranched individuals (albeit in the absence of extensive wild-sourced exports from Ghana prior to the uptake of ranching; Fig. 3). In all three countries, since approximately 2003 (up to 2017), an average of >90% of annual country-level importer-reported exports (excluding seizures) were documented "ranched" (source code R, or "born in captivity" [source code F]) and an average of

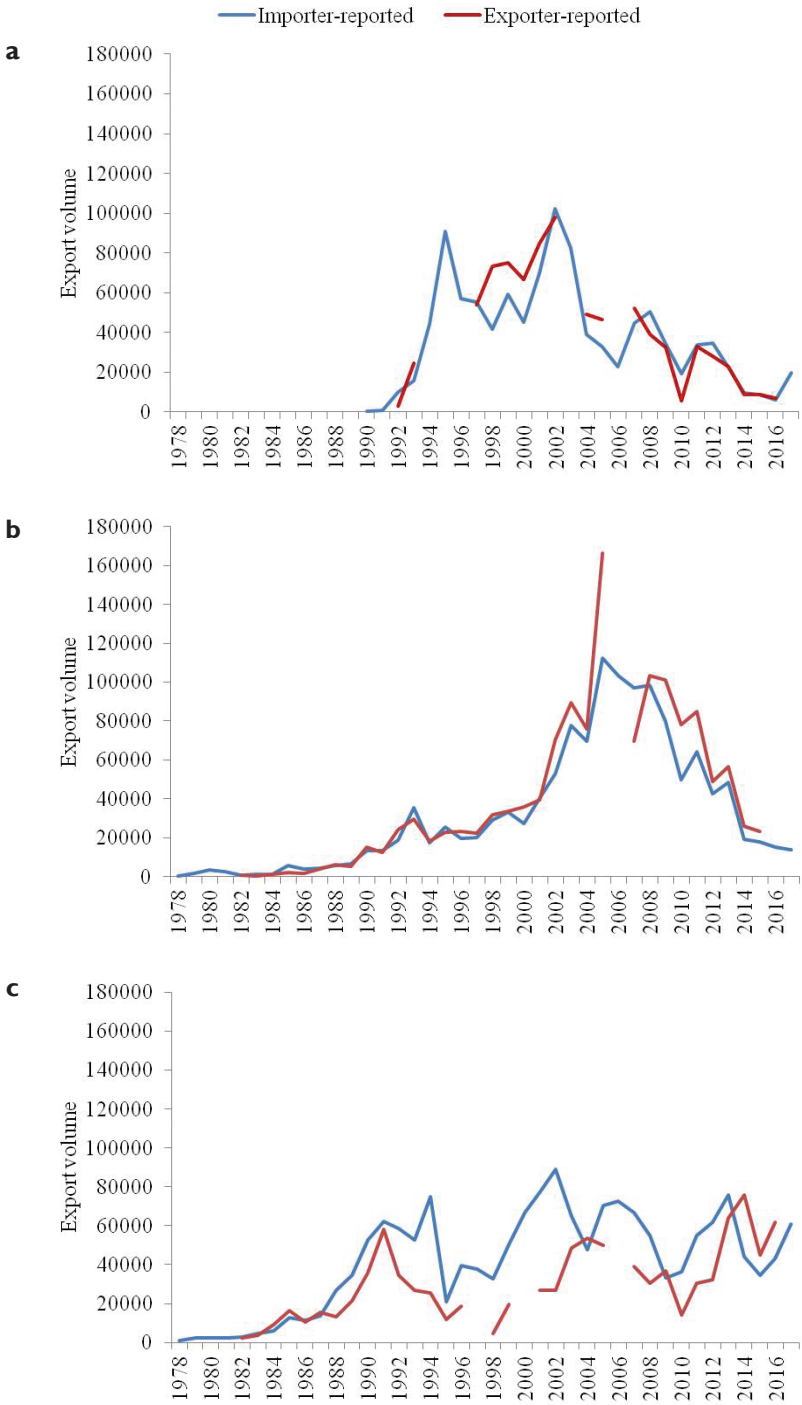

Figure 2. Annual export volume (number of individuals) of live ball pythons from Benin (**a**), Ghana (**b**) and Togo (**c**), as reported by both importing and exporting countries. Note: missing exporter-reported data in some years in all three countries. Source: CITES Trade Database (http://trade.cites.org/).

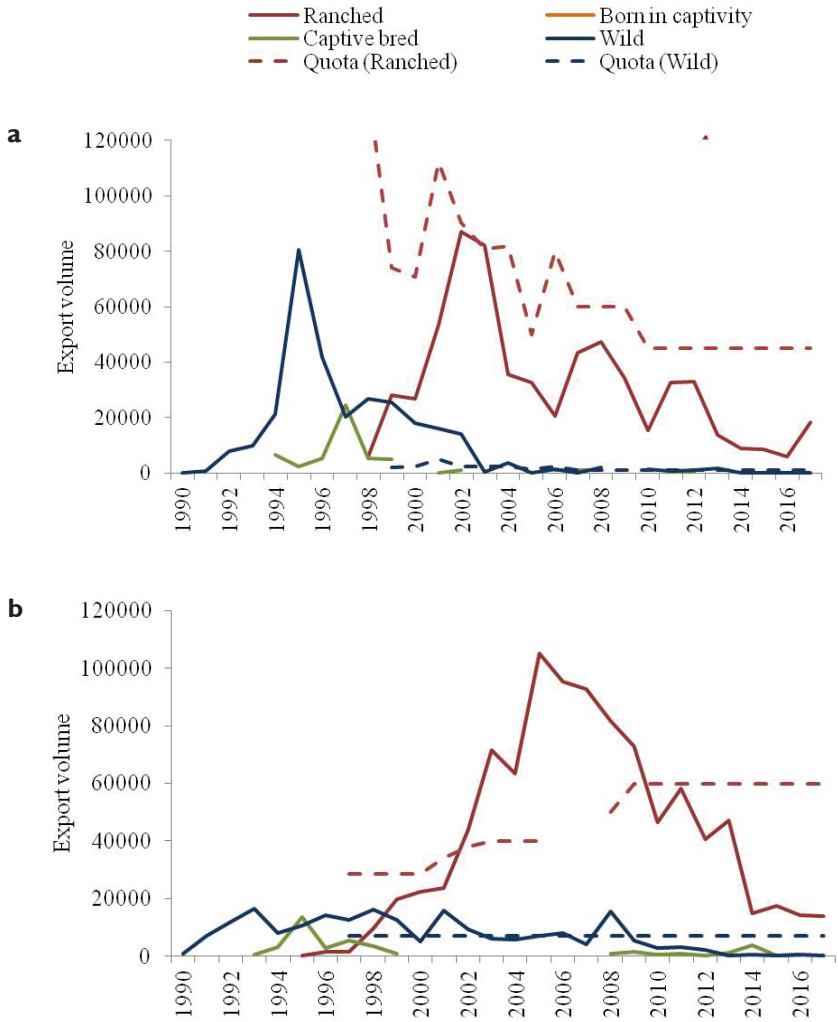

Figure 3. Source of annual exports (export volume, number of individuals) documented by Benin (**a**), Ghana (**b**) and Togo (**c**), based on importer-reported quantities in the period 1990–2017 (source codes were not recorded prior to 1990), together with export quotas (dashed lines) for ranched and wild specimens as communicated to CITES. Source codes defined as follows (Lyons et al. 2017): "Ranched" (R) = Specimens of animals reared in a controlled environment, taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood; "Bred in captivity" (C) = Animals bred in captivity in accordance with Resolution Conf. 10.16 (Rev.) (i.e. where parents mated in a controlled environment and where the breeding stock was established in a manner not detrimental to the survival of the species in the wild, and maintained [under normal circumstances] without the introduction of specimens from the wild); "Born in captivity" (F) = Animals born in captivity (F1 or subsequent generations) that do not fulfil the definition of "Bred in captivity" in Resolution Conf. 10.16 (Rev.) (e.g., parents did not mate in captivity, or the breeding stock/operations did not meet CITES requirements); Wild (W) = Specimens taken from the wild. Further detailed graphs in Suppl. material 1. Source: CITES Trade Database (http://trade.cites.org/) and UNEP (2019).

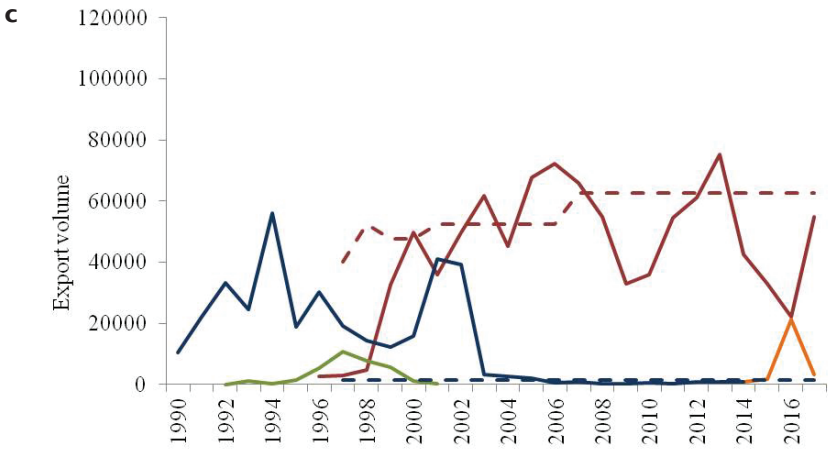

Figure 3. Continued.

< 6% were documented "wild-caught" (source code W; Fig. 3). However, the proportion of wild-caught individuals exported (post-2003) varied annually (ANOVA: $F_{1, 41} = 6.59$, $p = 0.014$) and among countries ($F_{2, 41} = 4.51$, $p = 0.017$), with Ghana, specifically, exporting a greater proportion of wild-caught individuals (maximum 15.9%, equating to 15,592 individuals, according to importer-reported quantities) as compared to Togo (annual maximum 5.2%, equating to 3,165 individuals; Tukey's post-hoc test, $p = 0.012$).

All three countries (post-2003) used additional alternative source codes representing different captive production systems; however, only Togo used the source code F, reporting a total of 26,782 individual ball pythons born in captivity exported between 2014 and 2017. All three countries used the source code C ("captive-bred") in the mid-1990s prior to the establishment of ranching (see Fig. 3); post-2003, use of this source code was limited (Fig. 3) but nevertheless accounted for up to 6% of Benin's exports (captive-bred individuals from Benin cannot be seen in the graph due to the low numbers and missing data in intermittent years but amounted to 1,000 individuals in each of 2006, 2007 and 2008, 600 in 2011 and 2012, and 1,200 in 2017) and up to 20% of Ghana's exports (in 2014, equating to 3,770 individuals).

U.S. Fish and Wildlife Service LEMIS import data listed a total of 51 unique export company names (inferred on the basis of similar spelling; n = 18, 22 and 11, respectively, in Benin, Ghana and Togo) that exported ball pythons to the USA between 2000 and 2017. Of the 51 export companies listed, only two to three (per country) operated in all years, and these were responsible for 64.8%, 71.8% and 88.3% (respectively, for Benin, Ghana and Togo) of total country exports to the USA. In Benin and Ghana, the number of export companies shipping ball pythons to the USA declined from eight and 11 in 2004 and 2005, respectively, to four and two in 2017. The number of export companies in Togo exporting to the USA in a single year was between two and six over the entire period (2000–2017; four in 2017).

Markets

CITES trade records document exports of ball pythons from Benin to 34 different countries/territories, and from both Ghana and Togo to each 57 different countries/territories (Suppl. material 2). The following is based on exporter-reported quantities. The USA was the largest importer from all three countries (Fig. 4). Over the most recent five years (2012–2016), the most significant importing countries/territories after the USA were: Hong Kong, the UK, France, Spain, and Ghana (albeit with each importing country/territory being the main importer for different exporting countries, Fig. 4). At a regional level, over the most recent five years, there was a statistically significant association between exporting country and importing regions (χ^2 = 65643, df = 8, $p<0.001$) such that Ghana appeared to be less dependent on the USA market than either Togo or Benin, exporting to a more diverse range of countries and, specifically, exporting significantly more snakes to Asian countries (36.6% exports compared with 6.8% and 10.4% for Benin and Togo, predominantly Hong Kong, but also Japan and Taiwan). Western European countries were prominent as importers from all three countries, comprising 9.1%, 18.3% and 15.1% of exports from Benin, Ghana and Togo, respectively (Fig. 4). There was little intra-continental trade within Africa originating from range states (except between Togo, Benin and Ghana, Fig. 4; see *Trade links* below). The only African non-range states to play a role as importers were Egypt (another major hub for the export of snakes from the African continent; see Jensen et al. 2019) and South Africa. Collectively, African countries were responsible for receiving only 6.4%, 1.5% and 3.7% of exports from Benin, Ghana and Togo.

For three of the main importers (the USA, the EU combined as a region, representing mainly Spain, UK, France and Italy, and Hong Kong), broad trends in the number of ball pythons imported over time suggest that whilst the role of the USA and the EU as importers declined over recent years, the role of Hong Kong as a major importer (supplied by Ghana, Togo and the USA) increased (Suppl. material 3). In parallel, the role of the USA as an exporter increased, and the USA is now the fourth largest global exporter of ball pythons responsible for the supply of ball pythons to a range of countries on all continents, including the UK, Germany, Japan, Hong Kong, Canada and Mexico (Suppl. material 3). The source of ball pythons exported from the USA shows a switch first from wild-sourced to ranched (as indicated by export source above), and then from ranched to captive-bred individuals, thus demonstrating a shift from the USA operating as a re-exporter of ball pythons documented as ranched in range states to an exporter of their own in-country production (in addition to the supply of their own [unquantified] domestic market, Suppl. material 3). Germany now also exports up to 1,600 captive-bred ball pythons each year, and is the largest exporter after range states, the USA and Canada.

Quotas and CITES Compliance

There was no statistically significant difference among the three countries in their tendency to exceed export quotas (the number of years in which quotas were exceeded)

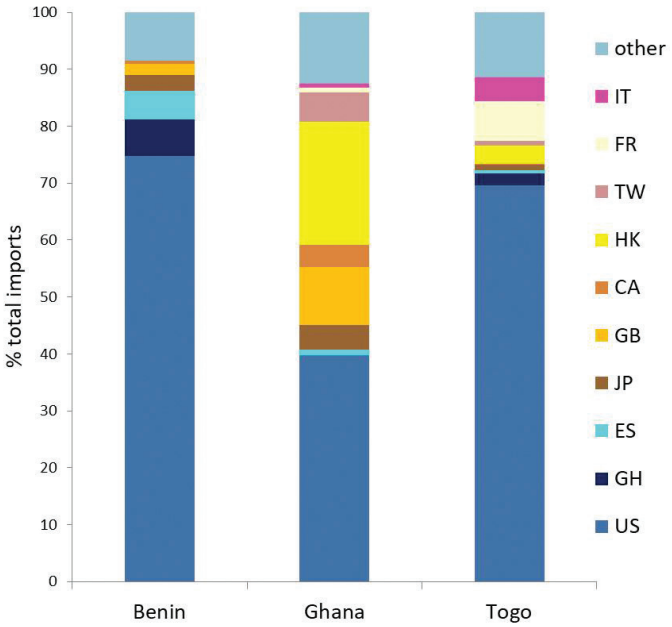

Figure 4. Countries importing live ball pythons exported from Benin, Ghana, and Togo, 2012–2016, shown as a proportion of total reported exports (based on exporter-reported quantities) from each country, including all countries responsible for importing 3% or more of total exports from at least one exporting country. US = USA, GH = Ghana, ES = Spain, JP = Japan, GB = UK, CA = Canada, HK = Hong Kong, TW = Taiwan, FR = France, IT = Italy. Note that for Ghana data are missing for 2016. Source: CITES trade database (http://trade.cites.org/).

over the 21 years included in the study for either ranched (number of years quotas exceeded = 1–8; $\chi^2 = 4.05$, df = 2, p = 0.132) or wild ball pythons (number of years quotas exceeded = 8–10; $\chi^2 = 0.96$, df = 2, p = 618; based on importer-reported quantities; Fig. 3). But the extent to which they exceeded quotas (the number of individuals in excess of specified quotas) was statistically significantly higher for Ghana, for ranched ball pythons, than Benin (ANOVA: $F_{2, 59} = 7.33$, p = 0.001, post-hoc pairwise test p < 0.001), and was statistically significantly higher for Togo, for wild-sourced pythons, than Ghana (although, for the latter, the country-wide comparison was not statistically significant [ANOVA: $F_{2, 57} = 2.98$, p = 0.059, post hoc pairwise test p = 0.047; based on importer-reported quantities; Fig. 3]). However, national export quotas communicated to CITES, and the rate and direction of quota adjustment over time, differed among countries. For example, the annual export quota in Ghana for ranched ball pythons was initially very low (28,500) and exports that were substantially higher than agreed quotas tended to occur during the period when the quota was low (Fig. 3b). During this period (in the early 2000s), the absolute volumes of live ball python exports from Benin and Ghana were not substantially different but the export quota in

Benin for ranched ball pythons was considerably higher (139,000, Fig. 3a). Ghana has since steadily increased its quota to 60,000 (Fig. 3b) whilst Benin has reduced its quota (Fig. 3a). Similarly, annual export quotas for wild specimens in Togo were exceeded on ten different occasions by up to 39,644 individuals (average 13,730) but Togo's quotas for wild-sourced pythons were the lowest of all three countries (1,500; Fig. 3c). Post-2003, wild caught quotas were exceeded rarely in any of the three countries, and by relatively small amounts (Suppl. material 1). In Ghana, animals declared to be captive-bred were exported in excess of quotas (200 individuals, established in 2009) in three of the last five years (2012–2016; Suppl. material 1). There is no quota in Togo for ball pythons born in captivity (source code F).

Trade links

Figure 5 summarises recent (2012–2016) ball python trade links among the three main range states of Benin, Ghana, and Togo. Most notable is the role of Ghana, that appears to operate as a trade hub, re-exporting ball pythons imported from Benin and Togo. Between 2012 and 2016, 6.4% of Benin's and 2.1% of Togo's exports were shipped to Ghana, most (>98%) were reported as ranched (although Ghana reported a proportion exported from Togo under source code F [born in captivity]), and most (c.90%) were then re-exported by Ghana to destinations outside Africa (Fig. 5). Since 2002 (and the broad-scale introduction of the ranching source code for ball pythons), 17% of ball pythons (n = 169,610, or 210–33,873 per year) exported from Ghana were reported to have originated from Benin, and 1.9% (n = 18,790, or 220–7,340 per year) from Togo. Reciprocal ball python trade from Ghana to Benin or Togo, and trade between Togo and Benin, was reported less frequently, inconsistently, and tended to involve smaller volumes. For example, Ghana reported the export of <1,000 (predominantly ranched) ball pythons to Benin since 2002 (800 between 2012 and 2016) but these were not reported as imports by Benin. In the year 2002, 2,000 ranched ball pythons exported by Benin were reported to have originated from Togo, but there were no recorded ball python exports from Togo to Benin. Similarly, 148 ranched ball pythons exported by Togo in 1981 (n = 70) and 2004 (n = 78) were reported to have originated from Ghana but there were no matching export/import records.

Other range states were represented (as animal origin or importers) in CITES trade records to a lesser extent, and the countries involved changed over time. In the 1990s, a proportion of ball pythons exported from Benin were reported to have originated from Burkina Faso (n = 2,987), Guinea (n = 2,200) and Mali (n = 2,850), c. 3,000 wild ball pythons exported by Togo were also reported to have originated from Mali, as did 440 exported by Ghana. More recently, in 2017, 75 ranched ball pythons exported by Togo reportedly originated from Senegal, and, in 2013, 4,000 ball pythons (also ranched) originated from Chad (although the latter is a regional country and not listed on the IUCN Red List as a range state per se). There were no export records for ball pythons from either Senegal or Chad to Togo. Togo also reportedly exported c. 800 ball pythons to Niger.

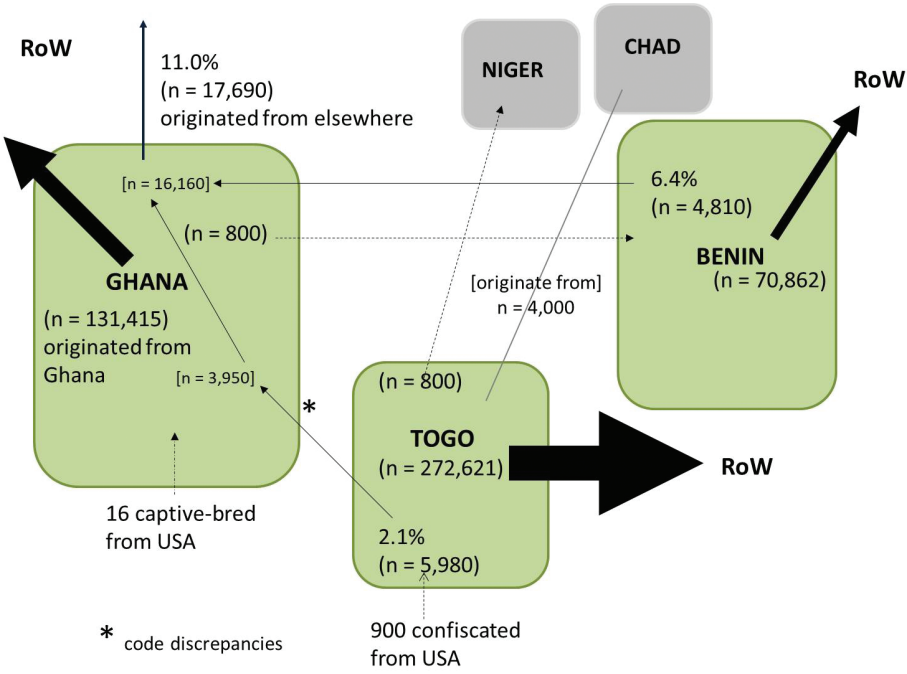

Figure 5. Exports to and from range states, 2012–2016 (including Chad which is a regional country but not listed on the IUCN Red List as a range state for ball pythons). Arrows show direction of export/import and are proportional to numbers exported. Note that 2016 figures were not available for Ghana. RoW = 'Rest of World'. Data source: CITES Trade Database (http://trade.cites.org), based on all live ball python trade records involving range states.

Discussion

Overview

Despite the relatively extensive geographical range of ball pythons, CITES trade data show that ball python production to supply the global pet trade, from within range states, remains almost exclusively sourced from three countries in the centre of their range–Benin, Ghana, and Togo (see also Toudonou 2015). Predominantly taken directly from the wild in the 1980s and 1990s, all three countries switched to ranching as the dominant reported production method in the early 2000s (see Gorzula et al. 1997). Togo has maintained relatively high levels of ranch production in recent years, but CITES trade records suggest that Benin and Ghana have not. It is not clear whether this decline in exports, from Benin, and to a lesser extent Ghana, was a conservation strategy designed to maintain exports within agreed quotas or a consequence of declining wild ball python populations required to supply production.

A significant proportion of exports from Ghana originate from Benin and Togo, implying that Ghana operates as a re-exporter (see also Pernetta 2009) in addition to a

harvester of its own resources. Ghana also supplies a slightly different and more diverse market than Togo and Benin (Fig. 4), relying more on the Asian than the USA market, and exports more reportedly captive-bred specimens (Suppl. material 1). Africa exports over 100 live reptile species to Asia (notably, in addition to ball pythons, leopard tortoises *Stigmochelys pardalis*), and whilst exports along this trade route have increased in recent years, there has also been an increase in reports of captive-bred specimens of a number of species (Outhwaite and Brown 2018). In Benin, production in recent years has been half to a third that of Ghana and Togo (Fig. 5) and management authorities of Benin have further reduced quotas of ranched specimens to 22,000 in 2018 (UNEP 2019), perhaps in response to reports from hunters and farmers that ball pythons in Benin have declined in number and may be extirpated in some areas (see Toudonou 2015). Export quotas for ranched individuals in Togo and Ghana (as of 2019) remain at 62,500 and 60,000 (UNEP 2019), respectively. However, in recent years Ghana has exported far fewer snakes than its quota allowed (Fig. 3), whilst Togo, despite being the smallest of the three countries, largely fulfils its quota (which may, in part, be supported by informal cross border trade at the local level, D'Cruze et al. 2020b, below).

At a range-wide level, differences in the use of source codes among exporting range states, and inconsistencies in reporting of trade among range states, as well as inconsistencies in the use of source codes between exporting and importing countries, may suggest irregular trading and/or improper ranching procedures, creates confusion in monitoring ball python trade, and represents an area of potential concern (CITES AC28 Com. 5). Since 2014, a proportion of ball python exports from Togo have been declared (by Togo, and by importing countries) as source code F (born in captivity, Fig. 3) rather than R (ranched). The CITES definition for animals "born in captivity" (see Lyons et al. 2017) may be a more appropriate descriptor of individual pythons from eggs of gravid females brought in to farms during what is otherwise described as "ranching" (see D'Cruze et al. 2020b); however, hunters in Togo sell eggs and snakes to farms in Benin and Ghana (D'Cruze et al. 2020b) but neither of these countries uses the source code F in their export reports to CITES (nor do countries importing from Benin or Ghana). Whilst it is possible that the source code F is being used in Togo, for example, by new staff, or under new guidance, that has not yet been adopted elsewhere, it is also possible that since there is no quota for ball pythons born in captivity it provides a useful loophole (i.e. classifying a proportion of exports as "F" may be a mechanism to maintain export volumes of ranched specimens within agreed quotas, see Fig. 3).

Domestic legislation

A brief review of the legislation in each of Benin, Ghana and Togo highlights some contradictory text regarding the legality of the practice of ranching in these countries. In Benin, for example, a license is required by Law No. 2002–16 (2004) for the harvest, market or export of "partially protected species" (which includes ball pythons), whether for personal or commercial purposes. Collection of the eggs of partially protected species

is also permitted on a limited basis but, under Article 154, it is an offence to hunt gravid females or juveniles. Since females and their juveniles of partially protected species are stated to be treated as fully protected species (Article 33), Article 36 also suggests that these animals should not be kept (except under exceptional cases for scientific purposes).

In Ghana, ball pythons are partially protected under Schedule II of the Wildlife Conservation Regulations of 1971 that prohibit hunting, capturing or destroying any species listed in Schedule II between August 1 and December 1 of each year, and hunting, capturing or destroying juveniles, or adults accompanied by juveniles, at all times. Outside of these restrictions ball pythons may be captured with possession of a valid licence according to Article 6 of the 1971 Regulations.

In Togo hunting permits are required by Articles 78, 79, 80 and 81 of Law No. 2008–09 of the Carrying Forest Code (2008) but discussions with hunters (D'Cruze et al. 2020b) suggest that not all hunters have them. Furthermore, as in Benin, it is unclear whether (even in the possession of a permit) the hunting of gravid females, juveniles, eggs and the associated destruction of ball pythons burrows (as described in D'Cruze et al. 2020b) can be carried out legally given that this activity is strictly forbidden under Article 78 of the Carrying Forest Code (2008). In Togo, sustainable management (see below) is required under Article 61 of the 005 Framework Law on the Environment (2008).

CITES compliance

At an international level, analysis of CITES trade records of ball pythons exported from range states reveals cases of missing data (e.g., missing export records for Benin, Fig. 2), potential mis-reporting (e.g., under-reporting of annual exports by Togo) and frequent exceeding of nationally established export quotas (Fig. 3, Suppl. material 1). In addition, there is evidence that snakes exported from a particular country were in fact sourced from neighbouring range states without adequare documentation (D'Cruze et al. 2020b) potentially negating conservation measures taken within other countries.

Discrepancies between export and import data in the CITES trade database are common (e.g., Phelps et al. 2010) and may arise for a number of legitimate reasons (e.g., specimens leaving a country at the end of one year, and arriving at their destination at the beginning of the following year, in which case the same shipment is recorded at export and import under different years, UNEP-WCMC 2013) – they may also reveal irregularities and deliberate under-reporting but these are not possible to distinguish from genuine reporting errors. Over-reporting by exporters can arise when the exporting country reports the number of specimens permitted for export rather than the actual number of specimens exported (UNEP-WCMC 2013).

All three countries exceeded quotas for both ranched and wild-sourced exports in at least one year, but vast differences in quotas mean that the implications of doing so differ. Exceeding a very low, conservatively established, quota, for example, may represent a lower risk in terms of potential over-exploitation than trading within the limits of a very high quota.

Trade links

Although some trade is formally documented between these three range states it is not clear to what extent CITES-recorded trade is representative of the actual inter-state trade that occurs in this region. For example, whilst Ghanaian exports include a proportion that originates from other range states there are discrepancies in the numbers traded between Benin and Ghana, and in source codes between Togo and Ghana, meaning that total regional exports (and their origins) are difficult to reliably quantify, and compliance with CITES trade quotas difficult to assess. For example, in 2012, Benin reports exporting 2,950 ball pythons to Ghana, whereas Ghana reports importing 10,500 Ball pythons from Benin–there are no transactions in either previous or later years that appear to account for the difference of almost 7,000 ball pythons.

Similarly, in 2014, Togo reported exporting 2,500 ranched ball pythons to Ghana but whilst Ghana reports importing 2,200 ranched ball pythons from Togo the following year (which may or may not be the same snakes) they also report importing 500 ball pythons born in captivity and 50 wild-sourced ball pythons in 2014 that do not appear in Togo's export records. The involvement of other range states, albeit at a relatively lesser level, and changes in the states involved over time, suggests that, as has been observed for other species in trade (e.g., pangolins, Heinrich et al. 2016), this regional network is dynamic and may be subject to change in response to relative abundance of snakes across their range, and economic interests of neighbouring states.

That ball python hunters also hunt in neighbouring countries, and sell pythons caught in one country to snake farms in another (D'Cruze et al. 2020b) introduces further complications. In reality CITES-reported exports from Benin, Ghana and Togo are composed to at least some degree of snakes sourced informally (or illegally) from neighbouring countries (see also Gorzula et al. 1997; Owusu-Nsiah 1999).

Sustainability

Sustainability is difficult to assess in the absence of wild population data. Although harvest size is often monitored in lieu of wild population size, harvest may be influenced by effort (hunting period, numbers of hunters, and area hunted) and the methods used, all of which may vary over time (Weinbaum et al. 2013 and references therein). Thus, whilst, for example, declining exports from Benin may indicate declining wild populations, it could also indicate shifting focus of local income opportunities, and it does not necessarily follow that relatively stable exports from Togo indicate stable wild populations. There are no accurate wild population data available (i.e. based on robust density estimates) for this species in any part of their range (Auliya and Schmitz 2010) but 75% of Togolese hunters surveyed by D'Cruze et al. (2020b) reported that there were fewer snakes than there were five years previously, and several commented that they had to travel further to find them.

Ball python exports from all three countries are dominated by a small number of export companies (although it is possible that there are more that export exclusively

to parts of the world other than the USA), and the number of companies operating in Benin and Ghana appears to have declined (although it is not possible to distinguish between a decline in the number of companies and a decline in the number of companies exporting to the USA). Further, shifting global trends (in market demand, Suppl. material 2) and increasing competition from captive-bred sources from the USA (e.g. Herrel and van der Meijden 2014) and some European countries, suggests that trade in individuals sourced directly from range states may itself not be sustainable in the long-term at present quantities.

The wider aspects of this trade: for example, risks of genetic pollution and/or disease introduction associated with improper re-release of snakes from ranches, and concerns regarding poor animal welfare at farms and in temporary holding facilities, together with documented additional uses in traditional medicine and bushmeat are discussed elsewhere (see Auliya et al. 2020, D'Cruze et al. 2020c, d, e). All such issues are potentially accentuated in a system whereby snakes are hunted and traded in convoluted trade chains throughout the region as described here (based on CITES trade patterns), and in D'Cruze et al. (2020b) (based on hunter questionnaires).

Recommendations

A multidisciplinary review in Togo (Auliya et al. 2020; D'Cruze et al. 2020b, c, d, e) has raised concerns as to whether ball python production methods are sustainable, humane and/or compliant with current legislation and other management protocols. To inform effective and evidence-based policy decision-making, D'Cruze et al. (2020e) recommended that the CITES scientific authorities in Togo should develop and initiate a scientific research programme to determine: (1) the distribution, population status, and population trends of ball pythons; and (2) demographic parameters of wild populations, including the reproductive output of wild and ranching-affected females, and (3) the morbidity and mortality rates of ball pythons during collection and transport from the point of harvest to the exporter's premises, prior to export. We suggest that the proposed research should be extended to Benin and Ghana.

Previous reviews of ball python production methods in West Africa have recommended a tripartite approach between the three main ball python exporters in West Africa (Benin, Ghana, and Togo) to ensure an effective regional-level conservation management plan for this species (e.g., de Buffrénil 1995). Key concerns highlighted here are: (1) improperly recorded cross-border ball python trade (together with unregulated cross-border hunting described in D'Cruze et al., 2020b); (2) inconsistent use of CITES codes; and (3) non-compliance with domestic legislation (see also D'Cruze et al. 2020e; Auliya et al. 2020). All of these potential issues could, arguably, be better addressed by CITES management authorities in Benin, Ghana, and Togo working together, coordinating efforts, and ensuring consistency in practices undertaken, regulation and legislation. To date, recommendations for this type of coordinated approach have not been officially acted upon.

At an international level, ball pythons have been included in a number of CITES driven processes in recent years but these have not taken the regional dynamics of py-

thon production into account. Specifically, at the CITES Conference of the Parties in 2016 (CoP17) Benin was encouraged to: (1) design and implement a management program for the species; (2) make non-detriment findings based on studies of the species, basic demographics, harvest and trade in the species; and (3) strengthen national regulations relating to trade control and monitoring, including stricter control policies for production systems (Dec. 17.276). However, at Animals Committee 30 the CITES Secretariat reported that Benin had not provided this information as requested. With regards to Togo, the ball python was not included in the Review of Significant Trade at Animals Committee 29 due to the incorrect assumption that it was already included in CITES Decisions (Dec. 17.276). To date, it appears that Ghana has not been requested to provide any similar information to CITES despite its prominent role in ball python production in West Africa. Instead of extending Decision 17.276 after the CoP18 in 2019, The United Nations Environment Programme – World Conservation Monitoring Centre was instead requested to "pay particular attention to *Python regius* from Benin, when performing its initial analysis of trade data" (CoP 18 Doc. 25). We recommend that this request be extended to Togo and Ghana. We also reiterate the recommendation that ball pythons in Benin, Togo, and Ghana should be considered for inclusion in future CITES procedures [e.g., Reviews of Significant Trade procedures (that would specifically delve into the sourcing of ball pythons from the wild), or Trade in Animal Specimens Reported as Produced in Captivity (that would focus on other issues related to those snakes reported as ranched)] at the next Animals Committee meeting.

Conclusions

This regional-level analysis reveals inconsistencies in management approaches among the three countries, and raises some concern regarding the sustainability of ball python trade in this region. It is clear that robust scientific information, that is currently lacking, is needed to determine the full current impact of the large scale international commercial trade on wild ball python populations and that this needs to be translated into policy in a cohesive way, particularly at a regional level in West Africa. Our findings, together with other recent studies in Benin and Togo (Toudonou, 2015; Auliya et al. 2020; D'Cruze et al. 2020b) suggest that export quotas and other management decisions are currently being made on an *ad hoc* commercial rather than scientific basis. The importance of this trade in terms of local livelihoods throughout the region (see e.g. D'Cruze et al. 2020b), particularly with respect to shifting global trade patterns and increasing competition for market supply from captive-bred sources in the USA and Europe (Suppl. material 1) (as well as the availability of, and adaptability to, alternative sustainable forms of income) also warrants further investigation. Ultimately, local livelihoods based on ball python exploitation are dependent on sustainability and continued market demand. The former depends on evidence-based and well-regulated wildlife management. The latter may (with increasing recognition of reptile sentience [Lambert et al. 2019], animal welfare [Baker et al. 2013], and public health risk [Moorhouse et al. 2017]) depend on evidence that the trade is sustainable, legal, safe (from a public health perspective), and humane.

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Supplementary material 1

Figure S1

Authors: Lauren A. Harrington, Jennah Green, Patrick Muinde, David W. Macdonald, Mark Auliya, Neil D'Cruze

Data type: CITES trade data

Explanation note: Annual ball python exports from range states shown against quotas.

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Supplementary material 2

Figure S2

Authors: Lauren A. Harrington, Jennah Green, Patrick Muinde, David W. Macdonald, Mark Auliya, Neil D'Cruze

Data type: CITES trade data

Explanation note: Global maps showing destination countries for ball pythons exported from Benin, Ghana and Togo.

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Supplementary material 3

Main importing countries: additional data

Authors: Lauren A. Harrington, Jennah Green, Patrick Muinde, David W. Macdonald, Mark Auliya, Neil D'Cruze

Data type: Additonal data for main importing countries and regions

Explanation note: Annual import volume and supply countries for ball python imports to the USA, the EU and Hong Kong, and annual export volume, countries supplied and source for ball pythons exported from the USA.

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RESEARCH ARTICLE

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The extent of the illegal trade with terrestrial vertebrates in markets and households in Khammouane Province, Lao PDR

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Abstract

Wildlife is one of the most important food resources in rural areas and popular among all social layers of Lao PDR. Numerous vertebrate species are sold at the local markets, but a comprehensive understanding of people's involvement and their impact on survival of local populations remains insufficient. This study provides the first interdisciplinary assessment using a questionnaire-based survey approach to investigate both markets and households in Khammouane Province in central Lao PDR. Data were recorded during the dry season (October and November 2017), as well as the rainy season (June and July 2018). We documented 66 traded species, mainly intended for consumption purposes, with more than half of them protected under either national law or international convention/red list. Furthermore, an evaluation of wildlife use from urban to the most accessible rural areas, indicated differences in affordability and trapping behavior. Our results suggest that wildlife availabilities can less and less satisfy the unchanged demands.

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Keywords
Biodiversity crisis, interdisciplinary, Southeast Asia, sustainability, wildlife trade

Introduction

Wildlife trade

The ongoing biodiversity crisis exceeds past rates drastically (Monastersky 2014; Thomsen et al. 2017) with an estimated loss of two to five species per hour (Singh 2002). The major driver for this loss is the over-exploitation of wildlife (Novacek and Cleland 2001) which can play an important role for local food security (Van Vliet et al. 2017, CI 2018) if handled sustainably. Millions of wild animal species and a broad variety of their products are traded every year (Rosen and Smith 2010), whereby the illegal trade alone is estimated to be worth 20 to 150 billion US dollars (Haken 2011), potential livelihoods for numerous people (Millennium Ecosystem Assessment 2005). Wildlife trade is considered the critical link between nature conservation and human sustainable development (Mascia et al. 2003, Broad et al. 2014). Wildlife is not only exploited for commercial trade, but also for self-subsistence (Redford 1992) of growing human populations. Lao PDR is located within the Indo-Burma Biodiversity Hotspot (Myers et al. 2000) and hosts a number of internationally important species (Duckworth et al. 1999). Simultaneously, it is one of the poorest countries in Southeast Asia (Government's Office 2014) measured by development and income (UNDP 2016). Lao PDR was identified as one major origin of seized illegally traded wildlife (Rosen and Smith 2010). Unsustainable trade poses a severe threat to Lao wildlife (Srikosamatara et al. 1992) and the current main challenge to preserve local fauna (Davies 2005, Gray et al. 2018, Srikosamatara et al. 1992).

The use of wildlife can be found in all classes of society, but a majority of Lao people lives under rural conditions (Silverstein et al. 2018) and benefits from wildlife for their income and as a food resource (Nooren and Claridge 2001, Roe et al. 2002). Other purposes are the common use for traditional or religious practices (Zuraina 1982), pet keeping (Chomel et al. 2007) and traditional medicines (Adeola 1992). Numerous species of terrestrial vertebrates are offered at Lao markets (Nijman 2010). Lao PDR holds a responsibility to implement nature conservation measures (Johnson et al. 2009), especially due to its large numbers of native mammals and birds (Giam et al. 2010). These taxonomic groups suffer from massive declines across the tropics with a modelled magnitude of 83% until 2050 (Benítez-López et al. 2017). Furthermore, wild-caught reptiles have occurred throughout Southeast Asian wildlife markets for more than 20 years (Klemens et al. 1995, Duckworth et al. 1999, Nijman et al. 2012) with Lao PDR regarded as a popular source (Stuart 2004). Similarly, the situation for amphibians must not be underestimated as one third of all amphibian species are already globally threatened (Whitfield et al. 2007) of which 70% are confined to tropical forests (Wilkie et al. 2011).

Legislation

Regulations and enforcements have been insufficient to control wildlife trade at both international or national level (Birnie et al. 2009; Rosen and Smith 2010). International cooperation against illegal trade is indispensable in order to effectively ensure conservation (Roe et al. 2002). Today's main wildlife trade regulation mechanism, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), relies on the capacities, resources, and efforts of signatory countries to implement its guidelines (Bennett 2011). However, the implementation is oftentimes not carried out properly or even fails due to contradictory laws or inadequate enforcement (Nurse 2015). To address this issue, CITES enlists national authorities for 183 states and regional economic organizations worldwide, including Lao PDR (last update: 31.07.2018, CITES 2016). CITES recorded significant trading activities in Appendix-II species in the country (CITES 2018).

Lao PDR's regulations on wildlife use and trade are mainly based on the Lao Wildlife and Aquatic Law (LWAL) (No.7, 2007), in which species are classified according to the Government's recognition of social values and protection requirements. Nonetheless, the regulations largely disregard international statuses and other biological factors. The LWAL lists three protection categories: species considered at risk of extinction and of high value to the society are listed in the Prohibition Category I; their use is prohibited without permission. Species in the Management Category II include those of national economic, social and environmental interest and importance for livelihoods; their use is attempted to be controlled (Schweikhard et al. 2019). A General Category III covers species with stable populations and are subject to a minimum of hunting restrictions. Due to its minor relevance for this study, this category is excluded here. The Prime Minister (No.05/PM, 8th of May 2018) directs authorities throughout the country to take commitments to international laws (The Laotian Times 2018). Additionally, a new Penal Code No. 26/NA issued on 17 May 2017 (effective 17th of October 2018) tightens prosecution of wildlife related violations. In addition, the Ministry of Agriculture and Forestry is instructed to investigate and take action against all businesses and individuals possessing or trafficking of wildlife (WWF Global 2018, The Laotian Times 2018). By limiting human interference within the animal's natural habitats, poaching is presumed to be controlled in a sustainable way (Peres 2002). Within these National Protected Areas (NPAs) and National Parks (NPs) it is easier to enforce restrictions, than trying to restrict people's habits overall (Milner-Gulland et al. 2003). With 24 NPAs and NPs, Lao PDR holds a significant number among Southeast Asian countries which results in a high likelihood for hunted wildlife to originate from these areas.

Khammouane / Hin Nam No Khammouane Province holds three protected areas, representing the remaining major forest areas: Phou Hin Poun NPA, Nakai Nam Theun NP and Hin Nam No NP (HNN), which is most relevant for this study. Covering an area of 92,000 hectares on the Lao-Vietnamese border in Boualapha District, HNN forms one of two largest protected continuous karst areas in the world (Williams 2018). On the 3rd of August 2016, it was submitted to UNESCO World Heritage Centre by the

Lao National Commission for UNESCO to be selected as a UNESCO Natural World Heritage site. It is the first NP in Laos with the status and can currently be found on the tentative list. The objectives of the HNN NP management include foremost the protection of wildlife. However, HNN NP's sensitive ecosystem is under steady pressure, since roughly 22 villages with a total population of 7,000 people (last update: 2005) live in the vicinity of the area. Alongside external stakeholders, many of the inhabitants live off the land and forests with poaching playing a key role (Magiera and De Koning 2013). Due to social factors, such as poverty and food insecurity, market demands and subsequent abandonment of other income (Pruvot et al. 2019), people have been driven to adopt ways of living that degrade the natural environment on which they depend (Broad et al. 2014). Currently, the expansion of land, encroaching into the protected area, is a way to secure basic livelihoods of villagers, especially in years of bad harvests (GIZ and Pro-CEEd 2014). Besides the constant struggle of linking sustainability and human survival, corrupt interests in Lao PDR hamper or render implementations ineffective, making it one of the latest countries failing to control illegal wildlife trade (Butler 2009).

Objectives

In a former study we provided data towards an annual overview and an evaluation of seasonal market fluctuations regarding offered species (Schweikhard et al. 2019). We assume that knowledge is lacking in the engagement of non-biological aspects in conservation. Therefore, the study combines an assessment of the current trade on-site, but also evaluates the trade drivers which are ultimately human. We aim not only to find out which species are affected but also to understand the role of wildlife in an average household. In addition to market surveys, we interviewed local households, which proved to be successful in portraying biological questions in a social context (White et al. 2005, Jones et al. 2005, Sirén et al. 2004, Drury 2011). This interdisciplinary approach allows the conclusion of a rough estimate of wildlife abundances around Khammouane Province. The assessment of traded species' vulnerability is based on respective categories of the IUCN Red List and CITES, as well as the LWAL Protection List in order to involve a local point of view.

Methods

We investigated the trade of wild-sourced terrestrial vertebrates, namely: mammals, birds, reptiles and amphibians. These four vertebrate classes cover the higher terrestrial fauna of the study area, Khammouane Province (~ 7,200 km^2). Located in central Lao PDR (17°30'N, 105°20'E) and bordering two other Lao provinces, as well as Vietnam and Thailand, the area connects important trade hubs and sets an ideal example for thriving trafficking (Fig. 1). Its capital, Thakhek, is situated along the Mekong River

which builds a natural border to Thailand. Due to its well-connected location, the town is a magnet for regional trading (Nooren and Claridge 2001).

To gain a year-round overview, the study took place in October and November 2017, as well as in June and July 2018, corresponding to dry and rainy season of the prevailing tropical monsoon climate. We surveyed local food markets (Fig. 2), documenting trade activities on-site, while household surveys addressed the consumer behavior (rainy season).

Market surveys

We conducted 66 observational surveys at 15 trade hubs (Fig. 1) (in two cases several markets at one site), which were at least visited twice, to address the main research question: Which species are traded to what extent? Findings with numbers over 100 individuals were rounded to each full ten count. As far as animals could be identified, the data was evaluated further regarding the corresponding conservation statuses based on CITES, the IUCN Red List, and the LWAL Protection List.

In addition to the permanent markets, temporary vending stalls along the highways *Route 12* and *Route 13* were documented, because they offered large amounts of wildlife (Nooren and Claridge 2001). These highways run through Khammouane

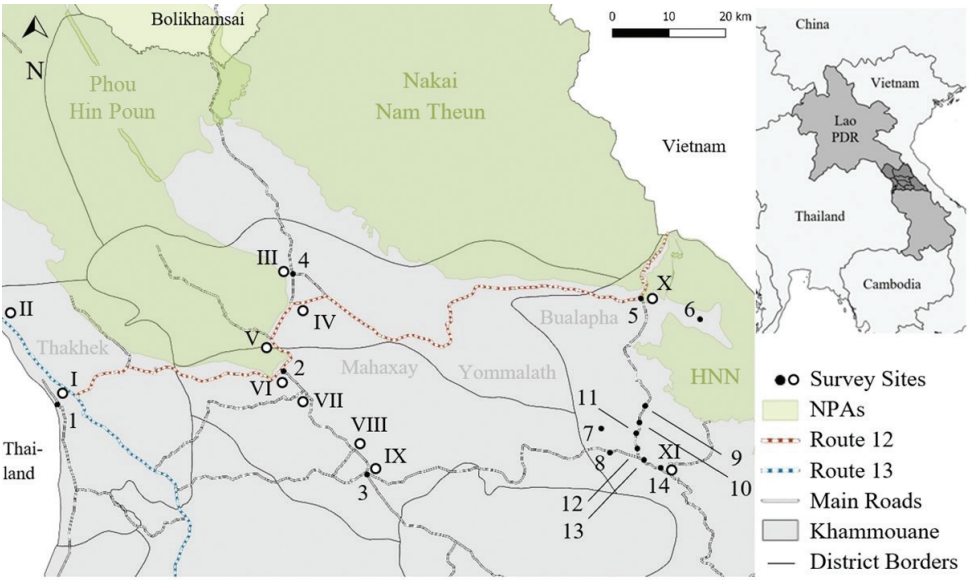

Figure 1. Map of Khammouane Province showing the locations of the household (black, 1–14) and market (white-filled, I–XI) survey sites. Source: Own map, compiled using self-collected GPS data and open source shapefiles ('Laos protected areas and heritage sites' by Open Development Laos (CC-BY-SA); 'Laos-Admin Boundaries' provided by Office for the Coordination of Humanitarian Affairs.

Figure 2. Local market facilities with cages and traps. Phot. T. Ziegler, K. Kasper.

Province into neighboring Vietnam, making them potential routes for the export trade. Eventually, one roadside stall, two convenience stores and one restaurant along these routes were also included in the study.

Household surveys

We conducted household surveys for a broader understanding of people's involvement in wildlife trade, use or trapping behavior. Here, trapping refers to a wide spectrum of wildlife harvesting including the use of snares, hunting with guns, slingshots or dogs, as well as the use of poisoned baits. We surveyed a total of 63 households at 14 sites (Fig. 1) within three different categories: rural areas, transition areas between these and urban areas, which reflect improving degrees of living conditions, infrastructure and trade accessibility with increasing urbanization. A majority of 44 surveys took place in the rural villages of Boualapha District around HNN NP, the main area of interest. In contrast, we included six interviews under urban conditions in the province capital, Thakhek, and 13 in transition areas between wildlife sources and main trade hubs. Participants were selected randomly and interviewed on a voluntary basis. A standardized questionnaire was used for data collecting (see Appendix 1). The interviewers, K. Kasper and J. Schweikhard, were introduced and accompanied by a GIZ employee as a direct translator, assuring the participant's anonymity and

immunity, as well as their understanding of the data being used for scientific purposes only. One interview was conducted for approximately 5–15 minutes and finally transcribed from voice recordings.

Data analysis

Statistical analyses were carried out in R environment for statistical computing (version 3.5.1, R Core Team 2017) using the libraries 'ggplot2' (Wickham 2016) and 'rcompanion' (Mangiafico 2018). To test for significant differences between findings of the dry and rainy season (up to 100 individuals per season), as well as area characteristics and other variables derived from household surveys, Fisher's exact test with a level of significance at $P < 0.05$ was applied. Ultimately, P-values were adjusted using the Holm method to correct the familywise error rate from multiple considerations of hypotheses (Holm 1979). Amphibian findings (with up to 2000 individuals per season) were analyzed using the χ^2-test with a level of significance at $P < 0.05$.

Results

Market surveys

Out of all listed terrestrial vertebrate species present in Lao PDR, large proportions could be found in trade, with majorities in highest conservation statuses (Tables 1, 2). Wildlife was found in all 15 surveyed trading sites (see also Figs 3, 4). A total of 66 species were documented with an average of 218.4 individuals per site. We refer to our previous study for further information (Schweikhard et al. 2019).

Household surveys

Approximately 90% of the surveyed households confirmed use of wildlife. However, differences became apparent in their own trapping involvement and the affordability of wild meat regarding the location of the household (Fig. 5). A majority of the rural population described wildlife harvesting as important for their livelihood and their involvement in trapping differed significantly from those in the urban area. Their trading activity on the other hand was comparably low, which illustrates a high self-consumption rather than trade interest when trapping wildlife. Whereas populations in the urban and transition areas almost entirely perceived wild meat as more expensive, results from rural villagers differed significantly as people found wild meat far more affordable. Also, wild meat was perceived cheaper in terms of sale units, since the cost per unit of domesticated meat was sometimes three times higher than wildlife products, e.g., a whole squirrel. During a survey in the transition area, one respondent claimed that although prices were rising, smaller species, such as rats, squirrels and frogs, were still affordable.

Table 1. Numbers of species native to Lao PDR and listed on CITES Appendices (CITES 2017), by categories of the IUCN Red List (IUCN 2020) and categories of the national LWAL Protection List (National Assembly 2008) in contrast to the proportion of observational data in the scope of the study.

Vertebrate	Conservation Status								
	LWAL		CITES		IUCN Red List				
Class	**I**	**II**	**I**	**II**	**CR**	**EN**	**VU**	**NT**	**LC**
Mammalia	44	15	35	24	11	23	19	8	172
Aves	36	21	6	77	7	6	18	36	656
Reptilia	8	13	3	26	5	10	17	5	121
Amphibia	1	0	0	0	0	6	11	6	112
% found	23.7	4.3	35.0	12.2	56.5	75.6	67.7	89.1	1.89

Table 2. Overview of observed species/genera at risk and their conservation status according to CITES Appendices, the IUCN Red List [Not Evaluated (NE), Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN) and Critically Endangered (CR)] and the Lao Protection List [Prohibition Category (P) and Management Category (M)], by taxonomic classes and orders. Large amphibian sales units were rounded to each full 5 or 10. Nat. = National Conservation Status; No.S.= number of sightings; Ind.= individuals.

Taxon	Species	Common Name	CITES	IUCN	Nat.	No.S.	Ind.
Mammalia							
Artiodactyla	*Muntiacus* sp.	Muntjac				1	1
	Muntiacus vaginalis	Northern Red Muntjac	none	LC	M	2	2
	Sus scrofa	Wild Boar	none	LC	none	5	10
	Tragulus kanchil	Lesser Mouse-deer	none	LC	none	1	1
Carnivora	*Helarctos malayanus*	Sun Bear	I	VU	P	1	1
	Herpestes javanicus	Javan Mongoose	none	LC	none	2	2
	Ursus thibetanus	Asian Black Bear	I	VU	P	2	2
	Lutrogale perspicillata	Smooth-coated Otter	II	VU	P	1	1
	Melogale personata	Burmese ferret-Badger	none	LC	M	1	1
	Paradoxurus hermaphroditus	Asian Palm Civet	none	LC	M	4	5
	Prionailurus sp.	Wild Cat				1	2
	Prionailurus bengalensis	Leopard Cat	II	LC	none	4	5
Chiroptera	*Cynopterus sphinx*	Greater Short-nosed Fruit Bat	none	LC	M	1	5
	Eonycteris spelaea	Cave Nectar Bat	none	LC	M	2	32
	Hipposideros armiger	GreatHimalayan Leaf-nosed Bat	-	LC	M	1	9
	Miniopterus sp.	Bent-winged Bat			M	1	4
	Rousettus amplexicaudatus	Geoffroy's Rousette	none	LC	-	1	1
Lagomorpha	*Lepus peguensis*	Burmese Hare	none	LC	M	1	1
Pholidota	*Manis* sp.	Pangolin	II	CR	P	2	5
Primates	*Nycticebus bengalensis*	Bengal Slow Loris	I	EN	P	3	3
	Pygathrix nemaeus	Red-shanked Douc Langur	I	CR	P	1	1
Proboscidea	*Elephas maximus*	Asian Elephant	I	EN	P	1	1
Rodentia	*Atherurus macrourus*	Asiatic Brush-tailed Porcupine	none	LC	M	2	2
	Bandicota savilei	Savile's Bandicoot Rat	none	LC	none	1	1
	Belomys pearsonii	Hairy-footed Flying Squirrel	none	DD	none	1	1
	Biswamoyopterus laoensis	Laotian Giant Flying Squirrel	none	DD	none	5	8
	Callosciurus erythraeus	Pallas's Squirrel	none	LC	none	14	26
	Callosciurus finlaysonii	Finlayson's Squirrel	none	LC	none	1	1
	Dremomys sp.	Red-cheeked Squirrel	-	-	none	1	2

Taxon	Species	Common Name	CITES	IUCN	Nat.	No.S.	Ind.
Rodentia	*Dremomys rufigenis*	Asian Red-cheeked Squirrel	none	LC	none	8	16
	Hylopetes sp.	Flying Squirrel	-	-	none	1	4
	Hylopetes alboniger	Particolored Flying Squirrel	none	LC	none	1	3
	Hystrix brachyura	Malayan Porcupine	none	LC	M	2	2
	Laonastes aenigmamus	Laotian Rock Rat	none	LC	P	2	3
	Leopoldamys edwardsi	Edwards's Long-tailed Giant Rat	none	LC	none	5	5
	Leopoldamys sabanus	Long-tailed Giant Rat	none	LC	none	12	80
	Menetes berdmorei	Berdmore's Ground Squirrel	none	LC	none	7	18
	Niviventer fulvescens	Chestnut White-bellied Rat	none	LC	none	3	4
	Petaurista sp.	Flying Squirrel	none	LC		5	7
	Petaurista elegans	Spotted Giant Flying Squirrel	none	LC	none	1	6
	Petaurista philippensis	Indian Giant Flying Squirrel	none	LC	P	1	2
	Ratufa bicolor	Black Giant Squirrel	II	NT	M	7	7
	Rhizomys pruinosus	Hoary Bamboo Rat	none	LC	none	2	2
	Rhizomys sumatrensis	Large Bamboo Rat	none	LC	M	1	1
Scandentia	*Tupaia belangeri*	Northern Treeshrew	II	LC	none	2	3
Aves							
Columbiformes	*Spilopelia chinensis*	Spotted Dove	none	LC	M	3	10
Cuculiformes	*Centropus sinensis*	Greater Coucal	none	NE	P	2	12
Passeriformes	*Acridotheres tristis*	Common Myna	none	LC	M	2	2
Strigiformes	*Ketupa ketupu*	Buffy Fish Owl	II	LC	M	1	1
Reptilia							
Squamata: Sauria	*Gekko gecko*	Tokay Gecko	II	NE	none	1	15
	Physignathus cocincinus	Chinese Water Dragon	none	NE	M	3	10
	Varanus nebulosus	Clouded Monitor	I	NE	M	4	5
	Varanus salvator	Asian Water Monitor	II	LC	M	4	4
Squamata: Serpentes	*Naja* sp.	Cobra	II	DD	M	1	1
	Ophiophagus hannah	King Cobra	II	VU	P	3	15
	Ptyas korros	Chinese Ratsnake	none	NE	none	4	8
Testudines	*Heosemys grandis*	Giant Asian Pond Turtle	II	VU	none	3	3
	Malayemys cf. *subtrijuga*	Mekong Snail-eating Turtle	II	VU	M	14	78
	Trionychidae sp.	Softshell Turtle				1	1
Amphibia							
Anura	*Babina chapaensis*	Chapa Frog	none	LC	none	1	10
	Fejervarya limnocharis	Asian Grass Frog	none	LC	none	19	2083
	Glyphoglossus guttulatus	Burmese Squat Frog	none	LC	none	2	110
	Hoplobatrachus rugulosus	East Asian Bullfrog	none	LC	none	41	2962
	Humerana miopus	Three-striped Frog	none	LC	none	5	395
	Kaloula pulchra	Banded Bullfrog	none	LC	none	3	107
	Leptobrachella sp.	Asian Leaf-litter Frog	none	LC	none	6	1162
	Occidozyga martensii	Round-tongued Floating Frog	none	LC	none	1	70
	Raorchestes parvulus	Karin Bubble-nest Frog	none	LC	none	1	6
	Sylvirana guentheri	Gunther's Amoy Frog	none	LC	none	9	575
	Sylvirana nigrovittata	Sap-green Stream Frog	none	LC	none	5	389

Roughly 84.1% of the respondents confirmed changes in the wildlife market over time. While a majority reported the general demand to remain the same, the availability of wild meats was reported to have decreased and accompanied by increasing prices (Fig. 6). In addition, it was repeatedly mentioned that prices for farmed meat were also rising with one informant speaking of increases up to one third in kilo prices.

Figure 3. Bengal Slow Loris (*Nycticebus bengalensis*) (left); squirrels (*Callosciurus erythraeus*, *Menetes berdmorei* or *Dremomys rufigenis*), bats (*Hipposideros armiger* and *Rousettus amplexicaudatus*) (right) offered at a local food market. Phot. C.L. Ebert.

Discussion

Socio-geographic diversity

Ultimately, the relationship between offer and demand as shown in the study can be a good indicator for wildlife use in the province as well as wildlife population status. By investigating local people's reflection on wildlife availability and accessibility in markets, we demonstrated that available wildlife products fail to satisfy the constant demand. This allows a disquieting view on the issue against the background of an escalating biodiversity crisis, as biodiversity especially in the study area faces an immense loss (Hughes 2017). Most frequently traded species mainly consisted of birds, squirrels, rats and frogs (Fig. 7). This intensity of pressure can negatively affect populations in the long term and driving bird species into extinction since prehistoric times (Duncan et al. 2002).

However, the use of wildlife as meat is no longer a matter of subsistence. This was confirmed by many of our interviewees in the urban areas, who described their preference of wild meat over domesticated meat. Studies from Africa and Asia indicated that an increase in wealth may cause a significant rise in demand, resulting in expanding wildlife markets in urban towns (Robinson and Bennett 2002). In urban areas where bushmeat is much pricier than domesticated meat (Bennett 2002), mainly people of high social status and income consume preferably rare and expensive sorts of wild meat (Shairp

Figure 4. Buffy fish owl (*Ketupa ketupu*) (top left), Mekong snail-eating turtle (*Malayemys* cf. *subtrijuga*) (bottom left), Chinese water dragon (*Physignathus cocincinus*) (top right), and East Asian bullfrogs (*Hoplobatrachus rugulosus*) with broken legs (bottom right) offered at a food market. Phot. K. Kasper, T. Ziegler.

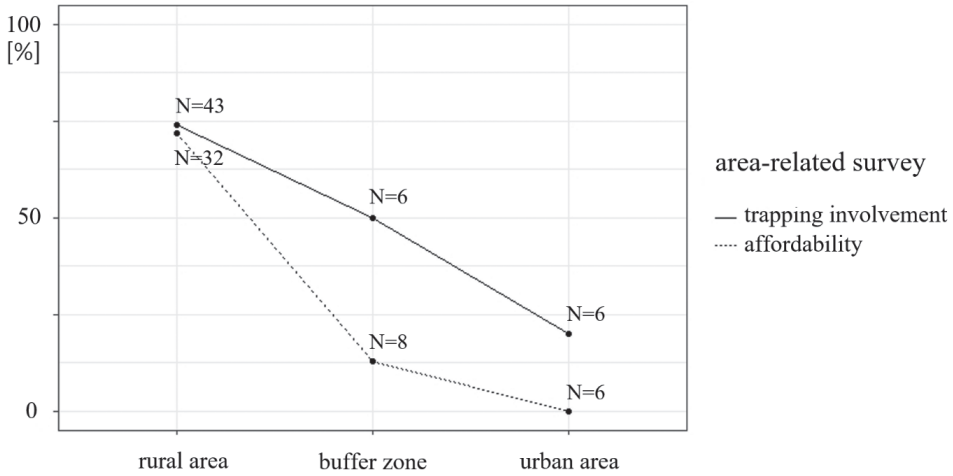

Figure 5. Households along an urbanization gradient (rural, transition and urban area) that depicts their involvement in wildlife trapping activities, and those that claimed rate wild meats on the market more affordable than meats from a farmed source (N = 55, 46). Trapping involvement of households in rural areas differs significantly from that in the urban area (Fisher's exact test, P = 0.032). Regarding wild meats' affordability, households in rural areas differ significantly from the other areas (Fisher's exact test, P=5.928-5), with a difference to transition areas by P = 0.007 and P = 0.005 to the urban area. Data was drawn from the respective interviews.

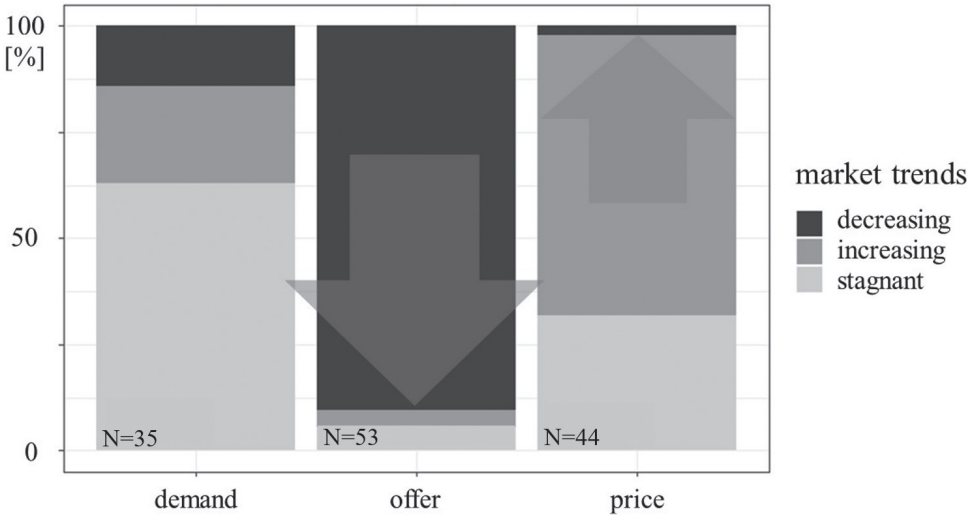

Figure 6. Households observing market changes regarding demand trends (N = 35), offer (N = 53) and prices (N = 44) for offered wildlife. There are differences between offer and demand (Fisher's exact test, $P = 4.08 \cdot 10^{-13}$), as well as offer and prices (Fisher's exact test, $P = 1.57 \cdot 10^{-20}$). Data was drawn from the respective interviews.

et al. 2016) to establish their social status among their peers. The high appreciation of wild meats, that many Lao people share was already documented in a prior study from Vientiane Province over 15 years ago: the majority of interviewed people stated that they would rather pay a higher price for a smaller amount of wild meat, than to pay a lower price for a larger amount of domesticated meat (Hansel 2004). Despite the risk of infectious diseases emerging from wildlife consumption (Zhang and Yin 2014, Kurpiers et al. 2016, Pruvot et al. 2019), our study reveals growing values of wild meats even today and similar statements were made in the urban environment and transition areas, whereas rural households were rather involved in trapping than paying high market prices. Minor but repeated information gathered about people's meat choice during our interviews indicates a preference for wild meats because of superstitious belief, its suspected health benefit and invigorating effect, as well as a status symbol that goes along with wild meat consumption. Other studies confirm this (Shairp et al. 2016, Sandalj et al. 2016).

Lao PDR

Wild meat remains a primary protein source in rural areas such as in Khammouane Province (Bennett 2002) and essential for people living in arboreous environments (Redford 1992, Milner-Gulland et al. 2002). The results of this study reflect this reality by showing a high level of participation by local people in hunting and trading activities in the rural areas of Khammouane Province. With the majority of Lao PDR´s citizens living in rural areas (Silverstein et al. 2018), their impact on local wildlife

Figure 7. Rats (*Leopoldamys sabanus*, *Niviventer* sp. or *Rattus* sp.) (upper left), squirrel (*Menetes berdmorei*) (upper right), and frogs (bottom) were most common traded wildlife. Phot. T. Ziegler.

populations is severe. The situation in Lao PDR resembles those in other regions in the world, such as Central Africa (Van Vliet et al. 2017), Indonesia (Harris et al. 2017) and Brazil (Chaves et al. 2019), with similar environmental, economic, and social settings.

The issue of unsustainable wildlife consumption in poor countries like the Lao PDR (Government's Office 2014) might even worsen in the future, as the global human population growth (Estrada 2016) and its demand for wildlife is estimated to increase the most in the world's least developed countries (FAO 2009). Furthermore, as Lao PDR is located in a biodiversity hotspot, the ultimate impact of the unsustainable hunting and trading of wildlife on global biodiversity and endemicity is unforeseeable.

Conclusions and recommendations

The current biodiversity crisis and the issue of its driving forces, such as the unsustainable usage of wildlife (GIZ and ProCEEd 2014), are complex problems which require multifaceted efforts to be implemented. A simple ban of trading wildlife is unlikely to succeed as it might shift the sales of wildlife and derived products from open market displays to the underground (Nooren and Claridge 2001). Without enforcing such

regulations, the law-obidiance is not ensured, as open sales of wildlife were observed during this study in close proximity to government signs stating the genereal ban of trading wildlife. If the trade shifted into the underground, it would dimmish the opportunity to monitor the trade and its trends. On the other hand, continuous trading observations would be aggravated. To get to the root of the issue, each driver for wildlife consumption needs to be addressed individually. Khammouane Province represented a hotspot of biodiversity as well as wildlife trade, and therefore requires appropriate attention by the local authorities. We recommend local authorities to assess the markets within the province capital Thakhek in particular, as they showed the highest quantity of wild meats. The markets at Namdik and Ban Kok turned out to be very active trade hubs for wildlife as well, regardless of the vertebrate group. This is presumably due to their advantageous location at Routes 12 and 13, which are the province's main connections across the international border between Lao PDR and Vietnam. Mahaxay May and Boualapha were found entirely inactive in terms of wildlife trade, although one household survey participant mentioned Boualapha as a place with occasional sales. Their market activities were probably replaced by the nearby market at Ban Kok. Thepsomebath and Ban Langkhang markets largely consisted of sellers from rural areas who are unable to afford stalls inside the market building. Rainy seasons seem to have a detrimental influence on sales activities, resulting in seasonal variation of documented activities. Additionally, Ban Langkhang is situated near the HNN NP where wildlife can be easily extracted. We gained information that before wildlife is offered here, it is mostly transported to Thakhek, where the demand is much higher.

Frogs, squirrels and rats were documented in almost every recorded instance, outnumbering other taxonomic groups by far (Fig. 7). Even though they are not in need of urgent protection now, further assessment of their natural populations in the NP should be conducted to better understand the hunting impact. Frequent trade of protected turtle and monitor lizard species on the other hand requires immediate interventions. Moreover, it is alarming that the trade and demand for keystone species like bears, civets and cat species could be easily witnessed.

The loss of certain species may cause a cascade of unforeseeable effects in the ecosystems. For example the loss of a species that others depend on can lead to subsequent extinctions of dependent taxa (Koh et al. 2004). Therefore, the biodiversity of tropical Southeast Asian countries like Lao PDR must be protected. Another known major obstacle in the conservation efforts is the lack of data (Novacek and Cleland 2001). Further investigations of the current level of diversity and distribution and population status of endangered species are urgently required.

Sharing a border with neighbouring China, one of the major wildlife consumers, significantly increases the species decline in Lao PDR (Srikosamatara et al. 1992). Strict and effective border controls should be established to reduce the amount of cross-border trade. During this study, many interviewees confirmed China as a main importer of wildlife poached in Lao PDR. The country's wildlife and their products are often trafficked across Vietnamese borders into China (WWF Indochina Program). New goals to better prevent wildlife associated crimes, including strengthening inter-

national cooperation, are supposed to be implemented until the year 2020 (Ministry of Natural Resources and Environment 2015). Rising awareness among Lao villagers could also support the conservation of wildlife, as a study on the effects of knowledge about wildlife laws in tropical Madagascar indicated that people with higher education/awareness are more likely to know about the wildlife laws and specific protected species and less likely hunt them (Keane et al. 2011). Not to mention that, to prevent wildlife trade and consumption implies a substantially reduced risk of wildlife-associated emergence of zoonotic parasites and pathogens in humans (Kapel and Fredensborg 2015, Greatorex et al. 2016, Borsky et al. 2020). As an immediate example, the outbreak of the coronavirus 2019 (COVID-19), primarily considered to be a consequence of consuming wildlife, has caused devastated damages on individual lives, society and economy (Galea et al. 2020, Fernandes 2020). Lastly, eco-tourism presents a great opportunity to combine conservation efforts and an alternative source of income. Former hunters with excellent knowledge of the forest and wildlife habitats can serve as professional wildlife tour guides. A similar approach is successful in the northern Lao Nam Et-Phou Loei NP (Butler 2009), where eco-tourism is operated, benefiting local villagers financially and motivating them to protect the forest and it's inhabiting species.

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Appendix 1

Standardized questionnaire for the household surveys, arranged by categories of interest.

		Consumption
1	**Is wildlife consumed or traded by themselves or in the household?**	94% yes; n=63
1.1	If no, in the past?	50% yes; n=4
1.2	If ever, which animals?	n=61
		Food Purposes
2	**How often is wild meat consumed?**	2% daily, 39% weekly, 48% monthly, 11% annually; n=54
3	**What kind of meat is preferred?**	40% wild meat, 44% domesticated meat; 16% uncertain; n=63
4	**Is wild meat more expensive or cheaper than domesticated meat?**	38% cheaper, 35% more expensive, 27% uncertain; n=63
5	**What are the reasons for choosing wild meat?** (multiple choice)	48% taste, 43% health, 9% beliefs, 18% status symbol, 21% food variety, 29% necessity; n=56
		Medicinal Purposes
6	**Is wildlife used for medicinal purposes?**	32% yes; n=56
7	**What kind of medicine is preferred?**	13% wildlife-based, 67% conventional, 20% uncertain; n=63
		Market Situation
8	**Has the wildlife trade changed over time?**	84% yes; n=63
8.1	Changes in prices?	22% none, 46% increase, 2% decrease, 30% uncertain; n=63
8.2	Changes in offer?	5% none, 3% increase, 76% decrease, 16% uncertain; n=63
8.3	Changes in demand?	35% none, 13% increase, 8% decrease, 44% uncertain; n=63
9	**Is open display on markets avoided (by direct connections between trappers and costumers)?**	32% yes, 2% no, 66% uncertain; n=63
		Trapping & Trading
10	**Is the household involved in wildlife trading?**	16% yes; n=57
10.1	Is trading important for their livelihood?	44% yes; n=9
11	**Is the household involved in wildlife trapping?**	65% yes; n=55
11.1	Is trapping important for their livelihood?	76% yes; n=33
		Conservation
12	**Is there awareness about conservation statuses?**	89% yes; n=63
12.1	Is the Lao Protection List known?	7% yes; n=59
12.2	If so, could protected and unprotected species be differentiated?	25% yes; n=4

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Distribution modelling of the Pudu deer (*Pudu puda*) in southern Chile

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Abstract

The Pudu deer (*Pudu puda*) is endemic to the temperate rainforest of Chile and Argentina and currently faces serious conservation problems related to habitat loss. However, studies undertaken on this species are not sufficient to identify suitable areas for conservation purposes across its distribution range. In order to estimate the current and future distribution of the Pudu deer in southern Chile, we modelled the potential distribution of this species, based on occurrence points taken from seven contiguous provinces of this area using the Maxent modelling method. The Pudu deer distribution covered an estimated area of 17,912 km^2 (24.1% of the area analysed), using a probability of occurrence above 0.529, according to the threshold that maximises the sum of sensitivity and specificity. In contrast to the Andes mountain range, areas with higher probabilities of occurrence were distributed mainly on the eastern and western slopes of the Coastal Mountain Range, where extensive coverage of native forest persists, as occurs in the provinces of Ranco, Osorno and Llanquihue. Projections to 2070, with global warming scenarios of 2.6 and 8.5 rcp, revealed that large areas will conserve their habitability, especially in the Coastal mountain range. Our results reveal that the Coastal mountain range has a high current and future habitability condition for the Pudu deer, a fact which may have conservation implications for this species.

Keywords

conservation, habitability, niche modelling, Pudu deer, temperate rainforest, threatened species

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Introduction

The Pudu deer, *Pudu puda* (Molina, 1782), is a cervid endemic to southern South America, characterised by being one of the smallest deer in the world due to its short shoulder height (30–40 cm) and lower body weight (< 15 kg) (Jiménez 2010). This species is distributed in Chile and Argentina from 35°10'S to 46°45'S (Jiménez 2010) and from 39°23' to 42°58'S (Meier and Merino 2007), respectively, occupying an area of 128,278 km^2 according to the International Union for Conservation of Nature (IUCN), mainly located in Chile. The Pudu deer characteristically inhabits the pristine temperate rainforest, particularly in areas of dense understorey growth and native bamboo thickets (Eldridge et al. 1987; Meier and Merino 2007), but can also be found in disturbed and secondary forest habitats (Jiménez 2010). Current conservation status of the Pudu deer is Near Threatened according to the IUCN or Vulnerable, based on the threatened species list of the Chilean Ministry of Environment. This conservation status is related to different threats that appear to have affected the viability of the species. Amongst these, local threats have been identified linked to the expansion of human activities, such as forest loss and fragmentation, predation by domestic dogs, competition with exotic species and poaching activities (Miller et al. 1973; Wemmer et al. 1998; Silva-Rodríguez et al. 2010; Silva-Rodríguez and Sieving 2012; Jiménez and Ramilo 2013). Global warming, as a result of the concentration of greenhouse gases, may constitute another threat that could reduce the future survival prospects of the Pudu deer. It is expected that this factor may affect the habitability conditions of current distribution areas of the species as a consequence of climate change and, therefore, affect its future geographic distribution. This is not an unlikely scenario, since global climate models predict precipitation pattern changes and increased frequency and severity of droughts by the end of the 21st century (IPCC 2014), a process that is expected to impact ecosystem structure and function. In fact, modelling studies have predicted that levels of species loss of all currently-known species will range from 0 to 54%, including an overall extinction risk of 7.9%, as a result of future climate change (Urban 2015).

Although the Pudu deer is under threat, few studies have been carried out on this species and available information is insufficient to clarify its density or identify suitable areas for conservation plans. With regard to density data, available estimations suggest that the Pudu deer population may be fewer than 10,000 individuals across its distribution range (Miller et al. 1973; Wemmer et al. 1998; Jiménez and Ramilo 2013). Another estimation from the IUCN suggests, however, that the Pudu deer population is likely to exceed this figure, based on the assumption of 10% occupancy by this species in the native rainforest. Nonetheless, it should be noted that, to date, no extensive field data-based estimation has been performed to support this. Recent studies on this issue have been performed mostly in small areas of southern Chile, particularly in natural reserves or areas with relatively well-preserved native forests (Delibes-Mateos et al. 2014; Sanino et al. 2016; Zúñiga and Jiménez 2018). Despite this sampling limitation, these studies have contributed important evidence that supports a reduced relative abundance of this species in these areas, since the number of detection events per 100 camera-trap days has been relatively low (0.16–3.4).

Osorno Province (40°13'–41°00'S) is a geographic area located in the northernmost part of the Los Lagos Region. Around 15 native terrestrial mammals have been described in this Province, with the Pudu deer being the only one native deer currently distributed in this area (Iriarte 2010). Large areas of pristine Valdivian temperate rainforest can be found in Osorno Province, mainly in the Coastal and Andes mountain ranges (Miranda et al. 2017). Although previous (Vanoli 1967) and recent (Pavez-Fox and Estay 2016) records support the presence of the Pudu deer in this geographic area, its current distribution and abundance are still unknown, particularly in areas with densely-vegetated forests or associated with different land uses. Since the Pudu deer is characterised by evasive behaviour, cryptic colour, considerable nocturnal activity and also because it inhabits dense forest habitats (Zúñiga and Jiménez 2018), the process of recording field data for the species is a complex task. Reliable data are important for the conservation of the species, for example, to define or potentiate new protected areas, such as national reserves, amongst others. This objective should be addressed as matter of priority, given that, in Chile's current system of protected areas, the most suitable habitat for the Pudu deer is under-represented (about 6%) (Pavez-Fox and Estay 2016), in addition to the increasing degradation of its habitat as a result of native forest loss (Miranda et al. 2017). Another conservation approach that could be implemented in Chile is to maintain viable populations within small areas, as has been suggested by some authors (Shaffer 1987; Simonetti and Mella 1997), given that the Pudu deer presents a restricted home range.

The conservation of the Pudu deer in Chile requires combined efforts in several research areas, such as ecology, genetic structuring of populations and determination of the relative effect of different threats affecting the species in its natural environment. Amongst these issues, a top priority is to determine the current status of populations distributed in areas affected by significant loss or fragmentation of native forest or where it has been replaced by grasslands for agricultural purposes or by exotic forest plantations (Silva-Rodriguez et al. 2011). In the case of Osorno Province, this is a matter of particular concern, given that, between 2006 and 2013, the area planted with exotic tree species increased significantly (+20.6%), a large part of this growth being at the expense of native forest (CONAF-UACh 2014). Given that the habitat of the Pudu deer is closely linked to the native forest, it is important to establish the viability or distribution of populations in those areas of Osorno Province, where original characteristics have been altered due to change of land use as a result of human activity.

Mathematical modelling of species distributions based on maximum entropy (Phillips et al. 2006) is an interesting tool with several applications in species conservation, especially when information about current and potential habitats is absent (Phillips et al. 2004; Papeş and Gaubert 2007). This method uses the environmental characteristics of areas a species is known to inhabit to estimate the environmental suitability of regions that currently lack record (Anderson et al. 2002). Thus, a predictive model is constructed showing the potential distribution map of the species. Based on this map, it is possible to assess the suitability of sites for conservation purposes (Chefaoui et al. 2005), to predict of geographic ranges of a species (Raxworthy et al. 2003; Anderson

and Martínez-Meyer 2004) and identify priority areas for conservation efforts (Peterson et al. 2000), amongst others. Pavez-Fox and Estay (2016) have applied this analysis to model the Pudu deer distribution range in Chile aimed at evaluating the effectiveness of the Chilean National System of Protected Areas to protect the habitat of this deer. Although these authors used several Pudu deer points of occurrence across Chile to model the distribution of this species (in total 73), data density in some geographic areas was low, as was the case for Osorno Province (n < 5). With the exception of the Andes mountain range, this analysis indicated that most areas of the Province presented low suitability for the species, based on the result of a suitability map. Thus, the predicted distribution of the Pudu deer for the Province of Osorno merits further analysis by using a larger dataset in order to confirm the previous modelling.

The objective of this paper was to determine the distribution of the Pudu deer in seven Provinces from southern Chile, including the Osorno Province, by using modelling of species distribution, based on several recent occurrence data. We also modelled the future distribution of the species in the climate change scenario to determine how this phenomenon could affect its potential geographical distribution in the study area. This analysis may provide important clues as to how the species could respond to climate change, for example, in terms of variations in geographic range.

Methods

Study area

The area used for modelling the Pudu deer geographical distribution corresponded to the terrestrial environments between 39° and 44° South latitude of Chile. This geographic area comprises 74,295.5 km^2 and includes, from north to south, the Provinces of Cautín (8,207.6 km^2), Valdivia (9,146.8 km^2), Ranco (9,053.1 km^2), Osorno (9,246.6 km^2), Llanquihue (14,706.8 km^2), Chiloé (8,982.8 km^2) and Palena (14,952 km^2). This region contains a significant remnant of native temperate rainforest that covers a large proportion of each Province, as occurs in Osorno (42.9%), Llanquihue (54.5%), Chiloé (68.3%) and Palena (65.7%) (CONAF-UACh 2014), mostly distributed in the Coastal and Andes mountain ranges (Miranda et al. 2017). Climate in this region is warm-temperate and rainy with a Mediterranean influence and mean annual precipitation and temperature of 2,490 mm and 12.0 °C, respectively (Errazuriz et al. 2000). The Coastal Mountain range located in this area is characterised by an average height of 500 m a.s.l., which tends to gradually decrease towards the south (Ramírez and San Martín 2005; Villagrán and Armesto 2005). Meanwhile, the Andes Mountains present a higher altitude, averaging 1500 m a.s.l., with some elevations above 3000 m a.s.l (Garreaud 2009). Both mountain ranges present a predominance of vegetational formations comprising temperate laurifoliar rainforest, that include the Valdivian, North Patagonian and Subantarctic types (Villagrán and Hinojosa 2005). The temperate rainforest of Chile encompasses the Valdivian Rainforest Ecoregion, which has been listed amongst the most endangered ecoregions of the world and has a critical conservation status (Dinerstein et

al. 1995; Olson and Dinerstein 1998; Miranda et al. 2017). In addition, the Valdivian Rainforest Ecosystem is considered a biodiversity hotspot and, therefore, a region of high conservation priority (Ormazabal 1993; Myers et al. 2000; Smith-Ramírez 2004).

Occurrence data

Occurrence records of the Pudu deer were retrieved from several sources, including national park records (Puyehue National Park), incidents of individuals found (alive or injured) in rural areas of the province and reported in the local newspaper supported by photographs (El Diario Austral of Osorno), from records of native fauna rescue operations compiled by the Agricultural and Livestock Inspection Service (Servicio Agrícola y Ganadero (SAG)) Osorno, from direct observation of free-ranging individuals detected by using a camera trap and the naked eye and from indirect signs of the species revealed by footprints (Fig. 1). In total, we considered 88 occurrence points, spanning the period between 2000 and 2019, almost all from the Osorno, Llanquihue, Chiloé and Palena Provinces (Región de Los Lagos) (Fig. 2). This data set also included occurrence points previously reported by Delibes-Mateos et al. (2014) (n = 1) and Pavez-Fox and Estay

Figure 1. Records of occurrence of the Pudu deer in the geographic area studied. Free-ranging individuals registered by using a camera trap at Los Riscos (Coastal mountain range, Purranque district) (**A**), near Hueyusca village (Coastal mountain range, Purranque district) (**B**), injured juvenile individual found in a rural area at Choroy (Coastal mountain range, San Juan de la Costa district) and reported in the local newspaper (**C**) and footprints registered in Puyehue National Park (Andes mountains, Puyehue district) (**D**).

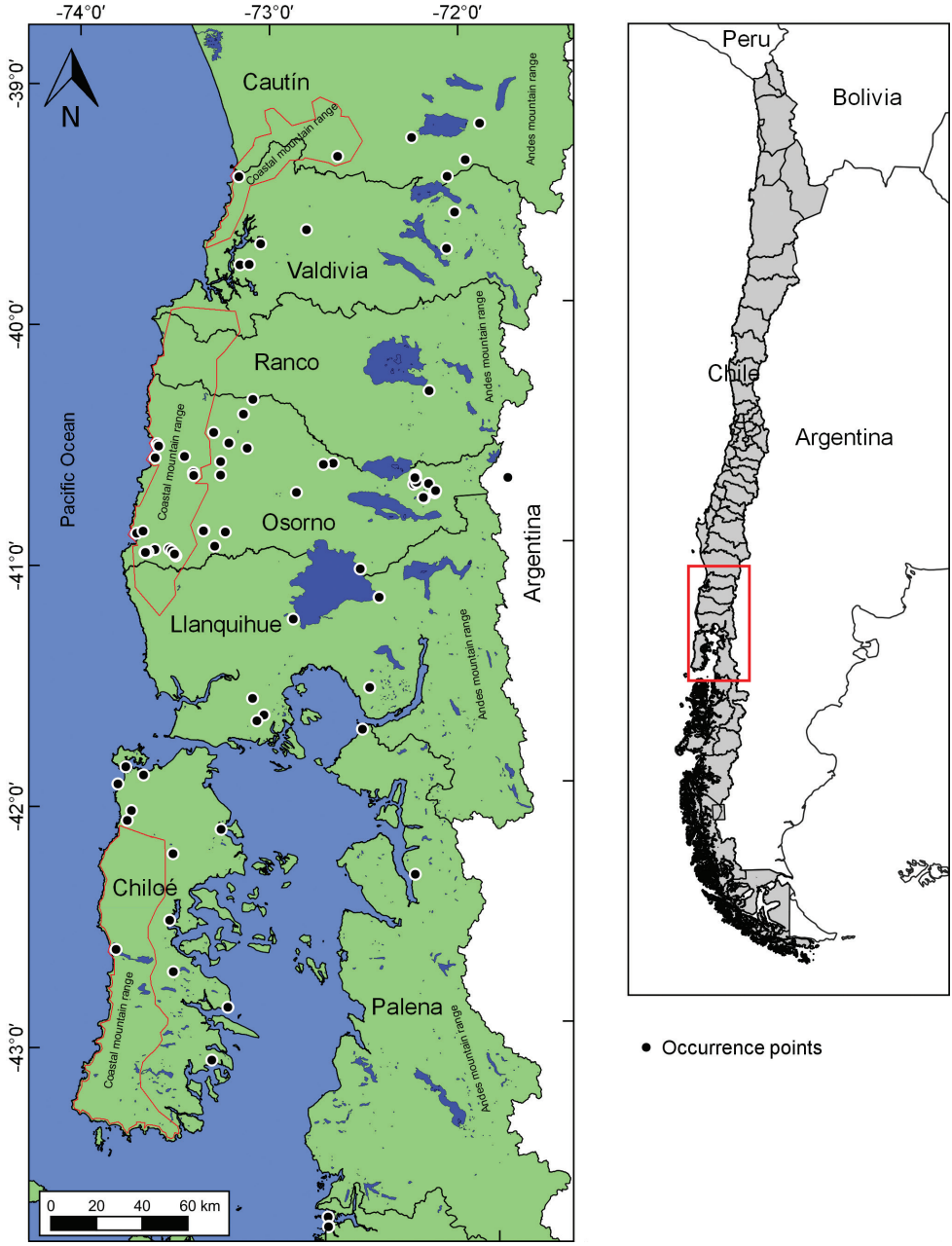

Figure 2. Georeferenced occurrence data of the Pudu deer used for model fitting in southern Chile. Black points indicate occurrence data. Names of each Province are indicated. The polygons with red lines indicate the location of the Coastal mountain range.

(2016) (n = 21), located either within this geographic area or in the nearby northern Provinces of Cautín and Valdivia. Further points located in the adjacent southern Province of Aysén, registered by Sanino et al. (2016) (n = 3), were also included.

Occurrences were georeferenced according to standard procedures whereby coordinates were assigned using Google Earth based on locality names. Details of the occurrence points recorded in this study (n = 63), including locality, coordinates, date, type of evidence and source, can be found in Suppl. material 1: Table S1; while, occurrence points from previous studies (n = 25) are provided in Suppl. material 2: Table S2. The occurrence points covered most of the Pudu's geographical distribution in this area, therefore, capturing almost the full niche of the species to calibrate the model. This aspect is important when modelling the potential future distribution of a species under climate change (Barbet-Massin et al. 2010). In addition, Moran's I index was also calculated to measure the overall spatial autocorrelation of the dataset, based on the estimation of observation independence within a dataset (Moran 1950).

Environmental data

To evaluate the potential geographical distribution of the Pudu deer in the study area and to identify suitable habitats currently occupied by the species, a set of bioclimatic variables from the WorldClim database (http://www.worldclim.org/) were used (Fick and Hijmans 2017). Initially, 19 bioclimatic variables of the Community Climate System Mode (CCSM) climate model (Gent et al. 2011) were considered. To reduce the multicollinearity effect, correlation coefficients were calculated between each pair of variables using the SDM toolbox extension implemented in ARCGIS 10.3 (Brown 2014). In those pairs with a high Pearson correlation value ($r \geq 0.7$), one of the variables was eliminated from the model. Thus, the following bioclimatic variables were selected for analysis: Isothermality (Bio3), Maximum Temperature of Warmest Month (Bio5), Minimum Temperature of Coldest Month (Bio6), Annual Temperature Range (Bio7), Precipitation of Wettest Month (Bio13), Precipitation Seasonality (Coefficient of Variation) (Bio15) and Precipitation of Warmest Quarter (Bio18). All the aforementioned environmental layers have a spatial resolution of 30 seconds of arc (approx.1 km). In addition, land cover and altitude variables obtained from the Diva-Gis database (http://www.diva-gis.org) were included (Hijmans et al. 2001), totalling nine variables for current distribution modelling. Land cover comprises different classes, such as tree cover evergreen and deciduous with broadleaved or mixed leaf type, shrub deciduous cover, herbaceous cover, sparse herbaceous or sparse shrub cover and cultivated and managed areas. Bio5, Bio6, Bio15 and Bio18 variables have previously been used in modelling distribution studies of the Pudu deer (Pavez-Fox and Estay 2016) and other deer species (Pease et al. 2009), since they provide important information that aids accurate determination of deer presence. The graphic results were compared with current land use reported in 2016 for southern Chile by the National Forestry Corporation (CONAF), available in Infraestructura de Datos Geoespaciales (IDE) database of the Ministerio de Bienes Nacionales, Gobierno de Chile (http://www.ide.cl/index.php/flora-y-fauna/item/1513-catastros-de-uso-de-suelo-y-vegetacion). On the other hand, to evaluate how future climate change may affect the potential geographic distribution of the species, the seven bioclimatic variables described above were used, but projected for 2.6 and 8.5 rcp (representative concentration pathways)

until the year 2070. These values indicate the increase in heat absorbed by the planet as a result of the concentration of greenhouse gases in each path, measured in Watts per square metre. In this case, 2.6 rcp represents the most optimistic scenario or least climate change (mean temperature rise of 1.0 °C) and 8.5 rcp is the most pessimistic and warmer scenario (mean temperature rise of 2.0 °C) (IPCC 2013; Taylor et al. 2011). Processing of the environmental layers was performed in QGIS 3.22 (QGIS Development Team 2018) and GRASS7 (GRASS Development Team 2016).

Modelling and statistical methods

To build geographical distribution models of the species under current and future environmental conditions, we used the MAXENT v.3.2.0 programme (Phillips 2017). The model was adjusted using 10,000 iterations, variable response curves, logistic output, generation of replicas with the bootstrap method and a regularisation multiplier value equal to 2. However, to maximise model fitting, we undertook tests under a range of regularisation coefficient values to choose the optimal value of this parameter, aimed at reducing overfitting (Merow et al. 2013). During this modelling process, the best model was evaluated by cross-validation using the Area Under Curve of test data, prior to splitting presence locations into training and test data. The logistic model output gives an estimate between 0 and 1 of probability of presence (Pearson et al. 2007). The relative importance of each variable to the model was estimated using the contribution percentage and the jackknife method. Each model (current, rcp 2.6 and rcp 8.5) was replicated 10 times, using a data ratio of 20% for training and 80% for evaluation, using a bootstrap framework (Hijmans 2012). Maxent models were evaluated using the Area Under Curve (AUC). The AUC measures the ability (probability) of the Maxent model to discriminate between presence sites and background sites (Phillips et al. 2006), thus, this parameter is useful to evaluate the geographical distribution of the species. Values of AUC range between 0 and 1.0, with values greater than 0.9 considered as an optimal threshold for species area predictions (Peterson et al. 2011). Pearson product-moment linear correlations were carried out to assess the relationship between the most important bioclimatic variables in the model and the probabilities of occurrence of the Pudu deer in the study area.

Post-processing

The fitted model, trained in the study area, was later projected to the terrestrial environments of provinces from southern Chile included between 39° and 44° south latitude, to estimate distribution of the species. The original map was converted to a binary map (0 = not suitable, 1 = suitable), applying a threshold, based on maximising the sum of sensitivity and specificity (SSS) (Liu et al. 2013). This method is recommended for threshold selection when only presence data are available, since it performs better than other threshold criteria (Liu et al. 2013).

Results

Current geographical distribution

Our dataset does not show significant ($P > 0.05$) spatial autocorrelation according to Moran's I index, either at the longitudinal ($I = 0.7790$, $P = 0.0845$) or latitudinal ($I = 0.0465$, $P = 0.8978$) geographic coordinates. Therefore, this result indicates that, in both cases, the occurrence points are randomly distributed. The best fitting model has a gain in AUC training of 0.910, an AUC of 0.908 and a standard deviation of 0.037. The evaluation value of AUC above 0.9 indicates that the model has a high ability to discriminate between sites with species presence versus sites where species is absent (background sites). Based on the seven WorldClim bioclimatic variables, in addition to the variables of land cover and altitude, the Maxent model predicts that the Pudu deer probabilities of occurrence in the study area varied between 0.0 and 0.9 (Table 1) and are shown in red scale in Fig. 3A. The Pudu deer distribution predicted by Maxent modelling covered an estimated area of 17,912 km^2 (24.1% of the area analysed), based on a probability of occurrence above 0.529, according to the threshold that maximises SSS (Table 1). These areas are highlighted in grey in the binary map (Fig. 3C). It can be observed that these areas are distributed mainly in the western sector of the Provinces of Valdivia, Ranco, Osorno and Llanquihue, on the eastern and western slopes of the Coastal mountain range, overlapping with sectors that currently contain extensive areas of native forest (Fig. 3B). There is also a high degree of overlap with extensive areas of either exotic tree plantations or mixed forest coverage (native and exotic). Furthermore, Ranco, Osorno and Llanquihue Provinces have areas with higher occurrence probability in the western slope of the Andes Mountain range. In the case of Chiloé and Palena Provinces, these higher occurrence areas are located in the northern part of the Province and in coastal areas, respectively. In addition, the SSS threshold value indicates that Osorno, Chiloé, Llanquihue and Ranco Provinces contain a large percentage of its total area, with higher occurrence probability of the Pudu deer, with 58.3%, 39.1%, 26.3% and 23.4%, respectively (Fig. 4). The environmental variables that most affect the current geographical distribution of the Pudu deer are Bio13 (relative contribution of 40.9%), Bio15 (34.5%) and Bio6 (11.2%) (Table 2). On the contrary, land cover and altitude variables combined contribute less than 4% of the model. A similar trend is observed for Bio13, Bio15 and Bio6 variables after jackknife analysis for model training gain reach a total maximum gain of 1.41, with variables Bio13 and Bio6 alone showing highest gains. When these variables are omitted, training gains are lowest, thus, revealing its importance in the model, i.e. the other variables provide scarce information (Table 2). The correlation analysis of variables that most affect the current geographical distribution of the Pudu deer and the probabilities of occurrence of the species in the study area indicates a strong significant positive association for Bio13 ($r = 0.654$, $df = 332991$, $P < 0.0001$), Bio15 ($r = 0.377$, $df = 332991$, $P < 0.0001$) and Bio6 ($r = 0.606$, $df = 332991$, $P < 0.0001$) variables.

Table 1. Occurrence probabilities and projected area for the current distribution of the Pudu deer in southern Chile.

Occurrence probabilities	Projected area (km^2)	Contribution (%)
0.0–0.1	6,401.0	8.6
0.1–0.2	5,438.3	7.3
0.2–0.3	8,652.6	11.6
0.3–0.4	13,628.9	18.3
0.4–0.5	17,545.7	23.6
0.5–0.6	13,676.0	18.4
0.6–0.7	7,056.0	9.5
0.7–0.8	17,14.4	2.3
0.8–0.9	182.6	0.2
0.9–1.0	0.0	0.0
Total	74,295.5	100
SSS threshold	17,912.0	24.1

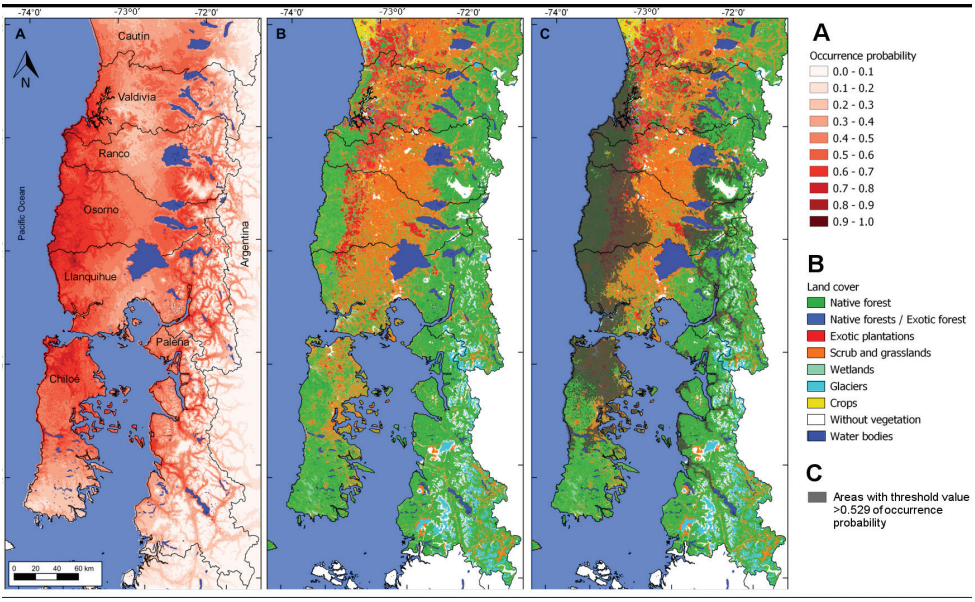

Figure 3. Projection of the fitted geographical distribution model of the Pudu deer in southern Chile. Projection under the current conditions (**A**), land use in the study area (**B**) and overlapping of suitable areas (grey areas) according to the SSS threshold value (> 0.529 of occurrence probability) on land use (**C**). In (**A**), red variations represent the predicted probability of suitable habitat conditions for the species.

Future geographical distribution

Since no projection data were available for land cover and altitude variable provided a limited contribution to the model, we excluded these variables when estimating the future geographical distribution of the Pudu deer. Thus, using the seven bioclimatic variables of WorldClim, Maxent predicts that the species currently develops over an area of 34,124.4 km^2 (SSS threshold value > 0.443), in environments whose habitat probabilities

Table 2. Estimates of relative contributions of the environmental variables to the Maxent model for the current geographical distribution of the Pudu deer in southern Chile.

Variable	Relative Contribution (%)	Permutation importance (%)	Jackknife of regularised training gain	
			With only variable	Without variable
Bio13	40.9	15.3	0.54	1.05
Bio15	34.5	11.0	0.37	1.00
Bio6	11.2	43.3	0.50	1.05
Bio18	4.0	14.2	0.51	1.08
Altitude	3.4	6.2	0.27	1.07
Bio3	2.8	7.4	0.18	1.03
Bio5	2.8	1.4	0.19	1.07
Land cover	0.5	1.3	0.02	1.08
Bio7	0	0	0.10	1.08

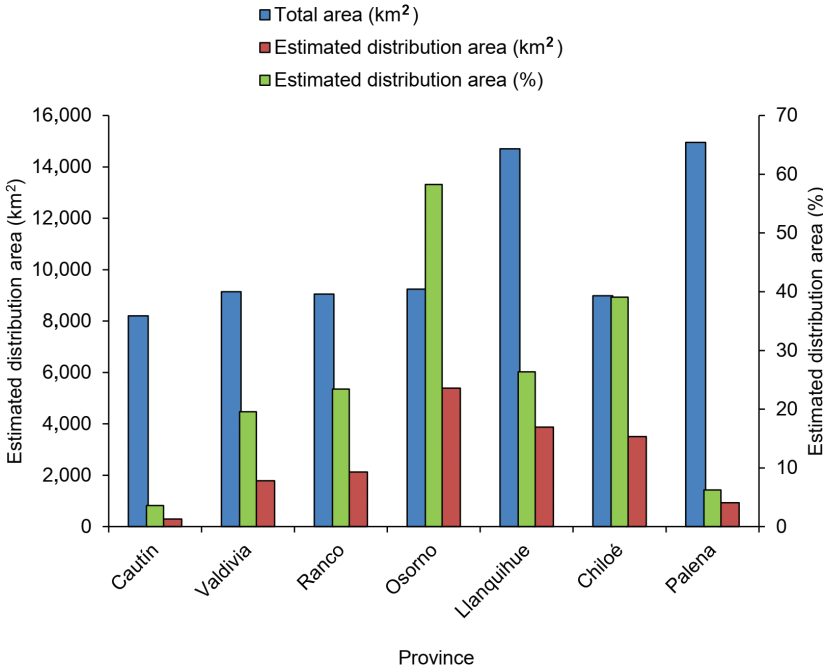

Figure 4. Bar chart representing the size of current Pudu deer distribution areas in different Provinces of southern Chile. The estimated distribution area was determined as the areas with high probability of occurrence (> 0.529), based on the threshold that maximises the sum of sensitivity and specificity. Total areas of each Province and size of estimated distribution areas (km^2) are shown. Percentage of estimated distribution area with respect to total area of the Province is also indicated.

of occurrence fluctuated between 0.0 and 0.9 (Table 3 and Fig. 5). For projections to 2070 with global warming scenarios of 2.6 and 8.5 rcp, the geographical distribution area comprises 35,717.8 km^2 (SSS threshold value > 0.435) and 20,056.3 km^2 (SSS threshold value > 0.540), respectively. Thus, there is a slight increase of 4.7% for 2.6 rcp, but a strong reduction of 41.2% for 8.5 rcp, with respect to the prediction of the model for current geographical distribution (Table 3). In addition, the predictions suggest that, in the

Table 3. Probability ranges of occurrence of the Pudu deer in southern Chile for current conditions and projections for 2070 in two global warming scenarios.

Occurrence probabilities	Current potential distribution (km²)	2.6 rcp scenario		8.5 rcp scenario	
		km²	Reduction (%)	km²	Reduction (%)
0.0–0.1	6,900.7	6,346.1	-8.0	6,596.0	-4.4
0.1–0.2	6,273.2	5,269.9	-16.0	5,921.6	-5.6
0.2–0.3	9,043.1	8,351.5	-7.6	8,874.2	-1.9
0.3–0.4	11,776.0	13,073.4	+11.0	13,227.3	+12.3
0.4–0.5	15,874.9	16,063.0	+1.2	12,622.2	-20.5
0.5–0.6	14,521.2	15,389.5	+6.0	16,397.3	+12.9
0.6–0.7	7,271.4	7,076.3	-2.7	7,905.3	+8.7
0.7–0.8	2,348.5	2,391.0	+1.8	2,384.0	+1.5
0.8–0.9	286.5	334.4	+16.7	367.5	+28.3
0.9–1.0	0.0	0.0	–	0.0	–
Total	74,295.4	74,295.0	0.0	74,295.4	0.0
SSS threshold	34,124,4	35,717.8	+4.7	20,056.3	-41.2

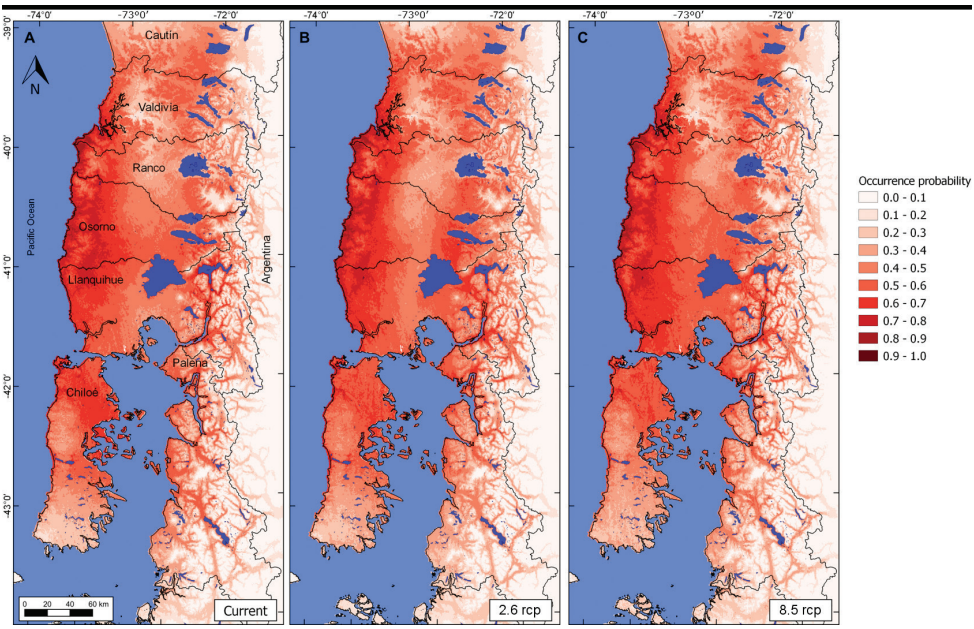

Figure 5. Future geographical distribution of the Pudu deer in southern Chile. Estimations for current conditions (**A**) and for projections to 2070 under 2.6 rcp (**B**) and 8.5 rcp (**C**). Red variations represent the predicted probability of suitable habitat conditions for the species.

future, areas with good habitability conditions will tend to increase. Thus, for example, under scenarios of 2.6 and 8.5 rcp and considering the highest probability of occurrence range from 0.8 to 0.9, there is an increase in area of 16.7% and 28.3% with respect to the 286.5 km² obtained in a similar probability of occurrence range with the current geographic distribution (Table 3). In addition, in both scenarios and taking into account the SSS threshold value, good habitability conditions currently observed in the west of Ranco, Osorno and Llanquihue Provinces and in the northern sector of the Province of Chiloé, will be maintained in the future (Fig. 6). These areas also coincide with sectors

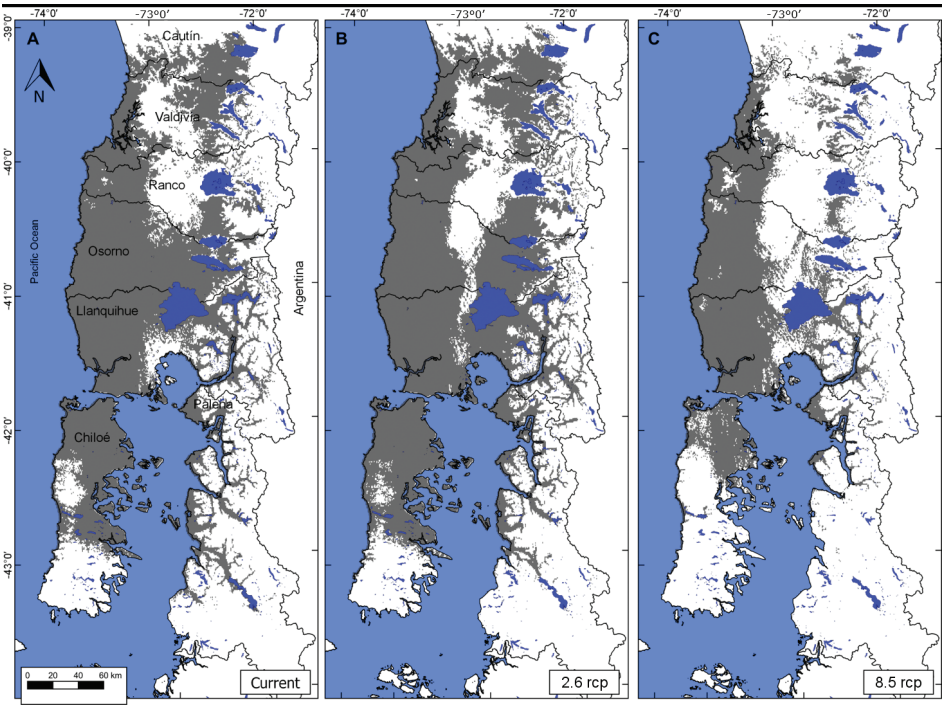

Figure 6. Binary maps showing future geographical distribution of the Pudu deer in southern Chile. Estimations for current conditions (**A**) and for projections to 2070 under 2.6 rcp (**B**) and 8.5 rcp (**C**). Grey areas represent the predicted probability of suitable habitat conditions for the species based on the SSS threshold value. SSS threshold values were as follows: > 0.443 for current scenario, > 0.435 for 2.6 rcp and > 0.540 for 8.5 rcp.

Table 4. Relative contribution of the environmental variables used to model the future geographical distribution of the Pudu deer in southern Chile.

Variable	Future 2070 with 2.6 rcp				Future 2070 with 8.5 rcp			
	Relative Contribution (%)	Permutation importance (%)	Jackknife of regularised training gain		Relative Contribution (%)	Permutation importance (%)	Jackknife of regularised training gain	
			With only variable	Without variable			With only variable	Without variable
Bio13	50.5	48.9	0.59	1.02	47.5	24.6	0.54	1.03
Bio15	28.1	4.7	0.31	1.03	26.3	5.1	0.26	1.02
Bio6	13.2	25.1	0.51	1.02	15.5	42.8	0.53	1.01
Bio3	3.3	7.1	0.11	1.02	6.3	17.3	0.12	0.98
Bio5	2.9	4.6	0.12	1.02	3.8	8.5	0.12	0.99
Bio7	1.2	0.8	0.10	1.07	0	0	0.12	1.04
Bio18	0.9	8.8	0.51	1.04	0.6	1.7	0.47	1.04

where native forest prevails. In contrast, towards the western sectors of the Andes mountain range (i.e. east of the Provinces), habitats for this species will present a low probability of occurrence. In order of importance, the variables that contribute most to the geographic distribution model for a scenario of 2.6 rcp are Bio13 (relative contribution of 50.5%), Bio15 (28.1%) and Bio6 (13.2%). The same variables contribute to the 8.5 rcp scenario as follows: Bio13 (47.5%), Bio15 (26.3%) and Bio6 (15.5%) (Table 4). The

results of the jackknife analysis on the training gain of the model for these variables reach a total maximum value of 1.41 and 1.33 for the scenarios of 2.6 rcp and 8.5 rcp, respectively. In both scenarios, the environmental variables with highest gains are Bio13 and Bio6, which therefore, appear to contribute the most useful information by themselves. When they are omitted, a great decrease in the total gain of the models occurs (Table 4).

Discussion

Our prediction of the current geographical distribution of the Pudu deer was consistent with the habitat hypothesis proposed for the species in southern Chile by Pavez-Fox and Estay (2016). However, our results differed in terms of habitability probabilities, given that these were higher in certain geographical areas compared to those reported by previous authors. For example, we observed that, between 40°00' and 41°30' South (approx.) (i.e. at the latitudinal section of Ranco, Osorno and Llanquihue Provinces), the eastern and western slopes of the Coastal mountain range show high habitability conditions for the species (occurrence between 0.5 and 0.9). This high probability may be related to the fact that extensive native forest coverage still exists in this mountain range. In the same latitudinal section, but circumscribed to the Andes mountain range, we obtained areas mainly with low to medium-level probability of occurrence (from 0.2 to 0.5), both in the precordilleran and higher altitude sectors. On the contrary, Pavez-Fox and Estay (2016) found low or medium habitability categories for the Pudu deer in similar areas of the Coastal mountain range, while the central valleys, along with the western and eastern slopes of the Andes mountain range, presented better environmental conditions for the species. We estimate that these discrepancies may be related to the inherent variables used in both studies, such as sampling effort, number of records of the species, amplitude of the geographical area analysed and selection of environmental variables. Regarding sampling effort, since data came from different sources, it is possible that observers' bias may have occurred, affecting the occurrence points in the dataset. However, this effect is likely to have been minimal since records were obtained from public (e.g. Agricultural and Livestock Inspection Service) and private (e.g. Puyehue National Park) agencies with a wide experience in the conservation of native fauna. These institutions maintain reliable records of this type of fauna, both regarding species identification and the date and place where sighted. Moreover, to avoid species misidentification from other sources (e.g. El Diario Austral of Osorno), occurrence records were only considered positive when photographs or videos of the species were available. Other records included in the dataset are very accurate, since they were compiled either directly by us, using camera-trap and footprints or from literature (Delibes-Mateos et al. 2014; Pavez-Fox and Estay 2016; Sanino et al. 2016). Future actions aimed at compiling Pudu deer occurrence points in an online public database, curated by experts, should benefit geographical distribution studies of this cervid in Chile.

The potential distribution model shows that the areas with the best habitability conditions were located in the western Provinces of Ranco, Osorno and Llanquihue, overlapping with areas where the vegetation formations of the Valdivian Laurifolio Forest

and Evergreen Forest of the Coastal Range predominate (Luebert and Pliscoff 2006). These native forest formations have been of great interest in terms of conservation efforts due to their status as a biodiversity hotspot and high level of endemism (Myers et al. 2000; Nahuelhual et al. 2007). However, despite their importance for the conservation of biodiversity in southern Chile, these forest formations have been exposed to a rapid rate of destruction and degradation due to anthropogenic causes (Myers et al. 2000; Echeverria et al. 2006, 2007). In fact, according to a study of historical reconstruction of vegetational cover and land use carried out by Lara et al. (2012), the loss of native forest, considering all vegetation formations registered in the regions of Los Ríos and Los Lagos, would be a consequence of its gradual replacement by grasslands and bushes (25% and 27%, respectively). This phenomenon would be more accentuated in the central valleys, located between the Andean and Coastal mountain ranges (Miranda et al. 2017). However, given that the eastern and the western slopes of the Coastal mountain range exhibit a lower degree of anthropic intervention, even though it is adjacent to valleys where there is greater agricultural and forestry activity, this geographical area, as identified in this paper, represents an ideal area for conservation of the Pudu deer. In fact, most of the records used in the modelling carried out in this study were taken from this area, which reflects its importance as an appropriate habitat for the survival of the species.

The AUC value above 0.9 suggests that our model describes the current potential of the Pudu deer distribution with a high degree of precision. Amongst the variables that mainly influenced probability of occurrence of the Pudu deer were precipitation of wettest month (Bio13), seasonality of precipitation (Bio15) and minimum temperature of coldest month (Bio6), which together contributed to 86.6% of the model. In contrast, Pavez-Fox and Estay (2016) reported that the most important variables in their prediction were seasonality in temperature (Bio4) and range of daytime temperatures (Bio7). This difference between the bioclimatic variables identified by both studies may be related to the size of the geographic area used in modelling. In our case, the area was smaller than that used by Pavez-Fox and Estay (2016), given that they analysed a geographic area spanning from 36° to 43° South latitude of Chile, including an adjacent area from Argentina and, therefore, lower environmental variability is to be expected. In addition, these authors used environmental variables obtained from modelling studies of other cervid species, whereas in our analysis, the environmental variables selected were those that presented low levels of collinearity in the study area. The considerable importance of the Bio13, Bio15 and Bio6 bioclimatic variables in our model could be related to some biological characteristics of the Pudu deer, such as habitat use and temperature tolerance. For example, wettest month precipitation could be related to vegetation availability throughout the year, since precipitation modulates the soil moisture and, therefore, the understorey growth in the temperate rainforest. Since this resource is used by the Pudu deer for feeding, cover and for escaping from threats (Jiménez 2010), the presence of temperate rainforest with a well-developed understorey throughout the year would enhance the Pudu deer abundance. In fact, Simonetti and Mella (1997) observed that stands with well-developed undergrowth in exotic plantations from central Chile, are important for Pudu deer abundance and that other medium-sized mammals. In the case of coldest month minimum temperature,

this variable indicates that low temperature is relevant to the probability of occurrence of the Pudu deer. In fact, a medium-high positive correlation was found amongst both variables. This result suggests that this cervid is better adapted to low, rather than high, temperatures. Pavez-Fox and Estay (2016) obtained a similar result, where mean diurnal temperate range was negatively related to habitat suitability, i.e. this species would be intolerant to sudden changes in temperature throughout the day. Moreover, this result also concurs with data on the Pudu deer activity pattern, since minimal activity occurred in the daytime, when temperatures are higher than other periods of the day, such as dawn, dusk and night (Eldridge et al. 1987, Zúñiga and Jiménez 2018).

The evaluated climate change scenarios suggest that, in the future, Pudu deer would be prone to maintain their presence in large areas where habitability conditions are currently appropriate. However, as has been reported in other studies (e.g. Ortíz-Yusty et al. 2014; Holloway et al. 2016; Bruneel et al. 2018), this trend should be considered with caution, because Maxent modelling only relates records of the species with environmental variables, but not with other variables that may also have an impact on the distribution of species, such as geographical barriers, ecological interactions or particular requirements (Guisan and Zimmermann 2000; Soberon and Peterson 2005). Taking these restrictions into account, distribution models, projected for global warming increases of 2.6 and 8.5 rcp, indicate that most habitability areas will be conserved to the west of Ranco, Osorno and Llanquihue Provinces and in the northern sector of the Chiloé Province, with probabilities of occurrence greater than 0.5. This scenario will be more evident in the Coastal mountain range. In contrast, by 2070, several areas in the western slopes of the Andes Mountain range, that currently represent suitable habitats for the species, are expected to decrease. This process would lead to loss native forest quality as a result of environmental homogenisation. This homogenisation could be the result of the increase in variables, such as minimum temperature of the coldest month (Bio 6) and the decrease in precipitation of the wettest month (Bio13). It should be noted that the negative effects of temperature increase in models of potential distribution have been reported for other cervids, such as the Himalayan Musk Deer (*Moschus leucogaster*) and the Alpine Musk Deer (*Moschus chrysogaster*) (Khadka and James 2017; Lamsal et al. 2018). This effect is considered to be due the fact that temperature increase can negatively influence the quality and productivity of vegetation that maintains equilibrium in terrestrial ecosystems (Klein et al. 2007). In addition to the combined effect of these environmental variables on the future distribution of the Pudu deer, we must consider the process of native forest loss occurring in the southern-central Chile due to anthropogenic activities, given that this variable plays a key role in the conservation of the species (Silva-Rodríguez et al. 2011). It has been suggested that the net loss of native forest was lower in recent years compared to the 1970−1990 period (Smith-Ramírez 2004; Miranda et al. 2017). However, this process is likely to continue in the future due to the persistence of factors that are difficult to control, such as forest fires (González et al. 2011), continuous and unregulated felling of forests (Donoso et al. 2014), increase in the use of native trees as firewood for domestic and industrial heating (Gómez-Lobo 2005; Marín et al. 2011) and land use change (Lara et al. 2012). In this sense, conser-

vation of the Pudu deer depends largely on the adoption of stricter regulations than those currently in existence in order to avoid future native forest degradation (Miranda et al. 2017), especially in those areas where habitability conditions for the species are optimal. This issue is especially important in areas where future distribution of the Pudu deer is projected, as is the case in western sectors of the Ranco, Osorno and Llanquihue Provinces, that include the Coastal range and to the north of the Province of Chiloé. Unfortunately, forest fragmentation in this geographic area is expected to continue in the future, based on the extrapolation to 2020 of the current deforestation rate recorded from 1976 to 1999 (Echeverria et al. 2008). Thus, this forest fragmentation process may constitute a major concern and could have potentially detrimental consequences for Pudu deer conservation under global climate change.

Conclusions

In contrast to the Andes mountain range, Maxent modelling predicted high probabilities of occurrence for the Pudu deer on the eastern and western slopes of the Coastal mountain range, located to the west of the Ranco, Osorno and Llanquihue Provinces, where extensive coverage of native forest persists, in addition to the northern sector part of the Province of Chiloé. In projections to 2070, with global warming scenarios of 2.6 and 8.5 rcp, this geographic area could conserve its habitability conditions that are currently appropriate for the species. Our prediction of potential Pudu deer geographical distribution is similar to the habitat identified for this species in southern Chile in a previous study. Since the Pudu deer is classified as Vulnerable in Chile, with a declining population size due to several factors, the distribution study performed here provides important data to identify specific geographic areas to develop conservation plans for this species. This is an important goal for the long-term conservation of the species.

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Supplementary material 1

Table S1

Authors: Nelson Colihueque, Aldo Arriagada, Andrea Fuentes

Data type: species data

Explanation note: Details of the occurrence points of the Pudu deer (*Pudu puda*) from southern Chile, including locality, coordinate, date, type of evidence and source.

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Supplementary material 2

Table S2

Authors: Nelson Colihueque, Aldo Arriagada, Andrea Fuentes

Data type: species data

Explanation note: Details of the occurrence points of the Pudu deer (*Pudu puda*) from southern Chile obtained from previous studies, including locality, coordinate, date and source.

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DATA PAPER

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Dataset of occurrences and ecological traits of amphibians from Upper Paraguay River Basin, central South America

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Abstract

There are many gaps in our biodiversity knowledge, especially in highly diverse regions such as the Neotropics. Basic information on species occurrence and traits are scattered throughout different literature sources, which makes it difficult to access data and ultimately delays advances in ecology, evolution, and conservation biology. We provide species occurrence and trait data for amphibian species in the Upper Paraguay River Basin, central South America. The compiled information is made available through two different datasets that hold (i) 17K species occurrence records and (ii) 30 species-level traits for 113 amphibian species. The first dataset includes the species occurrence records and informs specimen id, collection of housing, locality, geographical coordinates, geographic accuracy, collection date, and collector name. The second dataset covers species-level attributes on morphometry, diet, activity, habitat, and breeding strategy. These datasets improve accessibility to spatial and trait data for amphibian species in the Pantanal ecoregion, one of the largest wetlands on Earth.

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Keywords
Anura, Datapaper, Gymnophiona, life history traits, Pantanal floodplain, wetland

Introduction

The availability of species occurrences data is not uniform throughout the Earth and many gaps exist, especially in megadiverse regions such as the tropics (Collen et al. 2008, Meyer 2016). Georeferenced information is imperative for many basic and applied ecology fields (Whittaker et al. 2005), such as biogeography (Lomolino 2004, Silva et al. 2018), evolutionary biology (Holt 2003), and conservation planning (Whittaker et al. 2005, Chen et al. 2017). Although efforts to reduce knowledge gaps in species distribution have increased over the years, knowledge on species distribution is still incomplete (Lomolino et al. 2016). The accumulated occurrence data are not spatially uniform, with on-ground accessibility, economic development, and nature appeal largely affecting the inventory completeness of particular regions (Meyer et al. 2016, Moura et al. 2018). Inter- and intra-country variation in public policy may also add up to reduce the efficacy of initiatives to reduce sampling gaps (Beck et al. 2014, Troudet et al. 2017).

One way to reduce biodiversity knowledge gaps is through improving accessibility and data sharing networks (Chavan and Penev 2011). The Global Biodiversity Information Facility (GBIF), the largest online depository of occurrence records in the world, allows access to data from many natural history collections worldwide (GBIF.org 2019). However, the raw data fed to GBIF may include misidentifications or invalid species names due to outdated taxonomy provided by scientific collections (Beck et al. 2014). For example, it has been found that herbaria collections can hold up to 40% of Amazonian plant specimens with erroneous identifications (Hopkins 2007). To minimize this problem, researchers have relied on curated data papers (Chavan and Penev 2011). Data papers can also include information on species ecology to extend potential applications (Grimm et al. 2014, Oliveira et al. 2017, Gillings et al. 2019). Ecological traits determine species' ability to persist in a variety of environments and reflect the outcome of ecoevolutionary pressures on species interactions with abiotic and biotic factors (Ingram and Shurin 2009, Swenson and Weiser 2010). Spatial and trait data have been used to improve spatial models (Dubuis et al. 2013, D'Amen et al. 2015, Guisan et al. 2019), forecast community structure and dynamics (Cadotte et al. 2015, Blonder et al. 2018), predict population trends (Lips et al. 2003, Williams et al. 2010, Coulthard et al. 2019), and to understand potential impacts of climate change (Diamond et al. 2011, Foden et al. 2013). In spite of their importance, trait data are also scattered throughout literature, making its use difficult in comparative studies (Grimm et al. 2014).

Among those regions without proper biodiversity knowledge is the Upper Paraguay River Basin (UPRB), in the center of South America and home of the largest

wetland of the world (Alho et al. 1988, Junk and Wantzen 2004). The UPRB covers the Pantanal ecoregion, which is classified as an UNESCO World Heritage Site since 2000 (UNESCO 2020). It encompasses transition zones among Pantanal and other South American ecoregions, such as Cerrado, Amazonia, Chiquitano Dry Forest, and Dry and Humid Chaco (Olson et al. 2001), across the borders of Brazil, Bolivia, and Paraguay. The confluence of diverse fauna and flora from these different ecoregions is a peculiar characteristic of the UPRB (Silva et al. 2000, Piatti et al. 2019). Because of the spatially varying flooding regimes, many areas in the UPRB show low on-ground accessibility and therefore are still poorly sampled (Uetanabaro et al. 2008, Souza et al. 2017). Amphibian assemblages of some areas within the UPRB are completely unexplored, such as the Pantanal do Paiaguás and Pantanal do Nabileque, Central and South-west of the Pantanal, respectively (Souza et al. 2017), despite punctual efforts to catalogue amphibians in the Pantanal and surroundings. Given the present knowledge, the UPRB is characterized by higher amphibian richness in the surrounding plateaus, and fewer species in the floodplain, but with high abundance (Uetanabaro et al. 2008). Herein we make available more than 17,000 records for 113 amphibian taxa that occur in the basin. Whenever available and for each geographical record, we provide information on the collection of housing, locality, geographical coordinates, geographic accuracy, collection date, and collectors. For each species, we present trait information on the morphometry, diet, activity, habitat, and breeding strategy.

Methods

Data compilation

We compiled occurrence records for amphibians in the Upper Paraguay River Basin (UPRB) through specimens available in scientific collections and fieldwork. We visited five collections in Brazil: (i) Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande, Mato Grosso do Sul state; (ii) Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília, Federal District; (iii) Coleção Zoológica da Universidade Federal de Mato Grosso (ZUFMT-AMP), Cuiabá, Mato Grosso state; (iv) Coleção Herpetológica Célio F. B. Haddad, of the Universidade Estadual Paulista (CFBH), Campus of Rio Claro, São Paulo state; and (v) Coleção de Anfíbios do Museu Nacional of the Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, Rio de Janeiro state. We also verified amphibian specimens deposited at the Colección Herpetológica del Museo de Historia Natural Noel Kempff Mercado (MNKA), Santa Cruz de La Sierra, Santa Cruz Department, Bolivia; and in the Colección Herpetológica del Instituto de Investigación Biológica del Paraguay (IIBT-H), Asunción, Capital District, Paraguay. Fieldwork records were based on ongoing research developed by us and members of the Mapinguari Lab (voucher specimens from such research were housed in the ZUFMS collection). Specimens from fieldwork received the additional label MAP to allow their differentiation from speci-

mens previously available at the ZUFMS collection. In addition, we gathered information on morphometry, diet, activity, habitat, and breeding strategy for each amphibian species found in the UPRB based on the literature available.

Data description

Information on spatial and trait data for amphibian species of the Upper Paraguay River Basin (UPRB) is presented as two supplementary tables. Suppl. material 1: Table S1 includes the species occurrence data, whereas the Suppl. material 2: Table S2 contains the species-level trait data. We acknowledge that the spatial occurrence data is subject to taxonomic uncertainty, that is, the difficult of confirming the id of some species based on preserved specimens only. Therefore, for Suppl. material 1: Table S1, we provided 14,900 occurrence records for 89 amphibian species identified at the species-level, and additional 2,189 occurrence records for 24 taxonomic 'entities' of amphibians with taxonomic uncertainty (species identifications including "aff.", "gr.", "cf.", and "sp."). Taxonomy issues for some species are discussed in the session *Taxonomy deliberation*.

Suppl. material 2: Table S2 is defined at the species-level and therefore not subjected to the taxonomic uncertainty of preserved specimens. The trait data is presented for all 113 amphibian species that occur within the UPRB. References consulted to build the Table S2 are listed in the Suppl. material 2. In the following, we provide details on the fields represented in the two supplementary tables.

Suppl. material 1: Table S1 – Species occurrence records

Suppl. material 1: Table S1 contains the following fields:

Collection: The acronym of the scientific collection where the specimens are housed. ZUFMS-AMP = Zoological Collection of Reference of the Universidade Federal de Mato Grosso do Sul. CHUNB = Herpetological Collection of the Universidade de Brasília. ZUFMT-AMP = Zoological Collection of the Universidade Federal de Mato Grosso. CFBH = Herpetological Collection Célio F. B. Haddad, Universidade Estadual Paulista. MNRJ = Amphibian Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro. MNKA = Herpetological Collection of the Museo de Historia Natural Noel Kempff Mercado. IIBP-H = Herpetological Collection of the Instituto de Investigación Biológica del Paraguay. MAP = Fieldwork collection of the Mapinguari Lab (which will be housed in ZUFMS after).

Label number: number in the collection in which the specimen is housed. This information was extracted directly from the scientific collection records.

Family/Genus/Id._Level/Epithet/Species: Taxonomic data of the specimen (Family, Genus and Species). The column named "Id._Level" is related to the taxonomic level known ("cf.", "aff.", "gr." or "sp.", see "*Taxonomy deliberation*" session). If the "Id._Level" column is empty, the specimen was identified at the species level.

Locality: Name of the locality where the specimen was collected. It might refer to local designations, villages, or other locations below the municipality level. Locality information was extracted from the specimen catalogue available at each scientific collection.

Municipality/Adm_unit/Country: Information on the municipality, administrative unit (state level for Brazil, and department level for Bolivia and Paraguay), and country where the specimen was collected.

Latitude/Longitude: Geographic coordinates of the specimen, in decimal degrees. These data were made available mostly by the collectors. However, for specimens with missing data, we obtained the geographic coordinates of their respective locality via Google Earth Pro.

Geographic_Accuracy: Geographic accuracy of the record indicated by one of the three following levels. (i) "Exact_Location", for records of the exact place where the specimen was captured. (ii) "Nearby_Location", the collector did not provide the geographic coordinates but we obtained it through the locality description. And (iii) "Municipality_Centroid", records with unknown exact or nearby location were georeferenced based on the municipality centroid.

Collection_Day/Collection_Month/Collection_Year: Day, month, and year of the record. Data extracted from the catalogue of specimens available at each scientific collection.

Collector: The name of the collector who made the record, as informed in the catalogue of specimen. Only 37.5% of the records have this field filled.

Suppl. material 2: Table S2 – Species traits

Suppl. material 2: Table S2 contains 30 fields distributed in seven general topics: Identification (columns with gray background color), Conservation (brown background color), Morphometry (green background color), Diet (blue background color), Habitat (red background color), Activity period (yellow background color), Breeding strategy (orange background color), and References:

Identification-related fields

Family/Species/Year_of_description: Taxonomic level for family and species, followed by the year of description for each species.

Number_of_records: Number of georeferenced specimens in Suppl. material 1: Table S1 for each species.

Conservation-related fields

IUCN: Conservation status as provided by IUCN Red List Category and Criteria (2020) categorized as: Least Concern (LC), Data Deficient (DD), Near Threatened (NT), and Not Evaluated (NE).

IUCN_Pop: Current population trends as available at IUCN Red List Category and Criteria (2020): stable, unknown, decreasing, increasing, and Not Evaluated (NE).

Morphometry-related fields

Body_size: Mean Snout-Vent Length (SVL, in millimeters) for males, females, and for the species. In some cases, we found this value only for the species (not for male and female separately). If SVL was available only for males and females, we averaged both values to get the species mean.

Head_length: Mean Head Length (in millimeters) for males, females, and for the species. In some cases, we found this value only for the species (not for male and female separately). If head length was available only for males and females, we averaged both values to get the species mean.

Head_width: Mean Head Width (in millimeters) for males, females, and for the species. In some cases, we found this value only for the species (not for male and female separately). If head width was available only for males and females, we averaged both values to get the species mean.

Reference_Morphometry: References consulted for the morphometry of each species. All references are listed in the Suppl. material 3. Personal Communication were provided directly by us or by other colleagues consulted.

Diet-related fields

The different levels of prey type were organized in multiple columns, each column indicating the taxonomic group (up to Order, mostly) of the respective prey. See Table 1 for the complete list of preys categories. The Order Hymenoptera was registered into three different fields: "Hymenoptera_Formicidae" informs the percentage of ants among amphibian preys; "Hymenoptera_non_Formicidae" informs the percentage of others group of Hymenoptera, exemption of Formicidae; and "Hymenoptera" for the sum of "Hymenoptera_Formicidae" and "Hymenoptera_non_Formicidae", or when the author of the source provided information on Hymenoptera only. If prey identification was unavailable at the Order level, we used a higher taxonomic rank (e.g., Annelida, Insect_Pupa, Insect_Larvae) to differentiate preys. When two or more prey types belonged to the same higher taxon, but at different ranks, we did not sum those values. For example, Ixodida is an Order of Acari, but in our dataset, we did not add the values of "Ixodida" with those of "Acari" when the author provided these values separately. In each column, the data can be available in two different forms according to the information originally reported: (i) Presence/Absence data (dark blue background color) are classified as 0 (absence) and 1 (presence) if information on the Index of Relative Importance (IRI) was unavailable; and (ii) Percentage of IRI data (%IRI), the relative contribution of each prey category to the total IRI of each species. In these cases, items that could not be identified (e.g., fragmented bodies and advanced stages of digestion) were referred in the column "Not_Identified_raw_IRI" and they are not considered for the computation of the %IRI.

Table 1. Prey categories in the dataset. It includes Phylum, Class and the Prey Category (when available by the reference) and up the taxonomic level of Prey Category identified.

Phylum/Class/Prey_Category	Taxonomic level of Prey Category
Anellida	
Anellida	Phylum
Arthropoda	
Arachnida	
Acari	Subclass
Ixodida (Acari)	Order
Araneae	Order
Opiliones	Order
Pseudoscorpiones	Order
Scorpiones	Order
Chilopoda	
Chilopoda	Class
Scolopendromorpha	Order
Crustacea	
Crustacea	Subphylum
Diplopoda	
Diplopoda	Class
Spirostreptida	Order
Entognatha	
Collembola	Superorder
Insecta	
Blattodea	Order
Coleoptera	Order
Dermaptera	Order
Diptera	Order
Ephemeroptera	Order
Hemiptera	Order
Hymenoptera_Formicidae	Order
Hymenoptera	Order
Hymenoptera_non_Formicidae	Order
Insect_Larvae	Class
Insect_Pupa	Class
Isopoda	Order
Isoptera	Order
Lepidoptera	Order
Mecoptera	Order
Odonata	Order
Orthoptera	Order
Plecoptera	Order
Psocoptera	Order
Thysanoptera	Order
Trichoptera	Order
Malacostraca	
Decapoda	Order
Chordata	
Actinopterygii	
Synbranchiformes	Order
Amphibia	
Anura	Order
Gymnophiona	Order

Phylum/Class/Prey_Category	Taxonomic level of Prey Category
Aves	
Aves	Class
Mammalia	
Mammals	
Reptilia	
Lizards	
Mollusca	
Mollusca	Phylum
Others	
Vegetal_material	Kingdom
Not_identifield_raw_IRI	

Reference_Diet: References consulted for the diet of each species. All references are listed in Suppl. material 3. Personal Communication were provided directly by us or by other colleagues consulted.

Habitat-related fields

Major Habitat: Vegetational formation where the species is commonly present. We considered three major habitat types: (i) "Open" for species occurring in the Cerrado *sensu stricto*, grasslands, shrublands, and wetlands; (ii) "Forest" for species occurring in moist broadleaf forest, dry broadleaf forest, and riparian forest; and (iii) "Forest_Open" if the species is present in physiognomies of both major habitat type.

Habitat use: The microhabitat used by the post-metamorphic individuals. We classified microhabitats in four levels: (i) "Aquatic" when the species lives in the water body; (ii) "Arboreal" to species that use shrubs or trees for calling and live; (iii) "Fossorial" for species that lives underground or buried for some period; and (iv) "Terrestrial" for species that lives in the ground. As a note, a same species can use one or more level of habitat use.

Reference_Habitat: References consulted for the habitat of each species. All references are listed in Suppl. material 3. Personal Communication were provided directly by us or by other colleagues consulted.

Activity-related fields

Seasonality: Seasonal activity of the species was classified as (i) "Dry" when it breeds in the winter (also the dry season in the UPRB); and (ii) "Wet" if it breeds in the summer (wet season in the UPRB). Species that can breed in both seasons were classified as "Dry_Wet".

Habit: Period of activity of the species when it feeds and reproduces. We classified species as (i) "Diurnal" or (ii) "Nocturnal". Species active at both day and night were classified as "Diurnal_Nocturnal".

Reference_Activity: References consulted for the activity of each species. All references are listed in Suppl. material 3. Personal Communication were provided directly by us or by other colleagues consulted.

Breeding-related fields

Reproductive_mode: The reproductive mode characteristic of each species. The mode number for anurans follows the description provided by Haddad and Prado (2005) and Wells (2007) for caecilians.

Development: The mode of development after the egg hatching, classified as (i) "Indirect" development when the species has larval stages or (ii) "Direct" development of terrestrial eggs without larval stages.

Water_system: The water system in which species deposit their eggs, classified as (i) "Lotic" for species that breeds in flowing waters, such as rivers, streams, and rivulets; and (ii) "Lentic" water system for species breeding in still water like ponds and swamps. Species that reproduce in both water systems were classified as "Lentic_Lotic".

Eggs_Deposition: The substrate in which the species lay its eggs, classified as (i) "Water" for eggs laid directly in the flowing or still water; (ii) "Ground", when the eggs are laid directly on the ground, rocks or leaf on the ground; (iii) "Burrows", for eggs laid within a natural cavity or in a cavity built by the male or female of the species; (iv) "Basin", when eggs are laid in the water accumulated in a build basin nearby ponds, and (v) "Arboreal", eggs laid on leaves above the water system.

Nest: Some species use a foam nest to lay their eggs. We classified such species as "Foam". For the remaining species, we did not fill this field.

Reference_Breeding: References consulted for the breeding strategy of each species. All references are listed in Suppl. material 3. Personal Communication was provided directly by us or by other colleagues consulted.

Taxonomy deliberation

There are many taxonomy issues with amphibian species. Although taxonomists have improved their ability to unveil cryptic species, the cryptic diversity remains unknown in many tropical regions (Fouquet et al. 2007, Funk et al. 2012, Arteaga et al. 2016). Considering that some species in our dataset (Suppl. material 1: Table S1) are still unknown to science, we did not identify all occurrences at the species-level. For some occurrence records, we used *confer* ("cf.") to refer to species groups of either difficult identification based on preserved specimens or groups with high cryptic diversity. In the latter scenario, species would be distinguishable preferably through molecular analysis or bioacoustics parameters. For example, *Elachistocleis matogrosso* and *E. bicolor*, both occurring within UPRB, are diagnosed by the pattern of dorsal stripes (Caramaschi 2010). After specimen fixation and housing in museums, the efficacy of such diagnostic character is extremely reduced, which practically prevent the confirmation of species id based on preserved specimens

only. That is also in the case with *Adenomera* species (Angulo et al. 2003). Species belonging to *Adenomera* and *Elachistocleis* were named as "cf." in Suppl. material 1: Table S1.

Because the collector has more tools to identify specimens alive during fieldwork (e.g., bioacoustics, color in life), we also included the original identification for all specimens classified as "cf." in the column "Id._Level" in Suppl. material 1: Table S1, with only two exemptions. First, we joined all specimens identified as *Leptodactylus gracilis*, *L. jolyi*, and *L. sertanejo* as *Leptodactylus* cf. *jolyi*, due to the high morphological similarity observed among preserved specimens of these species, besides their overlapping distributions (Neves et al. 2017).

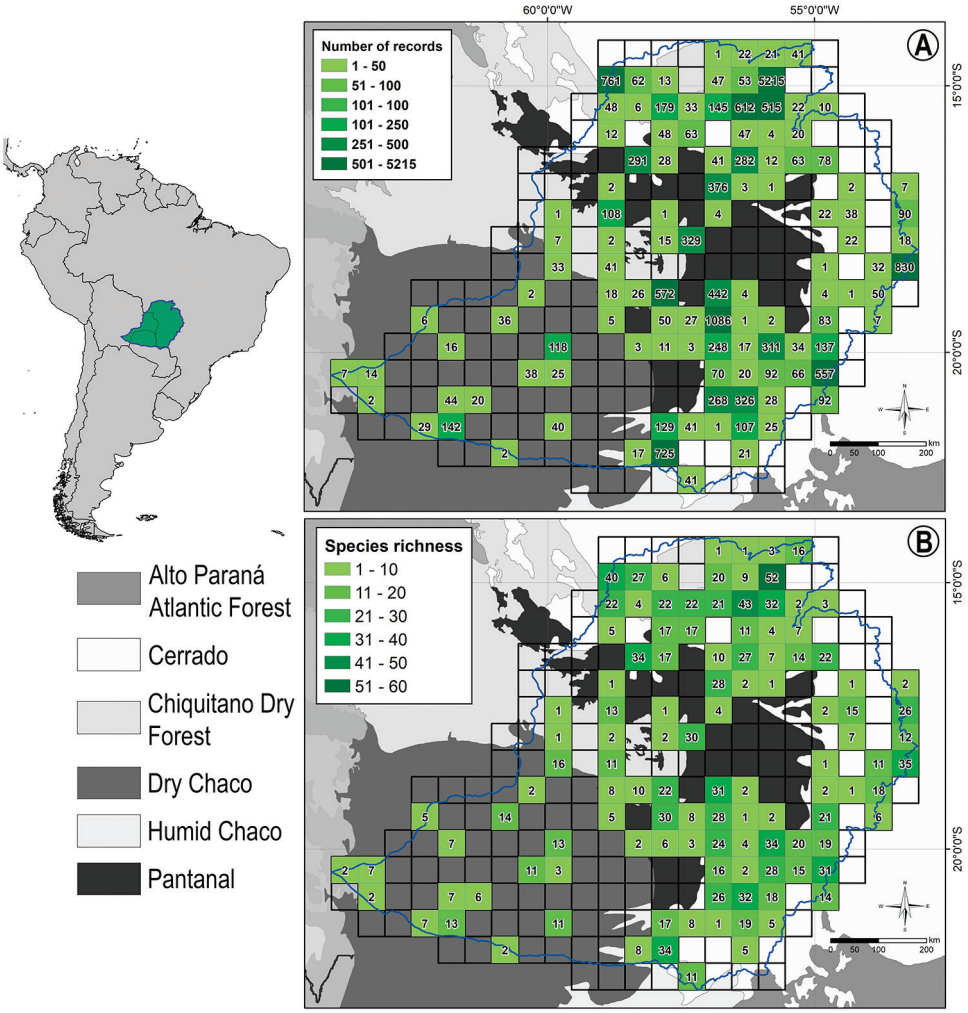

Figure 1. The insertion of Upper Paraguay River Basin (UPRB) within South America. **A** Number of amphibian occurrence records and **B** number of amphibian species recorded within each quadrat within the UPRB. Quadrats were drawn at the spatial resolution of 0.5 decimal degree. Background layer shows ecoregions in grayscale (Olson et al. 2001).

We also combined *Pithecopus azureus* and *P. hypocondrialis* under the name *P.* cf. *hypocondrialis*. *Pithecopus azureus* is distributed in the southern UPRB whereas *P. hypocondrialis* is an Amazonian species distributed in the northern UPRB (Frost 2020). However, the populations of *P.* cf. *hypocondrialis* found in the transitional zone of the distribution of these two species were not possible to identify using the diagnosis proposed by Caramaschi (2006). Therefore, we decided to place all the specimens as *P.* cf. *hypocondrialis*. In Suppl. material 2: Table S2, we provided the ecological traits for each species level named as "cf." in Suppl. material 1: Table S1. We also provide this information for *P. azureus* and *P. hypochondrialis*.

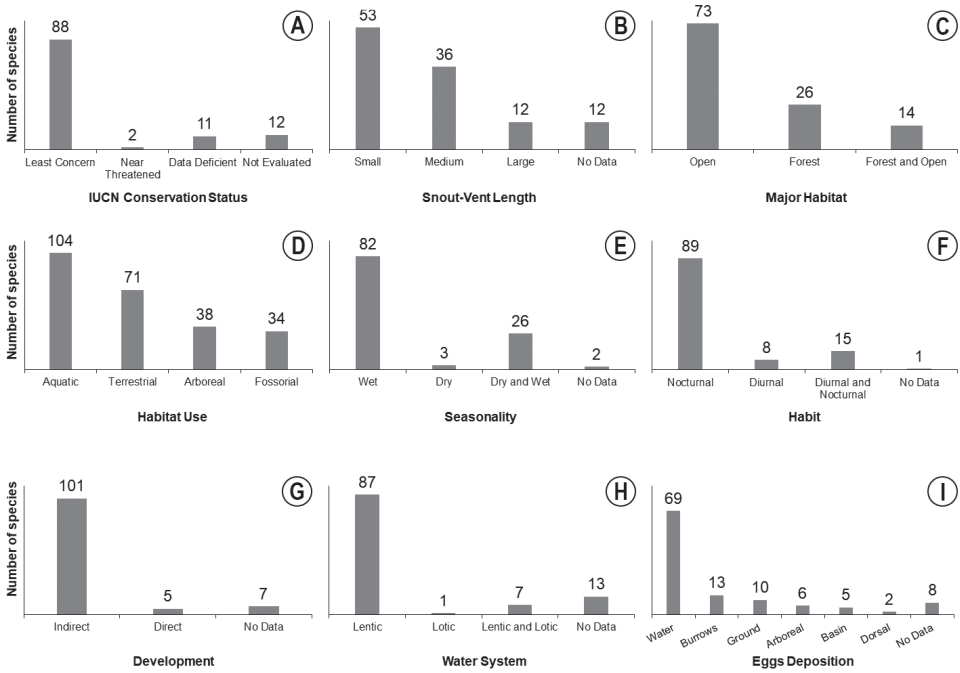

Figure 2. Species counting across levels of ecological traits for amphibians in Upper Paraguay River Basin. **A** Conservation status following International Union for Conservation of Nature and Natural Resources Red List of Threatened Species (2020) **B** body size category according to the snout-vent length (SVL) of each species. Categories correspond to row values (in millimeters – mm) from SVL divided in Small (0–39 mm), Medium (40–79 mm), Large (+ 80 mm), and No Data when the SVL was not available in the literature **C** major Habitat where the species is more associated and it can be Open areas, Forest or both **D** habitat Use for each species as Aquatic, Arboreal, Fossorial and/or Terrestrial **E** the seasonality of species showing which are more active in the Wet, Dry or both season **F** habit of the species classified according to their period of activity, which may be diurnal, nocturnal or both **G** larval Development with species showing direct (without tadpole stage) or indirect (with tadpole) development **H** water system used by the species breeding in Lotic environment, Lentic or both; and **I** the surface of the Eggs Deposition of each species and it can be in Water, Burrows, Ground, Arboreal, Basin or on the specimen Dorsal region. A total of 108 species were used for Water System and Eggs Deposition due to the Direct Development species.

For *Odontophrynus* species we kept the identification at the genus-level even knowing that two species within this genus occur in the UPRB (*O. americanus* and *O. lavillai*; Rosset and Baldo 2014). However, they are only distinguishable based on the ploidy of the cells or superficial characteristics, such as the pattern of dorsolateral spots (Rosset et al. 2006, 2009, Weiler et al. 2013), which makes their identification impossible with preserved species. Another issue concerns two *Oreobates* species that have their type localities within the UPRB. *Oreobates crepitans* was described from São Vicente, Cuiabá municipality, whereas *O. heterodactylus* was discovered in Cáceres municipality, both in Mato Grosso state, Brazil (Miranda-Ribeiro 1937, Bokermann 1965). However, the identity of these specimens in the collection is often unnamed and of difficult diagnosis based on specimens preserved (Padial et al. 2012). We thus placed *Odontophrynus* and *Oreobates* records as "sp." in Suppl. material 1: Table S1.

We use *group* ("gr.") for specimens from *Scinax ruber* and *Dendropsophus parviceps* species groups, for which we did not get the correct identification due to the high cryptic diversity among them. Other works have used the same nomenclature for both 'species group' in the literature (Kopp et al. 2010, São-Pedro and Feio 2010, Crivellari et al. 2014). Since the real identification of these species is untraceable, we removed them from Suppl. material 2: Table S2. The specimens named as *Leptodactylus* aff. *natalensis* is as yet an unknown species (Carvalho T., Personal communication).

Preliminary analyses and directions for future research

Datapapers compile well-curated species-level information on spatial and trait data of particular taxa and/or regions of interest (Chavan and Penev 2011). This datapaper comprises 17,089 records for 113 amphibian species, distributed in 14 families and 32 genera. Hylidae was the richest family (32 spp.) followed by Leptodactylidae (31 spp.), but this latter one showed the highest number of records (41.13% of total occurrence records), followed by Hylidae (35.77%). These two families are often the most common in short-term studies undertaken within the UPRB (e.g., Uetanabaro et al. 2007, 2008, Souza et al. 2010, 2017, Pansonato et al. 2011, Sugai et al. 2014).

The occurrence dataset is suitable to research on patterns of species diversity and distribution within UPRB (e.g., Valdujo et al. 2012, Roberto and Loebmann 2016, Silva et al. 2018). The regions with the highest number of species-occurrence records and apparent richness were concentrated in protected areas (e.g., Chapada dos Guimarães National Park, and Serra da Bodoquena National Park), target areas of environmental impact studies (e.g., Jauru and Manso hydroelectric plant regions), and around major urban centers (e.g., Campo Grande, Corumbá, and Cuiabá municipalities) (Suppl. material 1: Table S1; Fig. 1). Regions with striking sampling gaps or even without any species occurrence record are those showing low accessibility (e.g., Serra do Amolar, Pantanal do Paiaguás, and Pantanal do Nabileque). The Paraguayan portion of UPRB has a low number of records and only a few regions were sampled, nonetheless this may be biased due to the low number of Paraguayan scientific collections visited (Fig. 1B).

Species traits can greatly improve our understanding of ecological patterns and conservation planning (Grimm et al. 2014). Overall, species in the UPRB show prefer-

ence for open habitats and aquatic microhabitat, with predominance of species mostly nocturnal that breed in the wet season (Suppl. material 2: Table S2). The most common breeding strategy was indirect development in lentic water, with eggs laid directly on the water (Fig. 2). It is worth noting the high frequency of missing data for several traits explored in Suppl. material 2: Table S2, even for species that are common and abundant in the Pantanal and surroundings (e.g. *Boana lundii*, *Pseudis platensis*, *Scinax nasicus*, *Leptodactylus syphax*). Our ecological trait dataset helps identify which ecological aspects of what species are less known and therefore deserve further investigations.

In summary, it is necessary to encourage researchers to make available their unpublished data in order to minimize our biodiversity knowledge gaps (Chavan and Penev 2011). Amphibians are a highly threatened vertebrate group and the UPRB harbors the world's largest tropical wetland area. We hope the present data paper facilitates studies on ecology and conservation of amphibians from the Pantanal and surrounding plateaus.

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Supplementary material 1

Table S1

Authors: Matheus Oliveira Neves, Hugo Cabral, Mariana Pedrozo, Vanda Lucia Ferreira, Mário Ribeiro Moura, Diego José Santana

Data type: Species occurrence data

Explanation note: Data on occurrence records containing the collection of housing, locality, geographical coordinates, geographic accuracy, collection date, and collector name.

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Link: https://doi.org/10.3897/natureconservation.41.54265.suppl1

Supplementary material 2

Table S2

Authors: Matheus Oliveira Neves, Hugo Cabral, Mariana Pedrozo, Vanda Lucia Ferreira, Mário Ribeiro Moura, Diego José Santana

Data type: Species-level trait data

Explanation note: Data on ecological traits for each species, including morphometry, diet, activity pattern, habitat use, and breeding strategy.

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Link: https://doi.org/10.3897/natureconservation.41.54265.suppl2

Supplementary material 3

References from the citations contained in Suppl. material 2

Authors: Matheus Oliveira Neves, Hugo Cabral, Mariana Pedrozo, Vanda Lucia Ferreira, Mário Ribeiro Moura, Diego José Santana

Data type: References

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RESEARCH ARTICLE

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Threats from wildlife trade: The importance of genetic data in safeguarding the endangered Four-eyed Turtle (*Sacalia quadriocellata*)

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Abstract

Wildlife trade has been considered one of the largest threats to biodiversity in Southeast Asia. Many vertebrates, such as pangolins, elephants and turtles have been heavily hunted as a result of high demand from emerging markets in China and other countries in the region. In Vietnam, over-exploitation of turtles over several decades to supply the international trade has extirpated numerous populations and driven several species to the brink of extinction. To reverse this trend, conservation measures, such as re-introduction

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of confiscated or captive-bred animals to their native habitats, should be implemented to recover severely declined local populations. For species with a complex phylogeographic structure, however, it is crucial to understand geographic patterns of genetically-distinct populations to avoid releasing animals of unknown origin to wrong localities. In this study, we investigate the phylogeographic pattern of the Four-eyed Turtle (*Sacalia quadriocellata*), a widely traded species, which occurs in southern China, northern and central Laos and much of Vietnam, using samples with known localities and those collected from the local trade. Our range-wide phylogenetic and network study, based on the complete mitochondrial cytochrome *b* gene, recovered at least three major clades and seven subclades within the species range. Amongst these, two subclades, one from northern Annamites, Vietnam and the other from north-eastern Laos, are newly discovered. The fine scale phylogeographic analysis helped us to assign misidentified sequences from GenBank and those from confiscated animals with unknown origin to well-defined geographic populations. The results highlight the importance of incorporating samples collected from the local trade and the wild in genetic analyses to support both *ex-situ* and *in-situ* conservation programmes of highly-threatened species in accordance with the IUCN's One Plan Approach.

Keywords

conservation, cytochrome *b*, Lao PDR, *Sacalia quadriocellata*, Vietnam, wildlife trade

Introduction

Unsustainable exploitation of wildlife and their products has been recognised amongst the most serious threats to biodiversity conservation in Southeast Asia and to the survival of many globally-threatened wildlife species (Nijman 2010; Bennett 2011; Auliya et al. 2016; Krishnasamy and Zavagli 2020). According to Schneider (2008), the value from illicit trafficking of wildlife species and their products in the world is only ranked behind illegal trade in weapons and drugs. Experts estimate illegal global trade of wildlife to be worth at least $5 billion and possibly up to $20 billion per year (Wyler and Shikh 2013; Lawson and Vines 2014). This number can be converted into millions of wildlife individuals, particularly large mammals, birds and reptiles, which are poached and traded across national borders annually in the context of increasing demands for consumption and use.

In Vietnam, wildlife trade activities started to grow exponentially at the end of the 1980s when Vietnam opened its market to international trade, especially to China. In recent years, trade activities have still occurred widely and are likely to expand. The growth of wildlife trade has been illustrated through the number of export permits issued by Vietnam's CITES Office annually and documented illegal activities (WCS 2012; Janssen and Indenbaum 2019). In the global wildlife trade and consumption network, Vietnam is considered to play many roles, including three major ones: exporting, consuming and trafficking (Lawson and Vines 2014; Milliken 2014; Challender et al. 2015; Tran et al. 2016; Krishnasamy and Zavagli 2020).

Turtles have been collected and exported from Vietnam to China in large quantities since the late 1980s (Le and Broad 1995; Hendrie 2000; Le 2007). The trade has

continued to flourish in recent years with massive volumes recorded. For example, between 2005 and 2010, the Wildlife Crime Unit of the Education for Nature, Vietnam, an NGO focusing on countering wildlife trafficking, reported 163 smuggling cases of freshwater turtles and tortoises in the country, accounting for more than 25 tonnes or approximately 30,000 individuals (ENV 2010). As a result of a long period of overexploitation, many turtle populations in Vietnam have seriously declined and some species are considered virtually extinct or functionally extinct in the wild, such as the Vietnamese Three-striped Box Turtle (*Cuora cyclornata*), the Vietnamese Pond Turtle (*Mauremys annamensis*) and the Swinhoe's Softshell Turtle (*Rafetus swinhoei*).

Recently, the trade has taken advantage of the popularity of social networks in the country to sell turtles on these platforms. The number of turtles advertised for sale on Facebook, Myspace and Twitter has sharply escalated (Tran et al. 2016; Pham et al. 2019). According to a survey from March to May 2015, 346 turtle individuals of 15 different species were displayed on online markets nationally (Tran et al. 2016). An investigation into illegal trade activities on Vietnamese Facebook groups between 2013 and 2018 documented 481 turtle advertisements, involving almost 6000 individuals of 53 species and 12 families. Astoundingly, only around 42% or 22 species of those were native to Vietnam (Pham et al. 2019).

Vietnam is home to 29 species of freshwater turtles and tortoises (Turtle Taxonomy Working Group 2017) and as many as 24 of them are listed as Vulnerable or higher in the IUCN Red List (2020) (Fig. 1). One highly threatened, but largely neglected species is the Four-eyed Turtle (*Sacalia quadriocellata*), which occurs in China, Vietnam and Laos (Zhao 1998; Stuart et al. 2001; Shi et al. 2008; Suzuki et al. 2015; Turtle Taxonomy Working Group 2017). It is listed as Endangered by the IUCN Red List (2020) and included in Appendix II of the Convention on

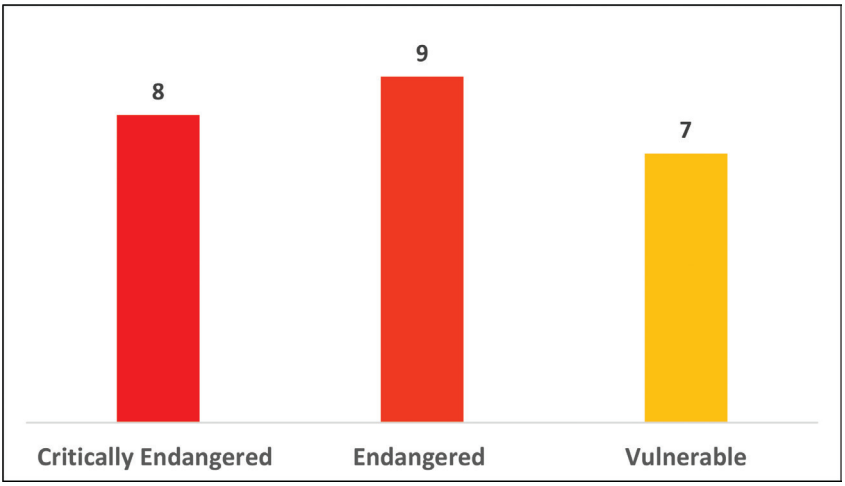

Figure 1. Conservation status of freshwater turtles and tortoises in Vietnam (IUCN Red List 2020).

International Trade in Endangered Species of Wild Fauna and Flora (CITES). The population of the species has been reportedly declining in China, Laos and Vietnam due to a high level of poaching and habitat loss (He et al. 2010; Suzuki et al. 2015; Tran et al. 2016), but no assessment is currently available for the species in any part of its range (IUCN Red List 2020). Over the last three years, 25 individuals of *Sacalia quadriocellata* were recorded in the trade in Vietnam by the Institute of Ecology and Biological Resources (Hanoi), comprising 22 turtles seized in Nghe An Province and three confiscated in Kon Tum Province.

The species has been shown to contain a high level of diversity with a number of genetically-distinct and geographically-isolated populations identified in a previous study (Shi et al. 2008). To our knowledge, these well-differentiated populations have not been recognised as separate conservation units in any conservation programme for the species, except for the population from Hainan of China, which has been proposed as a distinct species, *Sacalia insulensis* (Lin et al. 2018). This situation could lead to serious problems down the line, including mixing independently evolving lineages in conservation breeding facilities and releasing non-native animals to natural habitats, resulting in genetic pollution of local genetic pools. To further investigate the cryptic diversity and distribution of the poorly-studied species and clarify patterns of genetic differentiation of the species in Vietnam, we conducted both field and trade surveys across the range of the species with a focus in Vietnam and Laos. We sequenced the complete mitochondrial cytochrome *b* from collected DNA samples from wild populations and local trades and recovered their phylogenetic and network relationships using published and newly-generated sequences. Based on the results, we recommend options for conservation management of the species.

Materials and methods

Interview survey

A total of 2,758 interviews were conducted in 30 Provinces from northern to south central Vietnam from 2010–2018 with 79 turtle individuals observed. A short survey was also undertaken in Nam Xam Biodiversity Conservation Area, Xam Tai District, Houaphan Province in Laos from 25 May to 12 June 2015 with 118 interviews completed and 27 live specimens recorded. We conducted interviews using a semi-structured survey technique (Creswell and Poth 2018) with a focus on local people living around protected areas, as well as professional hunters and traders. Information on turtle origin, measurements and photos of individuals encountered during those interview surveys were recorded. Tissue samples for molecular analysis were collected from either tail tips or oral swabs and stored in 70% alcohol when possible. Geographic coordinates and elevation were recorded using a Garmin GPSMAP GPS 60CSx receiver in datum WGS 1984.

Field survey

Field surveys were conducted in three areas: 1) from 24 May to 3 June 2010 in Quang Nam Province at Cha Val Commune, Song Thanh Nature Reserve; 2) from 8 to 17 August 2008 at Khe Hua and Khe Phung Cam in Pu Huong Nature Reserve; and 3) from 9 to 19 June 2010 at Ban Bung – Khe Ca area in Pu Mat National Park, Nghe An Province, in north-central Vietnam (Fig. 2). The survey areas were characterised by evergreen forest and 30–50 degree slopes. Elevation ranged from 330 to 1000 m. We combined diurnal and nocturnal surveys, focusing on suitable habitats. Fifteen non-lethal aquatic turtle traps with chicken guts and rotten meat used as baits were also deployed to capture the Four-eyed Turtle in Khe Hua and Khe Phung Cam (Hua and Phung Cam Streams) in Pu Huong Nature Reserve and Khe Ca (Ca Stream) in Pu Mat National Park. Traps were checked every morning to change baits. The search in forests started at 6 am and ended around 5 pm. Measurements, information on habitat and ecology, tissue samples and photos were taken from turtles captured at the sites. Geographic coordinates and elevation were obtained using a Garmin GPSMAP GPS 60CSx receiver and recorded in datum WGS 1984.

Taxonomic sampling

In total, 20 new samples of *Sacalia quadriocellata* were incorporated in the analysis, including five wild collected samples with three from Pu Mat National Park and one each from Pu Huong Nature Reserve, Nghe An Province and Song Thanh Nature Reserve, Quang Nam Province. Another 13 were collected from local trade where the turtles were kept in local households from the area bordering with China, Cao Bang Province to the southern-most known localities of the species, Khanh Hoa Province in Vietnam and Houaphan Province, north-eastern Lao PDR. Two other samples were taken from confiscated animals in Pleiku City, Gia Lai Province in the Central Highlands, Vietnam (Table 1, Fig. 2). In addition, we obtained 34 sequences of the mitochondrial cytochrome *b* from GenBank for *Sacalia quadriocellata* and *S. bealei* and three others for outgroup taxa, *Cuora trifasciata*, *Cyclemys dentata* and *Mauremys annamensis*. Amongst these, 29 originated from Shi et al. (2008).

Molecular data

Total genomic DNA was extracted using the Dneasy Blood and Tissue Kit (Qiagen – Hilden, Germany) following the manufacturer's instructions for animal tissue. The genomic extraction was checked by electrophoresis. A negative control was used for every extraction.

We amplified the complete mitochondrial cytochrome *b* for all samples using HotStar Taq Mastermix (Qiagen – Hilden, Germany) and Dream Taq PCR Mastermix (Thermo

Table 1. GenBank accession numbers and associated voucher/laboratory numbers of ingroup taxa used in this study.

Species names	GenBank N	Voucher/Lab number	Reference	Origin	Locality
Sacalia bealei (5)	EU910982	HNUTSB0	Shi et al. 2008	Pet trade	–
Sacalia bealei (6)	EU910983	HNUTSB28	Shi et al. 2008	Pet trade	–
Sacalia bealei (7)	EU910984	HNUTSB19	Shi et al. 2008	Pet trade	–
Sacalia bealei (8)	AJ519501	MTD 41583	Barth et al. 2004	–	–
Sacalia bealei (9)	EU910981	HNUTSB25	Shi et al. 2008	Pet trade	–
Sacalia bealei (10)	EU910992	MVZ257748	Shi et al. 2008	Wild collected	Hong Kong, China
Sacalia bealei (11)	AY434585	HBS38403	Spinks et al. 2004	Pet trade	–
Sacalia bealei	GU183364	–	Nie and Jang 2016	–	–
Sacalia bealei	HQ442416	ANUM26080081	Xia et al. 2011	–	–
Sacalia bealei	NC016691	–	Nie and Jang 2012	–	–
*Sacalia bealei**	EF088646	–	Nie and Song 2016	–	–
Sacalia quadriocellata (12)	FJ211058	MVZ 258023	Shi et al. 2008	Wild collected	Quang Nam, Vietnam
Sacalia quadriocellata (13)	EU910995	FMNH 256542	Shi et al. 2008	Wild collected	Khammouane, Laos
Sacalia quadriocellata (14)	EU910994	FMNH 256543	Shi et al. 2008	Wild collected	Khammouane, Laos
Sacalia quadriocellata (15)	FJ211059	ZFMK 81536	Shi et al. 2008	Wild collected	Ha Tinh, Vietnam
Sacalia quadriocellata (16)	FJ211060	ZFMK 81535	Shi et al. 2008	Wild collected	Ha Tinh, Vietnam
Sacalia quadriocellata (17)	EU910974	HNU TSQ11	Shi et al. 2008	Pet trade –	
Sacalia quadriocellata (18)	AJ564465	MTD 42442	Barth et al. 2004	Pet trade	–
Sacalia quadriocellata (19)	EU910973	HNU TSQ8	Shi et al. 2008	Pet trade	–
Sacalia quadriocellata (20)	EU910993	ROM 28458	Shi et al. 2008	Local trade	Tuyen Quang, Vietnam
Sacalia quadriocellata (21)	EU910990	–	Shi et al. 2008	Pet trade	–
Sacalia quadriocellata (22)**	AY434614	HBS 38436	Spinks et al. 2004	Pet trade	–
Sacalia quadriocellata (23)	EU910988	HNU TSQ4	Shi et al. 2008	Pet trade	–
Sacalia quadriocellata (24)	EU910987	HNU TSQ3	Shi et al. 2008	Pet trade	–
Sacalia quadriocellata (25)	EU910991	MVZ 257747	Shi et al. 2008	Wild collected	Guangdong, China
Sacalia quadriocellata (28)	EU910985	HNU TSQ281	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (29)	EU910975	HNU TSQ224	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (30)	EU910989	HNU TSQ61	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (31)	EU910978	HNU TSQ264	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (32)	EU910986	R0520	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (33)	EU910980	HNU TSQ273	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (34)	EU910976	HNU TSQ231	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (35)	EU911001	MVZ 230485	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (36)	EU911000	MVZ 230484	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (37)	EU910977	HNU TSQ239	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata (38)	EU910979	HNU TSQ284	Shi et al. 2008	Wild collected	Hainan, China
Sacalia quadriocellata	GU320209	–	Nie and Jiang 2016	–	–
Sacalia quadriocellata	MT845096	SAC 7	This study	Local trade	Ha Tinh, Vietnam
Sacalia quadriocellata	MT845097	SAC 9	This study	Local trade	Ha Tinh, Vietnam
Sacalia quadriocellata	MT845098	SAC 10	This study	Local trade	Ha Tinh, Vietnam
Sacalia quadriocellata	MT845099	SAC 11	This study	Local trade	Quang Ninh, Vietnam
Sacalia quadriocellata	MT845100	SAC 12	This study	Local trade	Quang Ninh, Vietnam
Sacalia quadriocellata	MT845101	SAC 13	This study	Local trade	Quang Ninh, Vietnam
Sacalia quadriocellata	MT845102	SAC 15	This study	Local trade	Thua Thien Hue, Vietnam
Sacalia quadriocellata	MT845103	SAC 16	This study	Local trade	Houaphan, Laos
Sacalia quadriocellata	MT845104	SAC 17	This study	Local trade	Houaphan, Laos
Sacalia quadriocellata	MT845105	SAC 18	This study	Local trade	Houaphan, Laos
Sacalia quadriocellata	MT845106	SAC 19	This study	Confiscated	–
Sacalia quadriocellata	MT845107	SAC 20	This study	Confiscated	–
Sacalia quadriocellata	MT845108	SAC 21	This study	Wild collected	Nghe An, Vietnam
Sacalia quadriocellata	MT845109	SAC 22	This study	Wild collected	Nghe An, Vietnam
Sacalia quadriocellata	MT845110	SAC 23	This study	Wild collected	Nghe An, Vietnam
Sacalia quadriocellata	MT845111	SAC 24	This study	Wild collected	Nghe An, Vietnam
Sacalia quadriocellata	MT845112	SAC 25	This study	Local trade	Cao Bang, Vietnam

Species names	GenBank N	Voucher/Lab number	Reference	Origin	Locality
Sacalia quadriocellata	MT845113	SAC 26	This study	Local trade	Khanh Hoa, Vietnam
Sacalia quadriocellata	MT845114	SAC 27	This study	Local trade	Binh Dinh, Vietnam
Sacalia quadriocellata	MT845115	SAC 28	This study	Wild collected	Quang Nam, Vietnam

* This sequence record is labelled as *S. quadriocellata* on GenBank; ** This sequence record is labelled as *S. pseudocellata* on GenBank, a hybrid species, but still considered to be *S. quadriocellata* in Shi et al. 2008. Numbers in parentheses are marked following Shi et al. (2008).

Fisher Scientific – Vilnius, Lithuania). The standard PCR conditions were 95 °C for 15 min with HotStar Taq Mastermix (Qiagen – Hilden, Germany) or 95 °C for 5 min with Dream Taq PCR Mastermix (Thermo Fisher Scientific – Vilnius, Lithuania), 35 cycles at 95 °C for 30 s, 45 °C for 45 s, 72 °C for 60 s; a final elongation at 72 °C for 6 min. The PCR volume consisted of 2 µl of each primer at 10 pmol/µl, 5 µl water, 10 µl of Mastermix and 1–4 µl DNA template depending on the quantity of DNA. All primers used for this study are listed in Table 2. A negative and positive control was used for every PCR reaction. To confirm if the correct size was amplified, 5 µl of PCR product was run on a 1% agarose gel, 1X TBE buffer, stain with 2 pg/µl bromide and photographed under UV light. Successful PCR products were cleaned using Gene Jet PCR Purification Kit (Thermo Fisher Scientific – Vilnius, Lithuania) following manufacturer's instructions. Cleaned PCR products were sent to 1st Base (Malaysia) for sequencing.

Phylogenetic and network analyses. The sequences were edited using Sequencher v4.1.4 (Gene Codes Corp, Ann Arbor, MI, USA) then aligned using Bioedit v7.1.3 (Hall 1999) with default settings. Data were analysed using three phylogenetic methods, Maximum Parsimony (MP) as implemented in PAUP*4.0b10 (Swofford 2001), Bayesian Inference (BI) in MrBayes v3.2.7 (Ronquist et al. 2012) and Maximum Likelihood (ML) in IQ-TREE v1.6.8 (Nguyen et al. 2015), respectively. Intraspecific relationships amongst populations of *Sacalia quadriocellata* were also inferred using the NeighborNet algorithm (Bryant and Moulton 2004) using SplitsTree v4.14.2 (Huson and Bryant 2006). For MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch swapping algorithm, with no upper limit set for the maximum number of trees saved. All characters were equally weighted and unordered. For BI, we performed both single and multiple models by codon partitions to examine the robustness of the tree topology (Nylander et al. 2004; Brandley et al. 2005). Analyses were conducted with a random starting tree and run for 1×10^7 generations, four Markov chains (one cold, three heated) with default settings. Values of sample points were plotted against the number of generations to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. Two independent analyses were performed simultaneously. The cut-off point for the burn-in function was set to 25 and 28 in the single- and multiple-model Bayesian analyses, respectively, as $-\ln L$ scores reached stationarity after 25,000 and 28,000 generations in both runs of the two analyses. The posterior probability (PP) values for all clades in the final majority rule consensus tree were provided (Fig. 3). Nodal support was also evaluated using Bootstrap replication (BP) as calculated in PAUP (1,000

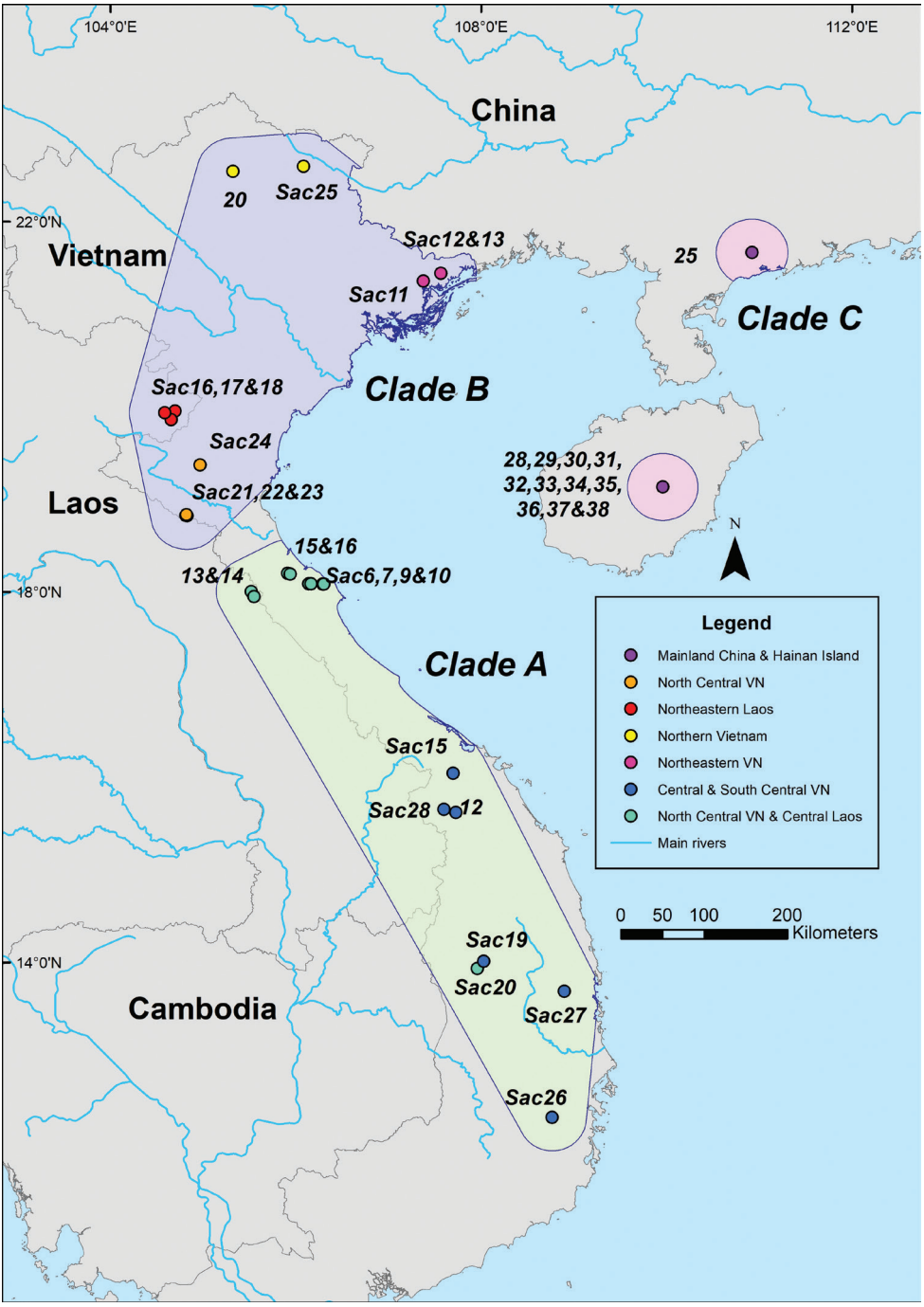

Figure 2. Samples of *Sacalia quadriocellata* collected from the field and local trade. Three delineated areas, A, B and C, represent distributions of three phylogeographic clades shown in Figs 3, 4. See Table 1 for more information of the samples.

Table 2. Primers used in this study.

Primer	Sequence (5' to 3')	Reference
CytbG (f)	AACCATCGTTGTWATCAACTAC	Spinks et al. (2004)
GLUDGE (f)	TGATCTTGAARAACCAYCGTTG	Palumbi et al. (1991)
CytbJSi (f)	GGATCAAACAACCCAACAGG	Spinks et al. (2004)
CytbJsr (r)	CCTGTTGGGTTGTTTGATCC	Spinks et al. (2004)
THR-8 (r)	GGTTTACAAGACCAATGCTT	Spinks et al. (2004)

(f): forward, (r): reverse

replications and 100 random taxon addition) and in SplitsTree (1000 replications) and employing ultrafast BP (10,000 replications) in IQ-TREE. We regarded BP value ≥ 70% and PP value ≥ 95% as strong support for a clade (Hillis and Bull 1993; Ronquist et al. 2012). For ML and BI analyses, we used the optimal model calculated by jModelTest v.2.1.10 (Darriba et al. 2012). The optimal model for nucleotide evolution was set to TVM+I for single model BI and ML analyses. For the Bayesian multiple model analysis, three selected models, TVMef+G, HKY+I and TrN+I, for three codon positions were assigned to these partitions in MrBayes using the command APPLYTO. Model parameters were inferred independently for each data partition using the UNLINK command. Uncorrected pairwise genetic divergences between different lineages of *Sacalia quadriocellata* were calculated in PAUP*4.0b10. The network analysis was performed in SplitsTree with the following settings: edge fitting as ordinary least squares, equal angle as chosen splits transformation, least squares to modify weights and four maximum dimensions as the filtering option. The generated split graph showed a visual representation of conflicting signals in the data by presenting them as a series of parallel edges. The programme computed the least squares fit (LSfit) between the pairwise distances from the graph and the distances from the matrix to produce a distance-based unrooted tree diagram by means of the neighbour-joining algorithm (Saitou and Nei 1987).

Results

We successfully sequenced the complete cytochrome *b* gene for 20 samples collected from eight Provinces in Vietnam, one Province in Lao PDR and two samples from confiscated animals. The final matrix consisted of 1143 aligned characters with no gap, of which 215 characters were parsimony informative and 822 were constant in the MP analysis. In total, 48 most parsimonious trees were found (Tree length = 504; Consistency index = 0.7; Retention index = 0.92). In the ML analysis, the log likelihood of the best tree found was -9838.332. The three analyses produced well-corroborated phylogenies, although the MP tree is less resolved, compared to those estimated by ML and BI. Most major nodes of the trees received high statistical support from at least two analyses (BP ≥ 70% and PP ≥ 95%) (Fig. 3).

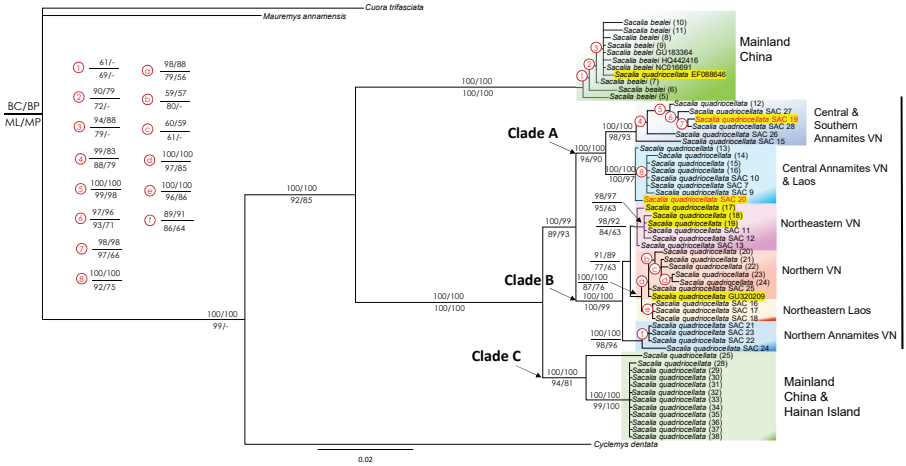

Figure 3. Bayesian cladogram, based on the complete mitochondrial cytochrome *b* gene. The numbers above and below branches are Bayesian posterior probability for single/multiple models and maximum likelihood/maximum parsimony bootstrap values, respectively (all in percentage). Highlighted samples in red and black letters denote those originating from confiscations and GenBank or the previous study with no reliable locality/identity, respectively.

Similar to results reported by Shi et al. (2008), our phylogenetic hypothesis strongly supports the monophyly of the genus *Sacalia* and its two species, *S. bealei* and *S. quadriocellata*. In addition, three main clades, Clade A, B and C, within *S. quadriocellata*, were well corroborated by all four analyses. Clade A included populations from north-central Vietnam and central Laos, including Ha Tinh and Quang Binh Provinces in Vietnam and Khammouane Province in Lao PDR, along with those from central Vietnam and south-central Vietnam, i.e. Thua Thien Hue, Quang Nam, Binh Dinh and Khanh Hoa Provinces (Figs 2, 3, Table 1). Both of the subclades within Clade A received strong statistical support from all four analyses. In this study, we expanded the north-central Vietnam and central Laos clade to include Ha Tinh Province (samples SAC 7, 9 and 10). One confiscated sample of unknown origin, SAC 20, was also grouped in this subclade.

The other subclade of Clade A contained a higher level of diversity than previously shown. The only sample incorporated in Shi et al. (2008), sample (12) from Song Thanh Nature Reserve, Quang Nam Province, was placed in a lineage (node 5) with another sample from the Reserve (SAC 28) and a sample from Binh Dinh Province (SAC 27) with high statistical support from all analyses. The second confiscated sample, SAC 19, also clustered in the group (Fig. 3). The populations from Thua Thien Hue Province (SAC 15), distributed north of Quang Nam and Binh Dinh Provinces and from Khanh Hoa Province (SAC 26), located in the southernmost locality of the species range, were divergent from the middle group (nodes 4 and 5) (Fig. 3).

In Clade B, four subclades were supported in our analyses instead of two in Shi et al. (2008). Two newly-discovered subclades included one from north-eastern Laos,

Houaphan Province and the other from north-central Vietnam, Nghe An Province (Table 1, Fig. 3). Both of the subclades received strong statistical support from all analyses. For two other subclades corroborated by Shi et al. (2008), our analyses were able to assign them to two separate biogeographic areas, the north-eastern Vietnam unit, consisting of samples from Quang Ninh Province (SAC 11, 12 and 13) and three samples of unknown origin (17, 18 and 19 from Shi et al. 2008) and the northern Vietnam unit, containing samples from Shi et al. (2008) with one from Tuyen Quang Province (sample 20) and a new sample (SAC 25) collected from neighbouring Cao Bang Province. A sample of unknown origin with GenBank accession number of GU320209 was also placed in this subclade (Figs 2, 3).

Clade C, basal to Clade A and Clade B, comprised one sample from mainland China, sample (25) and the rest from Hainan Island. This clade was the most divergent from those occurring in Vietnam and Laos with an average of approximately 3.5% in terms of genetic pairwise distance. The population from mainland China was around 2% differentiated from Hainan Island's population (Table 3). The network analysis generated the same results as those provided by phylogenetic estimations. In particular, three main clusters, Clade A, B and C, were also strongly supported by the Neighbor-Net algorithm and, amongst the clades, Clade A and B from Vietnam and Laos were more closely related to each other than to Clade C from mainland China and Hainan Island. In each main clade, subclades recovered by phylogenetic analyses, two in Clade A, four in Clade B and two in Clade C, were also corroborated, based on genetic distance in the network analysis (Fig. 4).

Discussion

The samples included in our study cover most of distribution localities of the Four-eyed Turtle reported in previous studies, for example, Shi et al. (2008) and Turtle Taxonomic Working Group (2017). Only those from several localities from mainland China,

Table 3. Uncorrected ("p") distance matrix showing percentage pairwise genetic divergence (cytochrome *b*) between different subclades within *Sacalia quadriocellata*. The highest pairwise distance within clades is italicised and shown in parenthesis.

Subclade name	CSAVN	CAVNLA	NEAVN	NORVN	NEALA	NORAN	HAINI	MACHI
CSAVN	(*1.8*)							
CAVNLA	1.5–2.5	(*0.5*)						
NEAVN	2.5–3.5	2.0–2.7	(*0.3*)					
NORVN	2.5–3.4	2.1–2.9	0.4–1.0	(*0.4*)				
NEALA	2.5–3.3	2.3–2.7	0.4–0.7	0.3–0.6	(*0*)			
NORAN	2.5–3.7	2.2–2.9	0.6–1.1	0.6–1.3	0.8–1.1	(*0.5*)		
MACHI	3.3–3.9	3.4–4.1	3.3–3.6	3.2–3.6	3.5–3.7	3.2–3.8	(*0.1*)	
HAINI	3.6–4.0	3.6–3.9	3.7–4.0	3.7–4.0	3.9–4.0	3.6–3.9	2.0–2.1	(*0*)

CSAVN: Central & southern Annamites, Vietnam; CAVNLA: Central Annamites, Vietnam and Laos, NEAVN: North-eastern Vietnam; NORVN: Northern Vietnam; NEALA: North-eastern Laos; NORAN: Northern Annamites; MACHI: Mainland China; HAINI: Hainan Island.

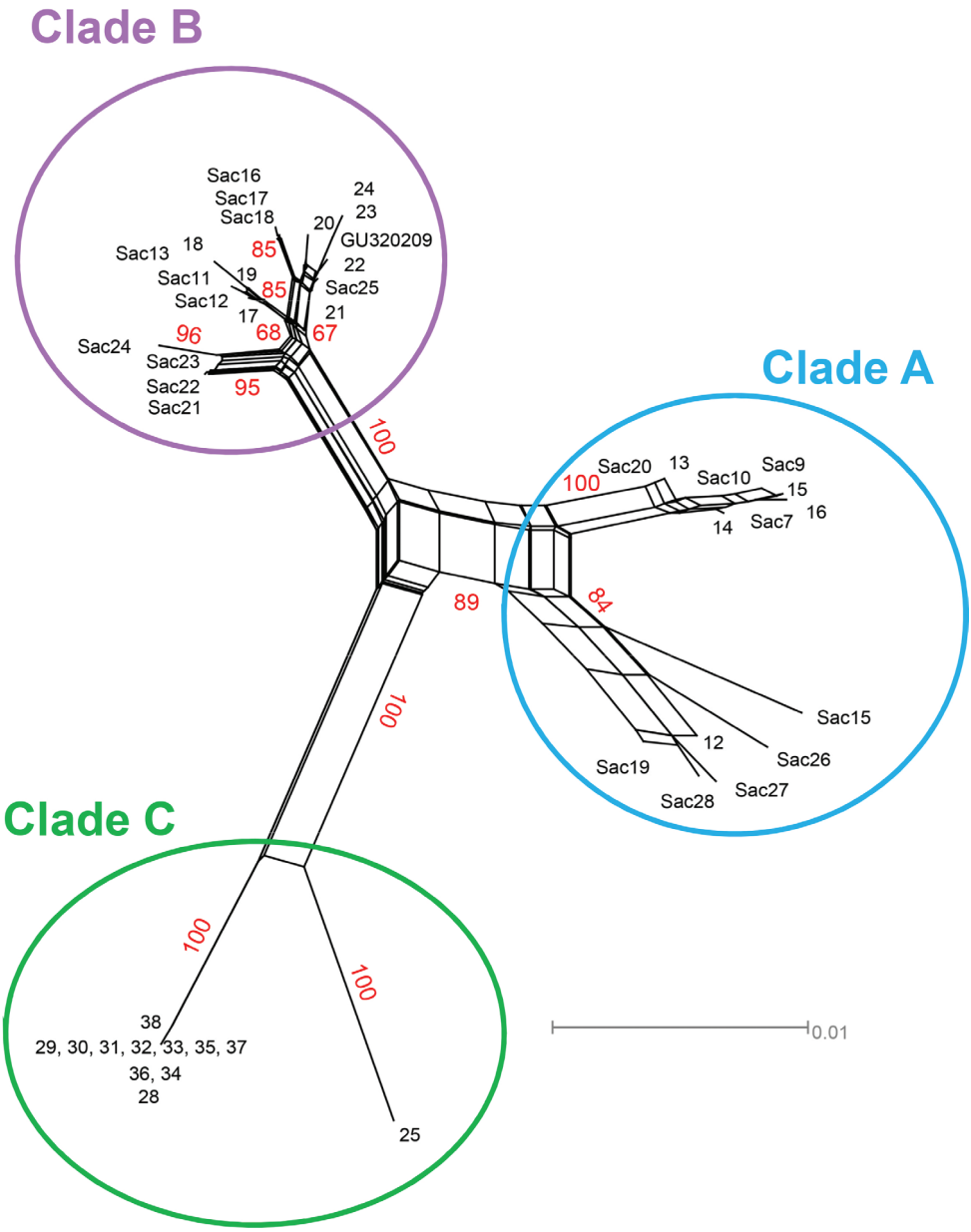

Figure 4. Split tree network, based on the complete mitochondrial cytochrome *b* gene. Red numbers at nodes are bootstrap values in percentage.

Hainan Island, northern Vietnam and possibly eastern Laos (Suzuki et al. 2015) were not included in the analysis. We also recorded the southernmost locality of the species in Khanh Hoa Province (Fig. 2), as, prior to this study, the southern end of the distribution of the Four-eyed Turtle was deemed to be in Binh Dinh Province (Turtle Taxonomic Working Group 2017). This population is also approximately 1.0–1.6% genetically

divergent from others distributed in central and south-central Vietnam. In general, the species shows substantial genetic diversity throughout its range and natural boundaries for each clade and subclade seem to follow river systems and mountain ranges.

The largely aquatic species exhibits a finer partitioned phylogeographic pattern compared to other species of turtles in the region (Stuart and Parham 2004; Blanck et al. 2006; Gong et al. 2009; Stuckas and Fritz 2011). The number of lineages recovered and their distribution in this study suggests the populations tend to be more isolated by natural boundaries compared to other reptiles and amphibians as demonstrated by Bain and Hurley (2011). Specifically, the population from north-western Laos is separated from that from northern Vietnam; both are found in the North-western Unit of Bain and Hurley (2011). In addition, populations in the central Annamites (Clade A) can be subdivided into at least two subclades. Within the central and southern Annamites subclade, three genetically-distinct populations were also identified. One is represented by sample SAC 15, which occurs north of Hai Van Pass, a well-known biogeographic boundary in the country. Another population denoted by sample SAC 26 is found in Khanh Hoa Province, southern Annamites and the remaining samples, SAC 27, SAC 28 and (12) occurring from Quang Nam to Binh Dinh Provinces (Table 1, Figs 2, 3). This subclade also exhibited the highest genetic divergence, around 1.8%, compared to other subclades in Vietnam and Laos (Table 3). Additional samples from this region will likely help to discover more diversity in this subclade.

Our new samples help to identify two novel subclades of *Sacalia quadriocellata*, one from northern Annamites, Nghe An Province, Vietnam and the other from north-eastern Laos, Houaphan Province, Lao PDR. Furthermore, two other subclades are more well-defined in terms of distribution with the addition of samples, SAC 11, 12 and 13 from this study. Previously, with only one known locality from sample (20), Shi et al. (2008) hypothesised that other samples of unknown locality, samples (17) to (24), share the same location. However, our analyses suggest that samples (20) to (24) belong to northern Vietnam's clade, whereas samples (17) to (19) originate from north-eastern Vietnam because they cluster with samples, SAC 11, 12 and 13 from Quang Ninh Province (Table 1, Figs 2, 3). The analyses also show that the subclade from central and southern Annamites, which was represented only by sample (12) from Quang Nam Province, is genetically much more diverse than previously determined. More samples from known localities in northern Vietnam, Hainan Island, mainland China and potentially eastern Lao PDR will likely reveal even higher diversity within the species. More surveys should therefore be conducted in the gap areas in China, Laos and Vietnam.

More detailed analyses can support assignment of unknown and/or misidentified samples to subclades with known origin. In addition to three samples, (17) to (19), from Shi et al. (2008), now revealed to be from north-eastern Vietnam, the identity of two samples from GenBank is also confirmed. One sample with accession number EF088646, which was misidentified as *Sacalia quadriocellata*, is, in fact, *S. bealei* as shown by our study. Another sample with accession number GU320209 belongs to northern Vietnam's subclade. Two samples, SAC 19 and 20, confiscated from Pleiku City, Gia Lai Province, were assigned to two different subclades, with SAC clustering

with other samples from central and southern Annamites and specifically most closely related to SAC 28 from Song Thanh Nature Reserve, Quang Nam Province and SAC 20 with those from central Annamites in Laos and Vietnam.

With the power to determine geographic provenance of unknown and misidentified samples, the application of phylogenetic and network analyses, based on informative barcoding genes, can help to track the origin of confiscated individuals from the trade. This is especially true when georeferenced samples are included, as shown in this and other studies (Welton et al. 2013; Siler et al. 2014; Zhang et al. 2015; Gaubert et al. 2016; Summerell et al. 2019; Kongrit et al. 2020; Ngo et al. 2020). Information of origin helps to identify hotspots of illegal hunting activities and provide better management strategies for conservation breeding programmes to avoid intermixing between animals from independently evolving lineages (Blair et al. 2017; Ngo et al. 2020). The population-level assignment can therefore assist monitoring of farmed animals to prevent laundering of wild turtles and other wildlife species (Ogden et al. 2009; Sigouin et al. 2017).

The key for proper assignment involves accurately georeferenced samples and selection of informative molecular markers, which can differentiate genetically-distinct populations (Ogden et al. 2009; Ogden and Linacre 2015; Blair et al. 2017). As turtles have declined drastically all over Asia (Horne et al. 2012; Rhodin et al. 2018), it is very challenging to sample animals from their native habitats. Historically, the Four-eyed Turtle had a low trade demand, but its market value has risen significantly, probably because natural populations of other turtle species have been extirpated (Tran et al. 2016). The recent trend has driven many turtle populations, including the Four-eyed turtles, in Vietnam to the brink of extinction (Tran et al. 2016; Pham et al. 2018). Our extensive field survey efforts in this study yielded only five wild-caught turtles. Due to this rarity, samples collected from the local trade, which are more readily available (Ly et al. 2011; McCormack et al. 2011; Pham et al. 2018), can substitute the field-collected ones in providing useful information on the local population. In this study, samples collected from the local trade helped to reveal at least one unknown subclade from north-eastern Laos. Others, i.e. SAC 11, 12 and 13, clarified the provenance of samples (17), (18) and (19) from Shi et al. (2008) or showed that populations from Thua Thien Hue Province (SAC 15) and Khanh Hoa Province (SAC 26), the southernmost locality of the Four-eyed Turtle's range, are genetically distinct from known populations.

These and other divergent populations clearly need more attention in future research to assess both their taxonomic and conservation status. Lin et al. (2018) suggested that the population from Hainan Island (samples 28–38 in Clade C of Fig. 3) represents a distinct species, but their morphological comparison included only specimens from Hainan and Guangdong and Guangxi and the results seemed to contradict the findings by Shi et al. (2008), specifically regarding the shape formed by the inner edges of pseudo-eyes on the back of the head and coloured patches in the lower jaw. The taxonomic revision is therefore unwarranted and the issue requires further investigation.

Population assignment can support releasing confiscated animals back to their natural range. Success of re-introduction programmes relies on a good understanding

of physiological demand of targeted species (IUCN/SSC 2013; Tarszisz et al. 2014). As an ectotherm, turtles are sensitive to changes of microclimate and cannot easily adapt if they are translocated to non-native habitat with different climate regimes (Butler 2019). In case of the Four-eyed Turtle, it would be detrimental to release animals originating from south-central Vietnam to northern Vietnam, because the two regions have very different climatic conditions (Sterling et al. 2006). In addition, the evolutionary differentiations between the populations, which have been likely facilitated by natural barriers of rivers and mountains, suggest they might have been isolated from each other for a long period of time with little evidence of gene flow. The release of animals with alien genotypes into a local population can lead to problems of outbreeding as they possess different adaptive potentials (Frankham et al. 2011; Weeks et al. 2011). The unwanted genetic pollution can result in extinction or compromise of local genetic pools (Butler 1994; Rhymer and Simberloff 1996; Fitzpatrick and Shaffer 2007). Genetic pollution through hybridisation between artificially-introduced and native turtles has been reported in several Asian countries (Fong and Chen 2010; Suzuki et al. 2014; Gong et al. 2018). Without sufficient guidance from genetic research, it would be almost impossible to reliably determine the provenance of four-eyed turtles confiscated from the trade, because only few morphological characters have been found to diagnose the populations (Shi et al. 2008).

The accelerated rate of wildlife trafficking has already complicated the issue of genetic pollution, especially in developing countries like Vietnam, where resources for keeping confiscated organisms are limited. Pressures to release the animals of unknown origin back to the wild can easily force errors in selecting appropriate sites. To date, the country has no specific regulation for translocation programmes and, in many cases, animals have been arbitrarily released to nearest sites of confiscated locations (Nguyen et al. 2017). However, this is often not where they come from, as our study clearly shows that two four-eyed turtles rescued from Pleiku have two different origins. In addition, the government has not provided any guidelines for rescuing and releasing confiscated animals back to the wild, which results in the re-introduction of unhealthy and non-native individuals (Nguyen et al. 2017). Government funding for combating wildlife trade is insufficient, let alone for genetic and disease screening. In our experience, resources for rescuing and releasing animals in accordance to the IUCN/SSC guidelines have largely come from international organisations and will likely to be the case in the foreseeable future.

Conclusion and recommendations

Pervasive international wildlife trade has resulted in a large volume of confiscated individuals with unknown origin. Consequently, there is a growing need for rescue centres and conservation breeding programmes around the world to take these animals into their facilities for rehabilitation and breeding. *Ex-situ* management should take into account the risks of mixing genetically-distinct lineages in captive facilities and

re-introducing non-native individuals to natural habitats. Detailed phylogeographic studies, employing field collected and local trade samples, can help to determine geographic provenance of confiscated animals and minimise impacts of the problems. Unfortunately, this kind of information is not available for many trade-targeted species, forcing managers to make difficult choices. To better manage the species, it is therefore a priority for conservation programmes to undertake phylogeographic works and genetically screen their captive colonies, especially when morphological characters to reliably diagnose geographically-distinct populations are lacking. To improve conservation of the Four-eyed Turtle in Vietnam, we recommend a genetic screening initiative to maintain genetic integrity of captive lineages. In addition, field and interview surveys should be conducted in gap areas in southern China, eastern Laos and northern Vietnam to clarify the genetic identity of the populations. Studies on population status and habitat suitability are also critically needed to establish areas for future releases of captive turtles. It is equally important that the government issues a set of criteria, including required standards for health and genetic profile of captive animals, to guide re-introduction activities. Without these comprehensive measures, biodiversity will be in great danger posed by genetic pollution from introduced non-native sources. Our research again underscores the IUCN's One Plan Approach, which aims to develop integrative strategies to combine *in situ* and *ex situ* measures with groups of experts for the purpose of species conservation.

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Differential responses of prairie rodents to edge effects from recreational trails

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Abstract

Edge effects are a common phenomenon in which an ecological variable changes with respect to distance from a habitat edge. Recreational trails may constitute a habitat edge for prairie rodents because of high human presence, high predator presence, or limited shelter compared to the prairie core. Despite the prevalence of trails in conservation parcels, their effect on wildlife distribution remains largely unstudied. We examined the impacts of recreational trails on small mammal activity in the restored prairies of the Cowling Arboretum at Carleton College. The prairies were restored from 1995 to 2008 and now comprise a contiguous prairie block of approximately 155 ha. Over 2 consecutive summers, we used infrared motion-sensing cameras to record the relative amount of time rodents spend at baited stations placed at different distances from the trail. The results varied between taxa: voles (*Microtus* spp.) avoided trail edges whereas mouse (Cricetidae and Dipodidae) and thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) activity was unaffected by trail proximity. Trails may therefore have species-specific effects on small mammals, with potential consequences for the connectivity and distribution of populations.

Keywords

camera, edge effects, thirteen-lined ground squirrels, mice, recreational trails, small mammals, voles

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Introduction

Habitat fragmentation leads to edge effects (Laurance and Yensen 1991, Flaspohler et al. 2001, Fahrig 1997, Haddad et al. 2015), which can affect species differently (Donovan et al. 1997, Debinski and Holt 2000, Miller and Hobbs 2000, Ries et al. 2004, Bock et al. 2002). Edge effects occur when ecological processes differ with respect to distance from a habitat edge (Donovan et al. 1997). Whereas some mobile organisms are attracted to edges, others avoid edges because of unfavorable interspecific interactions or resource availability (Ries et al. 2004). Such edge-aversion can change the functional area of a habitat patch from the perspective of that species (Laurance and Yensen 1991).

Recreational trails in natural areas are a nearly linear land use, creating long edges and potentially fragmenting habitat despite occupying minimal area. In addition to facilitating human activity (Reilly et al. 2017), trails may alter the abiotic environment by affecting light availability, soil temperature, soil moisture, erosion, litter depth, or other factors (Ballantyne and Pickering 2015). Insofar as trails induce edge avoidance, they may partially fragment otherwise contiguous patches. Thus, trails may decrease connectivity and core habitat area disproportionately to their footprint (McDonald and St. Clair 2004).

Responses to trail edges can vary widely among species (Benítez-López et al. 2010, Debinski and Holt 2000, Miller and Hobbs 2000, Reilly et al. 2017) due to differences in foraging behavior (Kerth and Melber 2009), vulnerability to predators (Flaspohler et al. 2001, Pardini 2004), or responses to human activity (George and Crooks 2006). A study of large mammal activity in an urban park found that bobcats (*Lynx rufus*), coyotes (*Canis latrans*), and mule deer (*Odocoileus hemionus*) were all less likely to cross trails during the day in places where there was high human activity (George and Crooks 2006), but the degree of responsiveness to human activity was species-specific. Deer were the most tolerant, while bobcats demonstrated a stronger aversion to areas of high human activity (George and Crooks 2006). In contrast, Reilly et al. (2017) found no correlation between wildlife occurrence and human activity for populations of 10 medium- to large-sized mammal species, including bobcats, coyotes, and mule deer. However, in areas of high recreational activity, coyotes were less active during the day and striped skunks (*Mephitis mephitis*) were more active in the late morning, perhaps to avoid coyotes (Reilly et al. 2017).

Smaller animals may also show species-specific responses to trail edges. The reduced cover and greater light on and near trails may put small mammalian prey at greater risk of predation (Kotler et al. 1991, Orrock and Danielson 2009), discouraging activity near trails. Cricetid species have been found to differ in their ability to cross highways and wooded medians; translocated deer mice (*Peromyscus maniculatus*) successfully crossed these boundaries and returned to their home ranges more often than meadow voles (*Microtus pennsylvanicus*) or red-backed voles (*Myodes californicus*) (McDonald and St. Clair 2004). Edge-aversion may also be a response to resource scarcity near edges. For example, both red-backed voles and their primary food source, truffles, decrease in abundance with increasing proximity to the clearcut edge of forest patches

(Mills 1995). When new edges were mowed in existing habitat, meadow voles showed no edge effect (Harper et al. 1993) or a slight preference for the edge (Nams 2012).

Whereas some studies have documented bird and large mammal responses to human trails, and others have observed species-specific responses of small mammals to other edges, the impact of recreational trails on small mammals is unknown. In this study, we examined the edge effects of trails on small mammals (< 0.5 kg) in a restored tallgrass prairie managed for conservation and recreation. Small mammals may be especially responsive to edge effects from trails because of their small size and vulnerability to terrestrial and aerial predators. Small mammals may avoid trails if they perceive them as barriers or unfavorable habitats, and these responses may vary with species.

We hypothesized that (1) small mammal activity would vary with respect to distance from recreational trails, (2) the presence or strength of this effect would vary among species, and (3) diurnal species would show the strongest edge avoidance.

Methods

Study site description

The Carleton College Cowling Arboretum (Northfield, MN, U.S.A., 44°28'N, 93°09'W) contained 155 ha of contiguous tallgrass prairie planted following conversion from agriculture between 1995 and 2008. The prairie was bordered by deciduous forest to the west and north, an agricultural field to the east (corn and soybean planted in annual rotation), and a state highway to the south (one lane in each direction, speed limit 45 mph). Over 100 plant species occur in the prairie, of which about 35 have >1% cover (Hernández et al. 2013). Previous studies at this site have examined the impacts of forest edge on white-tailed deer browsing (Nisi et al. 2015) and grassland bird distributions (Beck et al. 2016), but ours was the first study to examine the edge effects of recreational trails on animal movement. The relatively large size of this restoration and prevalence of recreational trails made this an ideal location to examine the impacts of trails on grassland small mammal activity.

The mammalian herbivore assemblage was a mix of prairie specialists (e.g., thirteen-lined ground squirrels [*Ictidomys tridecemlineatus*], plains pocket gophers [*Geomys bursarius*], and prairie voles [*Microtus ochrogaster*]) and generalists (*e.g.*, meadow voles, prairie deer mice [*Peromyscus maniculatus bairdii*], white-tailed deer [*Odocoileus virginianus*] and Eastern cottontail rabbits [*Sylvilagus floridanus*]) (Nisi et al. 2015). Predators of small mammals were common, and included red-tailed hawks (*Buteo jamaicensis*), great-horned owls (*Bubo virginianus*), barred owls (*Strix varia*), long-tailed weasels (*Mustela frenata*), short-tailed weasels (*Mustela ermine*), red foxes (*Vulpes vulpes*), coyotes, and feral house cats. Estimated home ranges for the focal species exhibit broad variation even within taxa, but movement of individuals is commonly greater than 20 m for *M. pennsylvanicus*, greater than 60 m for *P. maniculatus*, and greater than 100 m for *I. tridecemlineatus* (Getz 1961, Wood et al. 2010, Rongstad 1965).

Figure 1. A recreational trail through restored tallgrass prairie in the Cowling Arboretum at Carleton College, Northfield, Minnesota, USA.

The Arboretum contained a network of trails that were open to the public, most commonly used for walking and running. The trails consisted of dirt or gravel vehicle tracks in grass mowed to a width of 3–5 m (Fig. 1). Off-leash dogs, biking, and unofficial vehicles were not allowed, though the land managers drove on the trails with a pick-up truck and a small all-terrain utility vehicle several times per week.

Experimental design and methods

We measured animal activity using motion-sensing digital infrared cameras. We used cameras instead of live traps because we were primarily interested in whether activity, rather than presence or absence, was affected by the proximity of recreational trails. Camera stations were located along transects perpendicular to the trails, with stations at 0, 2, 4, 8, 16, and 64 m from the trail. Data was not collected from all distances for all transects due to occasional camera failures. To minimize the possibility of edge effects from habitat features other than trails, we placed transects in a core area of the prairie, beyond 100 m of forested areas, agricultural fields, roads, or other trails (Fig. 2). We set 12 unique transects in 2014 and re-sampled from 5 of those in 2015. Cameras were deployed along one transect at a time for approximately 48 h at each transect. Thus, each transect location represents an independent observation occurring at a unique time.

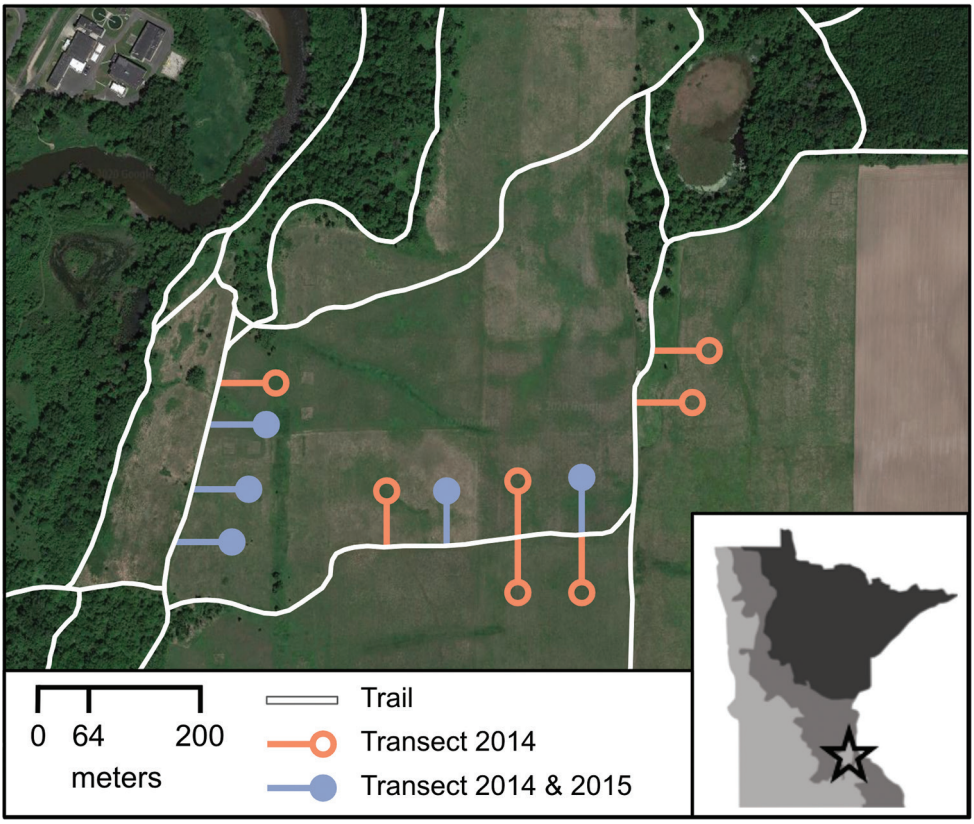

Figure 2. Approximate location of camera station transects (colored lines capped with circles) in relation to recreational trails (white lines) in the Cowling Arboretum at Carleton College, Northfield, Minnesota, USA, 2014-2015. Transect symbols have been elongated for visibility and are not to scale. The farthest camera station on each transect was always at least 64 m from any other trail or transect. Cameras and bait were only stationed along one transect at a time. Inset map shows the location of the Arboretum (star) in relation to Minnesota's major vegetative zones: conifer-hardwood zone (dark gray), deciduous forest-woodland zone (medium gray), and prairie zone (light gray).

At each station, we cleared a patch of vegetation about 50 × 80 cm with electric clippers or hand shears in order to ensure clear images of the animals (Fig. 3). At the edge of the clearing, we drove 2 rebar stakes into the ground, to which we attached a digital infrared camera (RECONYX RapidFire RM45, Holmen, WI, USA with SanDisk CF memory card, Milpitas, CA, USA) such that the lens was ~25 cm off the ground and its field of view was angled ~30° below horizontal (Fig. 3). Cameras were set at their default settings, programmed to take one black-and-white picture per second for 3 consecutive seconds following a motion trigger. Continued movement triggered further pictures with no lag time. Cameras operated continuously for the approximately 48-h sampling period at each transect.

We baited the stations with seeds of native prairie species common to the Arboretum: *Silphium laciniatum* (Asteraceae), *Desmodium canadense*, and *Dalea purpurea*

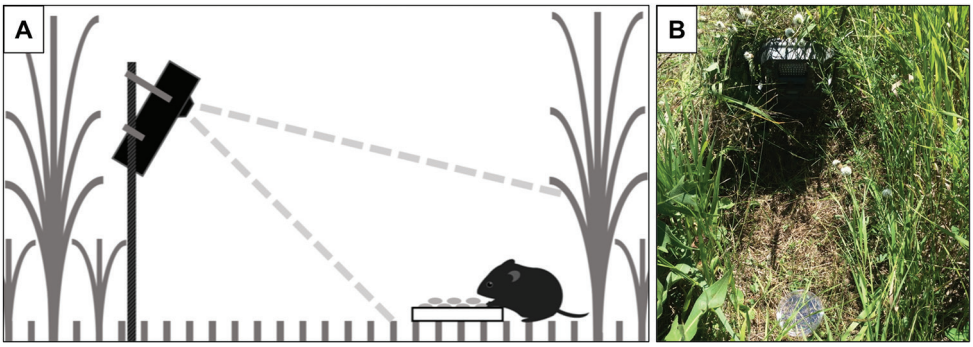

Figure 3. A Schematic of camera station seen from the side, depicting patch of cleared vegetation; digital infrared camera, zip-tied to rebar stakes, with its field of view angled approximately 30 degrees below horizontal (dashed lines represent approximate field of view); and petri dish of native prairie seeds for bait. Schematic not to scale **B** photo of camera station seen from above. Stations were deployed 2014–2015 in Northfield, Minnesota, USA, 2014–2015.

Vent. (Fabaceae)] (seeds were purchased from Prairie Moon Nursery, Winona, MN, USA). We autoclaved the seeds to prevent germination of non-local strains in the prairie planting. We mixed the seeds in roughly equal proportions, put them in petri dishes (roughly enough seeds to cover the bottom of the dish), and set one dish 50 cm in front of each camera. We did not collect data during periods of rain (4 d in July 2015, 6 d in August 2014, 6 d in August 2015) because in trial studies we observed that it influenced the effectiveness of the bait by washing it out of the petri dish.

We measured animal activity by counting all visits of each species to each camera at each transect, where a "visit" is defined as a set of 3 photos following a motion trigger, in which an animal appeared in at least one. Therefore, an animal feeding at the bait dish and triggering the camera multiple times counted for more "visits" than one that quickly passed in front of the camera. Thus, visit count is an approximate measure of the time members of each species spent at the bait station, rather than the number of unique occurrences. When a camera captured 2 animals simultaneously, we counted that photo set as 2 visits. Otherwise, we made no effort to distinguish between individuals. In our analyses, we controlled for differences in camera deployment times by calculating activity as the number of sightings per 24 h ([number of visits over deployment period]/[(hours the camera was deployed)/24]).

It is possible activity levels at the camera stations were different than those at undisturbed points in the prairie. Animals may have been attracted by the bait or repelled by the cut vegetation, the smell of humans, or the cameras and petri dishes. However, our study examined the relative activity levels at different distances, not the absolute activity level, and the degree of disturbance caused by the camera stations did not differ with respect to distance from trail.

The photos allowed for clear distinction among thirteen-lined ground squirrels, voles, and mice, but did not always allow for identification of species within the latter two taxa because of body orientation (e.g., tail not visible) or blurriness of the photo.

Based on previous live-trapping in the Arboretum prairie, voles are predominantly meadow voles, though prairie voles have occasionally been found in live traps (D. L. Hernández, personal observation). The local mouse species are prairie deer mouse, western harvest mouse (*Reithrodontomys megalotis*), meadow jumping mouse (*Zapus hudsonius*), and white-footed mouse (*Peromyscus leucopus*) (Angell and Braker 2010, Angell and Braker 2012). When we could not positively identify the animal in the photo, we excluded the visit from our analyses (< 1% of all visits).

Data analysis

For each taxon, we conducted a Kruskal-Wallis test for differences in activity with respect to distance from trail (m). We could not use an ANOVA because the data were not normally distributed. To avoid any potential issues with double counting, we aggregated data for points along transects that were used in both years (average weighted by the length of camera deployment in each year). We did not use a repeated measures approach because only 5 of the 12 transects were repeated in both years. When a Kruskal-Wallis test yielded a significant result ($P < 0.05$), we conducted a Dunn's test to identify which distances had significantly different activity while accounting for multiple comparisons. All statistics were done in R (R Version 3.3.2 with STATS package, www.r-project.org, accessed 30 Jun 2018).

Results

Trail edge effects on prairie rodent activity were apparent but varied among the species observed. Over 2 study seasons, our camera stations (n = 57 in 2014, n = 26 in 2015) captured 4358 visits by the focal taxa: 557 by mice (9.0 sightings station^{-1} in 2014; 1.7 sightings station^{-1} in 2015), 2494 by thirteen-lined ground squirrels (27; 36), and 1307 by voles (14; 20) (Fig. 3; Appendix 1: Table A1. In addition to the focal taxa, cameras occasionally captured pictures of Eastern cottontail rabbits, shrews (Soricidae), weasels, sparrows (Emberizidae), striped skunks, raccoons (*Procyon lotor*), an Eastern chipmunk (*Tamias striatus*), a Virginia opossum, and a domestic dog. Collectively, these non-focal taxa made up approximately 6% of the total visits (Appendix 1: Table A2). The sample size was too low to draw any meaningful conclusions about the distribution of these taxa. The influence of distance from the trail was not significant for either mouse activity ($\chi_5^2 = 1.03$, $P = 0.960$) or ground squirrel activity ($\chi_5^2 = 0.860$, $P = 0.973$). In contrast, vole activity was affected by distance from the trail ($\chi_5^2 = 16.0$, $P = 0.007$).

Over the 2-year study, we recorded only 13 visits by voles at 0 m (Fig. 4, Appendix 1: Table A1, available online in Supporting Information). All of the other distances had more than 130 recorded vole visits over the study period, with greater than 200 visits at 16 m and 64 m (Appendix 1: Table A1, Fig. 4). Vole activity at 0 m (average 0.474 visits day^{-1}) was less than at 16 m (20.7 visits day^{-1}, $P = 0.003$) and marginally significantly

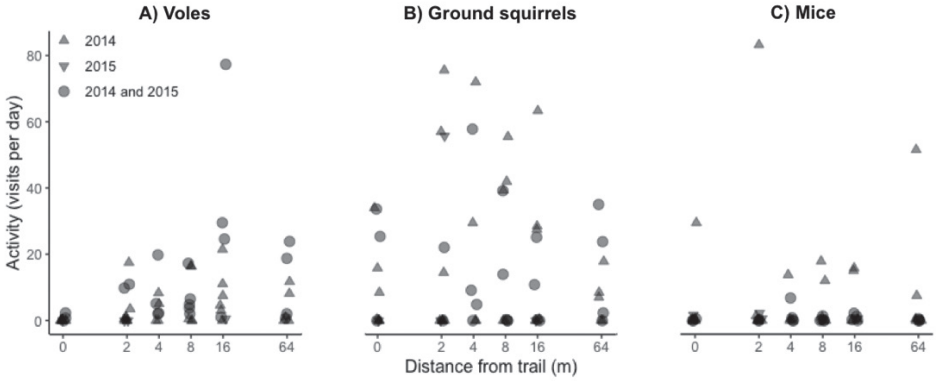

Figure 4. Small mammal activity at camera stations (visits day^{-1}) by distance (m) from recreational trails in Northfield, Minnesota, USA, 2014-2015. Panels show activity levels of **A** voles (*Microtus* spp.) **B** thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), and **C** mice (Cricetidae and Dipodidae). A "visit" is defined as a motion-triggered set of 3 photos in which an animal appeared in at least one photo. Observations were made at 57 camera stations over 12 transects in 2014 and 26 camera stations over 5 transects in 2015. Each data point represents the average activity at a camera station across the total observation period (approximately 48 hours when observed in one year and approximately 96 hours when observed in both years).

less than at 4 m (5.94 visits day^{-1}, $P = 0.078$), 8 m (6.74 visits day^{-1}, $P = 0.062$), and 64 m (8.49 visits day^{-1}, $P = 0.060$). These relationships were the same whether or not we combined data from the five transects that were repeated in both years. In 2014 alone (n = 12 transects), vole activity at 0 m was less than at all other distances except 2 m (0 – 4 m: $P = 0.041$; 0 – 8 m: $P = 0.020$; 0 – 16 m: $P = 0.005$; 0 – 64 m: $P = 0.046$). In 2015 alone (n=5 transects), vole activity did not differ with respect to distance from trail ($\chi_5^2 = 1.18$, $P = 0.946$).

Discussion

The edge effect of recreational trails was species-specific, affecting voles but not mice or ground squirrels. For voles, the effect was small in spatial extent but strong: camera stations immediately adjacent to trails never recorded more than 1 vole sighting per monitoring period (approximately 48 h). Our findings support our hypothesis that edge effects differ among species (Benítez-López et al. 2010, Debinski and Holt 2000, Miller and Hobbs 2000, Flaspohler et al. 2001, Pardini 2004, Reilly et al. 2017). Edge tolerance in white-footed mice has been found with road edges (Bissonette and Rosa 2009), but it is unknown whether all mouse species respond similarly to edges. Because photos were not high-enough resolution to differentiate among mouse species, it is possible there were additional species-specific differences in edge responses among mice that we did not detect.

We rejected our hypothesis that diurnal species would be more edge-averse than nocturnal species. Thirteen-lined ground squirrels showed no edge aversion despite being exclusively photographed during the day, when humans and dogs are most active on the trails. Voles were primarily nocturnal, largely eliminating the chance of direct disturbance by humans, yet strongly avoided the trail edge. Similarly, McGregor et al. (2008) found that white-footed mice and Eastern chipmunks avoided crossing roads but did not cross any more often in areas with low traffic, suggesting their behavior was not a direct response to human activity. Voles might be avoiding the scent of humans or domestic dogs, exposure to nocturnal mammalian predators using trails (Frey and Conover 2006, Harmsen et al. 2010, Reilly et al. 2017, Orrock and Danielson 2009), or exposure to owls (Kotler et al. 1991, Orrock and Danielson 2009).

Voles' edge aversion contradicts previous evidence that meadow voles may be edge-tolerant (Harper et al. 1993) or even edge-loving (Nams 2012). This may be due to the different nature of the edges in question: whereas these previous studies created new edges by mowing portions of the study area, our study focused on the impacts of trails that had been maintained for at least 9 years and consistently used by humans, vehicles, dogs, and possibly wild predators. This suggests the use of the trail may be more important than its physical structure.

Many studies of edge effects on small mammals have been conducted at much courser scales (e.g., Adams and Geiss 1983, Bock et al. 2002). By increasing our sampling intensity closer to the edge (including camera stations at 0 m, 2 m, and 4 m from the trail), we were able to detect an edge effect of <4 m – an effect size the studies above could not have detected. Even small edge effects could limit activity and thus species interactions near the edge, and we stress the importance of designing studies that can detect these fine-scale phenomena.

Although the majority of camera studies in recent years have focused on carnivores and ungulates (Burton et al. 2015, Reilly et al. 2017), cameras can be a powerful tool for studying small mammals, and have been used as such since the early days of animal-triggered remote photography (Kucera and Barrett 2011; see Gregory 1927, Gysel and Davis 1956, Pearson 1959, Osterberg 1962). While cameras cannot be used when marking individuals or the collection of tissue samples is required, camera traps can be left out in the field for longer than live traps, yielding more data per deployment. The disadvantages of cameras include the price, the possibility of malfunction, and the inability to distinguish very similar-looking species. Yet recent years have seen camera quality climb and price drop (O'Connell et al. 2011). In addition to technical advantages, camera traps offer a more humane alternative to live traps, which can stress, injure, or kill study subjects (Slade et al. 1993, Anthony et al. 2005). We hope to see their expanded use to conduct humane and detailed research on small animals in the field.

If small mammals avoid trail edges, trails could disproportionately reduce the size of their functional habitat, in turn affecting the connectivity and distribution of their populations. This would put recreation at odds with conservation in lands managed for both purposes. Fortunately, we found little to no evidence of conservation threats posed by trails in the study site. Edge effects were small in scale (<4m) and limited to meadow

voles, a species of least concern (Cassola 2017). However, because similarly small and species-specific effects could exist elsewhere, small mammal conservation efforts should be supported by fine-scale and species-specific research into potential trail edge effects. Managers should also consider that reducing foot or vehicle traffic may not be sufficient to limit the effect of trails (or roads; see McGregor et al. 2008), given that the voles in our study were primarily nocturnal and therefore not responding directly to humans.

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Appendix 1

Table A1. Average mammal activity level [visits/(d of observation)] at baited camera stations at varying distances from recreational trails (m) in Minnesota, USA, in 2014 and 2015. Where points in the same location were used in two years, activity is listed as the average activity of the two years, weighted by the observation period in each year. See Table A2for disaggregated observations listed independently in each year. A "visit" is defined as a motion-triggered set of 3 photos in which an animal appeared in at least one photo. The first four digits of transect codes refer to the restoration year of the prairie in that location, not the year in which data were collected.

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	1999.1	1.87	0	Chipmunk	0.00
2014	2003.1	2.00	0	Chipmunk	0.00
2014	2004.1	1.96	0	Chipmunk	0.00
2014	2004.2	2.00	0	Chipmunk	0.00
2014	2005.1	2.00	0	Chipmunk	0.00
2014	2008.1	1.96	0	Chipmunk	0.00
2014	2008.2	1.96	0	Chipmunk	0.00
2014 and 2015	1999.2	3.89	0	Chipmunk	0.00
2014 and 2015	2003.2	3.93	0	Chipmunk	0.00
2014 and 2015	2005.2	3.98	0	Chipmunk	0.00
2015	1998.1	1.83	0	Chipmunk	0.00
2014	1998.2	2.17	2	Chipmunk	0.00
2014	1999.2	2.04	2	Chipmunk	0.00
2014	2003.1	2.00	2	Chipmunk	0.00
2014	2004.1	1.96	2	Chipmunk	0.00
2014	2004.2	2.00	2	Chipmunk	0.00
2014	2005.1	2.00	2	Chipmunk	0.00
2014	2008.1	1.96	2	Chipmunk	0.00
2014	2008.2	1.96	2	Chipmunk	0.00
2015	2003.2	1.97	2	Chipmunk	0.00
2014 and 2015	1998.1	3.83	2	Chipmunk	0.00
2014 and 2015	2005.2	3.98	2	Chipmunk	0.00
2015	1999.1	1.82	2	Chipmunk	0.00
2014	1999.2	2.04	4	Chipmunk	0.00
2014	2003.1	2.00	4	Chipmunk	0.00
2014	2004.1	1.96	4	Chipmunk	0.00
2014	2008.1	1.96	4	Chipmunk	0.00
2014 and 2015	1998.1	3.83	4	Chipmunk	0.26
2014 and 2015	1999.1	3.69	4	Chipmunk	0.00
2014 and 2015	2003.2	3.93	4	Chipmunk	0.00
2014 and 2015	2005.2	3.98	4	Chipmunk	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	2003.1	2.00	8	Chipmunk	0.00
2014	2004.1	1.96	8	Chipmunk	0.00
2014	2004.2	2.00	8	Chipmunk	0.00
2014	2005.1	2.00	8	Chipmunk	0.00
2014	2008.1	1.96	8	Chipmunk	0.00
2014 and 2015	1998.1	3.83	8	Chipmunk	0.00
2014 and 2015	1999.2	3.89	8	Chipmunk	0.00
2014 and 2015	1999.1	3.69	8	Chipmunk	0.00
2014 and 2015	2003.2	3.93	8	Chipmunk	0.00
2014 and 2015	2005.2	3.98	8	Chipmunk	0.00
2014	1998.2	2.17	16	Chipmunk	0.00
2014	2003.1	2.00	16	Chipmunk	0.00
2014	2004.1	1.96	16	Chipmunk	0.00
2014	2004.2	2.00	16	Chipmunk	0.00
2014	2005.1	2.00	16	Chipmunk	0.00
2014	2008.1	1.96	16	Chipmunk	0.00
2014 and 2015	1999.2	3.89	16	Chipmunk	0.00
2014 and 2015	1999.1	3.69	16	Chipmunk	0.00
2014 and 2015	2003.2	3.93	16	Chipmunk	0.00
2014 and 2015	2005.2	3.98	16	Chipmunk	0.00
2015	1998.1	1.83	16	Chipmunk	0.00
2014	2003.1	2.00	64	Chipmunk	0.00
2014	2004.1	1.96	64	Chipmunk	0.00
2014	2004.2	2.00	64	Chipmunk	0.00
2014	2005.1	2.00	64	Chipmunk	0.00
2014	2008.1	1.96	64	Chipmunk	0.00
2014 and 2015	1998.1	3.83	64	Chipmunk	0.00
2014 and 2015	1999.2	3.89	64	Chipmunk	0.00
2014 and 2015	2003.2	3.93	64	Chipmunk	0.00
2014 and 2015	2005.2	3.98	64	Chipmunk	0.00
2014	1999.1	1.87	0	Dog	0.00
2014	2003.1	2.00	0	Dog	0.00
2014	2004.1	1.96	0	Dog	0.00
2014	2004.2	2.00	0	Dog	0.00
2014	2005.1	2.00	0	Dog	0.00
2014	2008.1	1.96	0	Dog	0.00
2014	2008.2	1.96	0	Dog	0.00
2014 and 2015	1999.2	3.89	0	Dog	0.00
2014 and 2015	2003.2	3.93	0	Dog	0.25
2014 and 2015	2005.2	3.98	0	Dog	0.00
2015	1998.1	1.83	0	Dog	0.00
2014	1998.2	2.17	2	Dog	0.00
2014	1999.2	2.04	2	Dog	0.00
2014	2003.1	2.00	2	Dog	0.00
2014	2004.1	1.96	2	Dog	0.00
2014	2004.2	2.00	2	Dog	0.00
2014	2005.1	2.00	2	Dog	0.00
2014	2008.1	1.96	2	Dog	0.00
2014	2008.2	1.96	2	Dog	0.00
2015	2003.2	1.97	2	Dog	0.00
2014 and 2015	1998.1	3.83	2	Dog	0.00
2014 and 2015	2005.2	3.98	2	Dog	0.00
2015	1999.1	1.82	2	Dog	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	1999.2	2.04	4	Dog	0.00
2014	2003.1	2.00	4	Dog	0.00
2014	2004.1	1.96	4	Dog	0.00
2014	2008.1	1.96	4	Dog	0.00
2014 and 2015	1998.1	3.83	4	Dog	0.00
2014 and 2015	1999.1	3.69	4	Dog	0.00
2014 and 2015	2003.2	3.93	4	Dog	0.00
2014 and 2015	2005.2	3.98	4	Dog	0.00
2014	2003.1	2.00	8	Dog	0.00
2014	2004.1	1.96	8	Dog	0.00
2014	2004.2	2.00	8	Dog	0.00
2014	2005.1	2.00	8	Dog	0.00
2014	2008.1	1.96	8	Dog	0.00
2014 and 2015	1998.1	3.83	8	Dog	0.00
2014 and 2015	1999.2	3.89	8	Dog	0.00
2014 and 2015	1999.1	3.69	8	Dog	0.00
2014 and 2015	2003.2	3.93	8	Dog	0.00
2014 and 2015	2005.2	3.98	8	Dog	0.00
2014	1998.2	2.17	16	Dog	0.00
2014	2003.1	2.00	16	Dog	0.00
2014	2004.1	1.96	16	Dog	0.00
2014	2004.2	2.00	16	Dog	0.00
2014	2005.1	2.00	16	Dog	0.00
2014	2008.1	1.96	16	Dog	0.00
2014 and 2015	1999.2	3.89	16	Dog	0.00
2014 and 2015	1999.1	3.69	16	Dog	0.00
2014 and 2015	2003.2	3.93	16	Dog	0.00
2014 and 2015	2005.2	3.98	16	Dog	0.00
2015	1998.1	1.83	16	Dog	0.00
2014	2003.1	2.00	64	Dog	0.00
2014	2004.1	1.96	64	Dog	0.00
2014	2004.2	2.00	64	Dog	0.00
2014	2005.1	2.00	64	Dog	0.00
2014	2008.1	1.96	64	Dog	0.00
2014 and 2015	1998.1	3.83	64	Dog	0.00
2014 and 2015	1999.2	3.89	64	Dog	0.00
2014 and 2015	2003.2	3.93	64	Dog	0.00
2014 and 2015	2005.2	3.98	64	Dog	0.00
2014	1999.1	1.87	0	Ground squirrel	0.00
2014	2003.1	2.00	0	Ground squirrel	8.50
2014	2004.1	1.96	0	Ground squirrel	15.83
2014	2004.2	2.00	0	Ground squirrel	34.00
2014	2005.1	2.00	0	Ground squirrel	0.50
2014	2008.1	1.96	0	Ground squirrel	0.00
2014	2008.2	1.96	0	Ground squirrel	0.00
2014 and 2015	1999.2	3.89	0	Ground squirrel	0.00
2014 and 2015	2003.2	3.93	0	Ground squirrel	25.40
2014 and 2015	2005.2	3.98	0	Ground squirrel	33.67
2015	1998.1	1.83	0	Ground squirrel	0.00
2014	1998.2	2.17	2	Ground squirrel	0.00
2014	1999.2	2.04	2	Ground squirrel	0.00
2014	2003.1	2.00	2	Ground squirrel	57.00
2014	2004.1	1.96	2	Ground squirrel	75.57

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	2004.2	2.00	2	Ground squirrel	14.50
2014	2005.1	2.00	2	Ground squirrel	0.00
2014	2008.1	1.96	2	Ground squirrel	0.00
2014	2008.2	1.96	2	Ground squirrel	0.00
2015	2003.2	1.97	2	Ground squirrel	55.73
2014 and 2015	1998.1	3.83	2	Ground squirrel	0.00
2014 and 2015	2005.2	3.98	2	Ground squirrel	22.08
2015	1999.1	1.82	2	Ground squirrel	0.00
2014	1999.2	2.04	4	Ground squirrel	0.00
2014	2003.1	2.00	4	Ground squirrel	29.50
2014	2004.1	1.96	4	Ground squirrel	72.00
2014	2008.1	1.96	4	Ground squirrel	0.00
2014 and 2015	1998.1	3.83	4	Ground squirrel	0.00
2014 and 2015	1999.1	3.69	4	Ground squirrel	4.88
2014 and 2015	2003.2	3.93	4	Ground squirrel	9.14
2014 and 2015	2005.2	3.98	4	Ground squirrel	57.82
2014	2003.1	2.00	8	Ground squirrel	42.00
2014	2004.1	1.96	8	Ground squirrel	39.32
2014	2004.2	2.00	8	Ground squirrel	55.50
2014	2005.1	2.00	8	Ground squirrel	0.00
2014	2008.1	1.96	8	Ground squirrel	0.00
2014 and 2015	1998.1	3.83	8	Ground squirrel	0.00
2014 and 2015	1999.2	3.89	8	Ground squirrel	0.00
2014 and 2015	1999.1	3.69	8	Ground squirrel	0.27
2014 and 2015	2003.2	3.93	8	Ground squirrel	13.97
2014 and 2015	2005.2	3.98	8	Ground squirrel	39.20
2014	1998.2	2.17	16	Ground squirrel	0.00
2014	2003.1	2.00	16	Ground squirrel	28.50
2014	2004.1	1.96	16	Ground squirrel	63.32
2014	2004.2	2.00	16	Ground squirrel	27.50
2014	2005.1	2.00	16	Ground squirrel	0.50
2014	2008.1	1.96	16	Ground squirrel	0.00
2014 and 2015	1999.2	3.89	16	Ground squirrel	0.26
2014 and 2015	1999.1	3.69	16	Ground squirrel	10.85
2014 and 2015	2003.2	3.93	16	Ground squirrel	0.00
2014 and 2015	2005.2	3.98	16	Ground squirrel	25.15
2015	1998.1	1.83	16	Ground squirrel	0.00
2014	2003.1	2.00	64	Ground squirrel	8.50
2014	2004.1	1.96	64	Ground squirrel	17.87
2014	2004.2	2.00	64	Ground squirrel	7.00
2014	2005.1	2.00	64	Ground squirrel	0.00
2014	2008.1	1.96	64	Ground squirrel	0.00
2014 and 2015	1998.1	3.83	64	Ground squirrel	0.00
2014 and 2015	1999.2	3.89	64	Ground squirrel	2.31
2014 and 2015	2003.2	3.93	64	Ground squirrel	35.05
2014 and 2015	2005.2	3.98	64	Ground squirrel	23.82
2014	1999.1	1.87	0	Mouse	0.00
2014	2003.1	2.00	0	Mouse	0.00
2014	2004.1	1.96	0	Mouse	0.00
2014	2004.2	2.00	0	Mouse	29.50
2014	2005.1	2.00	0	Mouse	0.50
2014	2008.1	1.96	0	Mouse	0.00
2014	2008.2	1.96	0	Mouse	0.51

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014 and 2015	1999.2	3.89	0	Mouse	0.51
2014 and 2015	2003.2	3.93	0	Mouse	0.00
2014 and 2015	2005.2	3.98	0	Mouse	0.00
2015	1998.1	1.83	0	Mouse	1.64
2014	1998.2	2.17	2	Mouse	1.38
2014	1999.2	2.04	2	Mouse	0.00
2014	2003.1	2.00	2	Mouse	0.00
2014	2004.1	1.96	2	Mouse	83.23
2014	2004.2	2.00	2	Mouse	0.50
2014	2005.1	2.00	2	Mouse	0.00
2014	2008.1	1.96	2	Mouse	0.00
2014	2008.2	1.96	2	Mouse	0.00
2015	2003.2	1.97	2	Mouse	0.51
2014 and 2015	1998.1	3.83	2	Mouse	0.00
2014 and 2015	2005.2	3.98	2	Mouse	0.00
2015	1999.1	1.82	2	Mouse	2.20
2014	1999.2	2.04	4	Mouse	0.00
2014	2003.1	2.00	4	Mouse	0.00
2014	2004.1	1.96	4	Mouse	13.79
2014	2008.1	1.96	4	Mouse	0.51
2014 and 2015	1998.1	3.83	4	Mouse	0.78
2014 and 2015	1999.1	3.69	4	Mouse	6.78
2014 and 2015	2003.2	3.93	4	Mouse	0.00
2014 and 2015	2005.2	3.98	4	Mouse	0.00
2014	2003.1	2.00	8	Mouse	0.00
2014	2004.1	1.96	8	Mouse	17.87
2014	2004.2	2.00	8	Mouse	12.00
2014	2005.1	2.00	8	Mouse	0.00
2014	2008.1	1.96	8	Mouse	1.02
2014 and 2015	1998.1	3.83	8	Mouse	0.00
2014 and 2015	1999.2	3.89	8	Mouse	0.00
2014 and 2015	1999.1	3.69	8	Mouse	1.35
2014 and 2015	2003.2	3.93	8	Mouse	0.00
2014 and 2015	2005.2	3.98	8	Mouse	0.25
2014	1998.2	2.17	16	Mouse	0.00
2014	2003.1	2.00	16	Mouse	0.00
2014	2004.1	1.96	16	Mouse	15.83
2014	2004.2	2.00	16	Mouse	15.00
2014	2005.1	2.00	16	Mouse	0.50
2014	2008.1	1.96	16	Mouse	0.00
2014 and 2015	1999.2	3.89	16	Mouse	0.00
2014 and 2015	1999.1	3.69	16	Mouse	2.16
2014 and 2015	2003.2	3.93	16	Mouse	0.25
2014 and 2015	2005.2	3.98	16	Mouse	1.26
2015	1998.1	1.83	16	Mouse	0.55
2014	2003.1	2.00	64	Mouse	0.00
2014	2004.1	1.96	64	Mouse	51.57
2014	2004.2	2.00	64	Mouse	7.50
2014	2005.1	2.00	64	Mouse	0.00
2014	2008.1	1.96	64	Mouse	0.51
2014 and 2015	1998.1	3.83	64	Mouse	0.00
2014 and 2015	1999.2	3.89	64	Mouse	0.51
2014 and 2015	2003.2	3.93	64	Mouse	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014 and 2015	2005.2	3.98	64	Mouse	0.00
2014	1999.1	1.87	0	Opossum	0.00
2014	2003.1	2.00	0	Opossum	0.00
2014	2004.1	1.96	0	Opossum	0.00
2014	2004.2	2.00	0	Opossum	0.00
2014	2005.1	2.00	0	Opossum	0.00
2014	2008.1	1.96	0	Opossum	0.00
2014	2008.2	1.96	0	Opossum	0.00
2014 and 2015	1999.2	3.89	0	Opossum	0.00
2014 and 2015	2003.2	3.93	0	Opossum	0.00
2014 and 2015	2005.2	3.98	0	Opossum	0.00
2015	1998.1	1.83	0	Opossum	3.82
2014	1998.2	2.17	2	Opossum	0.00
2014	1999.2	2.04	2	Opossum	0.00
2014	2003.1	2.00	2	Opossum	0.00
2014	2004.1	1.96	2	Opossum	0.00
2014	2004.2	2.00	2	Opossum	0.00
2014	2005.1	2.00	2	Opossum	0.00
2014	2008.1	1.96	2	Opossum	0.00
2014	2008.2	1.96	2	Opossum	0.00
2015	2003.2	1.97	2	Opossum	0.00
2014 and 2015	1998.1	3.83	2	Opossum	0.00
2014 and 2015	2005.2	3.98	2	Opossum	0.00
2015	1999.1	1.82	2	Opossum	0.00
2014	1999.2	2.04	4	Opossum	0.00
2014	2003.1	2.00	4	Opossum	0.00
2014	2004.1	1.96	4	Opossum	0.00
2014	2008.1	1.96	4	Opossum	0.00
2014 and 2015	1998.1	3.83	4	Opossum	0.00
2014 and 2015	1999.1	3.69	4	Opossum	0.00
2014 and 2015	2003.2	3.93	4	Opossum	0.00
2014 and 2015	2005.2	3.98	4	Opossum	0.00
2014	2003.1	2.00	8	Opossum	0.00
2014	2004.1	1.96	8	Opossum	0.00
2014	2004.2	2.00	8	Opossum	0.00
2014	2005.1	2.00	8	Opossum	0.00
2014	2008.1	1.96	8	Opossum	0.00
2014 and 2015	1998.1	3.83	8	Opossum	0.00
2014 and 2015	1999.2	3.89	8	Opossum	0.00
2014 and 2015	1999.1	3.69	8	Opossum	0.00
2014 and 2015	2003.2	3.93	8	Opossum	0.00
2014 and 2015	2005.2	3.98	8	Opossum	0.00
2014	1998.2	2.17	16	Opossum	0.00
2014	2003.1	2.00	16	Opossum	0.00
2014	2004.1	1.96	16	Opossum	0.00
2014	2004.2	2.00	16	Opossum	0.00
2014	2005.1	2.00	16	Opossum	0.00
2014	2008.1	1.96	16	Opossum	0.00
2014 and 2015	1999.2	3.89	16	Opossum	0.00
2014 and 2015	1999.1	3.69	16	Opossum	0.00
2014 and 2015	2003.2	3.93	16	Opossum	0.00
2014 and 2015	2005.2	3.98	16	Opossum	0.00
2015	1998.1	1.83	16	Opossum	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	2003.1	2.00	64	Opossum	0.00
2014	2004.1	1.96	64	Opossum	0.00
2014	2004.2	2.00	64	Opossum	0.00
2014	2005.1	2.00	64	Opossum	0.00
2014	2008.1	1.96	64	Opossum	0.00
2014 and 2015	1998.1	3.83	64	Opossum	0.00
2014 and 2015	1999.2	3.89	64	Opossum	0.00
2014 and 2015	2003.2	3.93	64	Opossum	0.00
2014 and 2015	2005.2	3.98	64	Opossum	0.00
2014	1999.1	1.87	0	Rabbit	0.00
2014	2003.1	2.00	0	Rabbit	0.00
2014	2004.1	1.96	0	Rabbit	0.00
2014	2004.2	2.00	0	Rabbit	2.50
2014	2005.1	2.00	0	Rabbit	0.00
2014	2008.1	1.96	0	Rabbit	0.00
2014	2008.2	1.96	0	Rabbit	0.00
2014 and 2015	1999.2	3.89	0	Rabbit	0.00
2014 and 2015	2003.2	3.93	0	Rabbit	1.52
2014 and 2015	2005.2	3.98	0	Rabbit	0.00
2015	1998.1	1.83	0	Rabbit	0.00
2014	1998.2	2.17	2	Rabbit	0.00
2014	1999.2	2.04	2	Rabbit	0.00
2014	2003.1	2.00	2	Rabbit	0.00
2014	2004.1	1.96	2	Rabbit	4.60
2014	2004.2	2.00	2	Rabbit	0.00
2014	2005.1	2.00	2	Rabbit	0.00
2014	2008.1	1.96	2	Rabbit	0.00
2014	2008.2	1.96	2	Rabbit	0.00
2015	2003.2	1.97	2	Rabbit	0.00
2014 and 2015	1998.1	3.83	2	Rabbit	0.00
2014 and 2015	2005.2	3.98	2	Rabbit	0.00
2015	1999.1	1.82	2	Rabbit	0.00
2014	1999.2	2.04	4	Rabbit	0.00
2014	2003.1	2.00	4	Rabbit	0.00
2014	2004.1	1.96	4	Rabbit	0.00
2014	2008.1	1.96	4	Rabbit	0.00
2014 and 2015	1998.1	3.83	4	Rabbit	0.00
2014 and 2015	1999.1	3.69	4	Rabbit	0.00
2014 and 2015	2003.2	3.93	4	Rabbit	0.00
2014 and 2015	2005.2	3.98	4	Rabbit	0.00
2014	2003.1	2.00	8	Rabbit	0.00
2014	2004.1	1.96	8	Rabbit	0.51
2014	2004.2	2.00	8	Rabbit	0.00
2014	2005.1	2.00	8	Rabbit	0.00
2014	2008.1	1.96	8	Rabbit	0.00
2014 and 2015	1998.1	3.83	8	Rabbit	0.00
2014 and 2015	1999.2	3.89	8	Rabbit	0.00
2014 and 2015	1999.1	3.69	8	Rabbit	0.00
2014 and 2015	2003.2	3.93	8	Rabbit	0.00
2014 and 2015	2005.2	3.98	8	Rabbit	0.00
2014	1998.2	2.17	16	Rabbit	0.00
2014	2003.1	2.00	16	Rabbit	0.00
2014	2004.1	1.96	16	Rabbit	19.40

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	2004.2	2.00	16	Rabbit	0.00
2014	2005.1	2.00	16	Rabbit	0.00
2014	2008.1	1.96	16	Rabbit	0.00
2014 and 2015	1999.2	3.89	16	Rabbit	0.00
2014 and 2015	1999.1	3.69	16	Rabbit	0.00
2014 and 2015	2003.2	3.93	16	Rabbit	6.35
2014 and 2015	2005.2	3.98	16	Rabbit	0.00
2015	1998.1	1.83	16	Rabbit	0.00
2014	2003.1	2.00	64	Rabbit	0.00
2014	2004.1	1.96	64	Rabbit	0.00
2014	2004.2	2.00	64	Rabbit	0.00
2014	2005.1	2.00	64	Rabbit	0.00
2014	2008.1	1.96	64	Rabbit	0.00
2014 and 2015	1998.1	3.83	64	Rabbit	0.00
2014 and 2015	1999.2	3.89	64	Rabbit	0.00
2014 and 2015	2003.2	3.93	64	Rabbit	1.52
2014 and 2015	2005.2	3.98	64	Rabbit	0.00
2014	1999.1	1.87	0	Shrew	0.00
2014	2003.1	2.00	0	Shrew	0.00
2014	2004.1	1.96	0	Shrew	0.00
2014	2004.2	2.00	0	Shrew	0.00
2014	2005.1	2.00	0	Shrew	0.00
2014	2008.1	1.96	0	Shrew	0.00
2014	2008.2	1.96	0	Shrew	0.00
2014 and 2015	1999.2	3.89	0	Shrew	0.00
2014 and 2015	2003.2	3.93	0	Shrew	0.00
2014 and 2015	2005.2	3.98	0	Shrew	0.00
2015	1998.1	1.83	0	Shrew	0.00
2014	1998.2	2.17	2	Shrew	0.00
2014	1999.2	2.04	2	Shrew	0.00
2014	2003.1	2.00	2	Shrew	0.00
2014	2004.1	1.96	2	Shrew	0.00
2014	2004.2	2.00	2	Shrew	0.00
2014	2005.1	2.00	2	Shrew	0.00
2014	2008.1	1.96	2	Shrew	0.00
2014	2008.2	1.96	2	Shrew	0.00
2015	2003.2	1.97	2	Shrew	0.00
2014 and 2015	1998.1	3.83	2	Shrew	0.00
2014 and 2015	2005.2	3.98	2	Shrew	0.00
2015	1999.1	1.82	2	Shrew	0.00
2014	1999.2	2.04	4	Shrew	0.00
2014	2003.1	2.00	4	Shrew	0.00
2014	2004.1	1.96	4	Shrew	0.00
2014	2008.1	1.96	4	Shrew	0.00
2014 and 2015	1998.1	3.83	4	Shrew	1.30
2014 and 2015	1999.1	3.69	4	Shrew	0.00
2014 and 2015	2003.2	3.93	4	Shrew	0.00
2014 and 2015	2005.2	3.98	4	Shrew	0.00
2014	2003.1	2.00	8	Shrew	0.00
2014	2004.1	1.96	8	Shrew	0.00
2014	2004.2	2.00	8	Shrew	0.00
2014	2005.1	2.00	8	Shrew	0.00
2014	2008.1	1.96	8	Shrew	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014 and 2015	1998.1	3.83	8	Shrew	0.00
2014 and 2015	1999.2	3.89	8	Shrew	0.26
2014 and 2015	1999.1	3.69	8	Shrew	0.27
2014 and 2015	2003.2	3.93	8	Shrew	0.25
2014 and 2015	2005.2	3.98	8	Shrew	0.00
2014	1998.2	2.17	16	Shrew	0.00
2014	2003.1	2.00	16	Shrew	0.00
2014	2004.1	1.96	16	Shrew	0.00
2014	2004.2	2.00	16	Shrew	0.00
2014	2005.1	2.00	16	Shrew	0.00
2014	2008.1	1.96	16	Shrew	0.00
2014 and 2015	1999.2	3.89	16	Shrew	0.00
2014 and 2015	1999.1	3.69	16	Shrew	0.00
2014 and 2015	2003.2	3.93	16	Shrew	0.00
2014 and 2015	2005.2	3.98	16	Shrew	0.00
2015	1998.1	1.83	16	Shrew	4.37
2014	2003.1	2.00	64	Shrew	0.00
2014	2004.1	1.96	64	Shrew	0.00
2014	2004.2	2.00	64	Shrew	0.00
2014	2005.1	2.00	64	Shrew	0.00
2014	2008.1	1.96	64	Shrew	0.00
2014 and 2015	1998.1	3.83	64	Shrew	0.00
2014 and 2015	1999.2	3.89	64	Shrew	0.26
2014 and 2015	2003.2	3.93	64	Shrew	0.00
2014 and 2015	2005.2	3.98	64	Shrew	0.00
2014	1999.1	1.87	0	Total Carnivora	0.00
2014	2003.1	2.00	0	Total Carnivora	0.00
2014	2004.1	1.96	0	Total Carnivora	0.00
2014	2004.2	2.00	0	Total Carnivora	0.00
2014	2005.1	2.00	0	Total Carnivora	0.00
2014	2008.1	1.96	0	Total Carnivora	0.00
2014	2008.2	1.96	0	Total Carnivora	0.00
2014 and 2015	1999.2	3.89	0	Total Carnivora	0.00
2014 and 2015	2003.2	3.93	0	Total Carnivora	0.25
2014 and 2015	2005.2	3.98	0	Total Carnivora	0.00
2015	1998.1	1.83	0	Total Carnivora	3.82
2014	1998.2	2.17	2	Total Carnivora	0.00
2014	1999.2	2.04	2	Total Carnivora	0.00
2014	2003.1	2.00	2	Total Carnivora	0.00
2014	2004.1	1.96	2	Total Carnivora	0.00
2014	2004.2	2.00	2	Total Carnivora	0.50
2014	2005.1	2.00	2	Total Carnivora	2.50
2014	2008.1	1.96	2	Total Carnivora	0.00
2014	2008.2	1.96	2	Total Carnivora	0.00
2015	2003.2	1.97	2	Total Carnivora	0.00
2014 and 2015	1998.1	3.83	2	Total Carnivora	0.00
2014 and 2015	2005.2	3.98	2	Total Carnivora	1.01
2015	1999.1	1.82	2	Total Carnivora	0.00
2014	1999.2	2.04	4	Total Carnivora	0.00
2014	2003.1	2.00	4	Total Carnivora	0.00
2014	2004.1	1.96	4	Total Carnivora	0.00
2014	2008.1	1.96	4	Total Carnivora	0.00
2014 and 2015	1998.1	3.83	4	Total Carnivora	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014 and 2015	1999.1	3.69	4	Total Carnivora	0.00
2014 and 2015	2003.2	3.93	4	Total Carnivora	0.00
2014 and 2015	2005.2	3.98	4	Total Carnivora	0.00
2014	2003.1	2.00	8	Total Carnivora	0.00
2014	2004.1	1.96	8	Total Carnivora	0.00
2014	2004.2	2.00	8	Total Carnivora	0.00
2014	2005.1	2.00	8	Total Carnivora	2.00
2014	2008.1	1.96	8	Total Carnivora	0.00
2014 and 2015	1998.1	3.83	8	Total Carnivora	0.00
2014 and 2015	1999.2	3.89	8	Total Carnivora	0.00
2014 and 2015	1999.1	3.69	8	Total Carnivora	0.00
2014 and 2015	2003.2	3.93	8	Total Carnivora	0.00
2014 and 2015	2005.2	3.98	8	Total Carnivora	0.75
2014	1998.2	2.17	16	Total Carnivora	0.00
2014	2003.1	2.00	16	Total Carnivora	0.00
2014	2004.1	1.96	16	Total Carnivora	4.09
2014	2004.2	2.00	16	Total Carnivora	0.00
2014	2005.1	2.00	16	Total Carnivora	0.00
2014	2008.1	1.96	16	Total Carnivora	0.00
2014 and 2015	1999.2	3.89	16	Total Carnivora	0.00
2014 and 2015	1999.1	3.69	16	Total Carnivora	0.00
2014 and 2015	2003.2	3.93	16	Total Carnivora	0.00
2014 and 2015	2005.2	3.98	16	Total Carnivora	0.50
2015	1998.1	1.83	16	Total Carnivora	0.00
2014	2003.1	2.00	64	Total Carnivora	0.00
2014	2004.1	1.96	64	Total Carnivora	6.13
2014	2004.2	2.00	64	Total Carnivora	0.00
2014	2005.1	2.00	64	Total Carnivora	0.00
2014	2008.1	1.96	64	Total Carnivora	0.00
2014 and 2015	1998.1	3.83	64	Total Carnivora	0.00
2014 and 2015	1999.2	3.89	64	Total Carnivora	0.00
2014 and 2015	2003.2	3.93	64	Total Carnivora	0.25
2014 and 2015	2005.2	3.98	64	Total Carnivora	0.00
2014	1999.1	1.87	0	Vole	0.00
2014	2003.1	2.00	0	Vole	0.00
2014	2004.1	1.96	0	Vole	0.00
2014	2004.2	2.00	0	Vole	0.00
2014	2005.1	2.00	0	Vole	0.50
2014	2008.1	1.96	0	Vole	0.00
2014	2008.2	1.96	0	Vole	0.00
2014 and 2015	1999.2	3.89	0	Vole	2.31
2014 and 2015	2003.2	3.93	0	Vole	0.76
2014 and 2015	2005.2	3.98	0	Vole	0.00
2015	1998.1	1.83	0	Vole	0.00
2014	1998.2	2.17	2	Vole	17.54
2014	1999.2	2.04	2	Vole	0.00
2014	2003.1	2.00	2	Vole	0.00
2014	2004.1	1.96	2	Vole	1.02
2014	2004.2	2.00	2	Vole	3.50
2014	2005.1	2.00	2	Vole	1.00
2014	2008.1	1.96	2	Vole	0.51
2014	2008.2	1.96	2	Vole	0.00
2015	2003.2	1.97	2	Vole	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014 and 2015	1998.1	3.83	2	Vole	10.95
2014 and 2015	2005.2	3.98	2	Vole	9.82
2015	1999.1	1.82	2	Vole	0.00
2014	1999.2	2.04	4	Vole	8.33
2014	2003.1	2.00	4	Vole	0.00
2014	2004.1	1.96	4	Vole	0.00
2014	2008.1	1.96	4	Vole	5.11
2014 and 2015	1998.1	3.83	4	Vole	19.83
2014 and 2015	1999.1	3.69	4	Vole	5.14
2014 and 2015	2003.2	3.93	4	Vole	2.29
2014 and 2015	2005.2	3.98	4	Vole	2.01
2014	2003.1	2.00	8	Vole	0.00
2014	2004.1	1.96	8	Vole	0.00
2014	2004.2	2.00	8	Vole	0.50
2014	2005.1	2.00	8	Vole	16.50
2014	2008.1	1.96	8	Vole	16.34
2014 and 2015	1998.1	3.83	8	Vole	6.52
2014 and 2015	1999.2	3.89	8	Vole	2.06
2014 and 2015	1999.1	3.69	8	Vole	17.31
2014 and 2015	2003.2	3.93	8	Vole	4.84
2014 and 2015	2005.2	3.98	8	Vole	3.78
2014	1998.2	2.17	16	Vole	11.08
2014	2003.1	2.00	16	Vole	21.50
2014	2004.1	1.96	16	Vole	4.60
2014	2004.2	2.00	16	Vole	0.00
2014	2005.1	2.00	16	Vole	7.50
2014	2008.1	1.96	16	Vole	3.06
2014 and 2015	1999.2	3.89	16	Vole	77.34
2014 and 2015	1999.1	3.69	16	Vole	24.61
2014 and 2015	2003.2	3.93	16	Vole	29.54
2014 and 2015	2005.2	3.98	16	Vole	1.01
2015	1998.1	1.83	16	Vole	0.55
2014	2003.1	2.00	64	Vole	1.00
2014	2004.1	1.96	64	Vole	11.74
2014	2004.2	2.00	64	Vole	0.00
2014	2005.1	2.00	64	Vole	0.00
2014	2008.1	1.96	64	Vole	8.17
2014 and 2015	1998.1	3.83	64	Vole	18.78
2014 and 2015	1999.2	3.89	64	Vole	23.89
2014 and 2015	2003.2	3.93	64	Vole	2.04
2014 and 2015	2005.2	3.98	64	Vole	0.75
2014	1999.1	1.87	0	Weasel	0.00
2014	2003.1	2.00	0	Weasel	0.00
2014	2004.1	1.96	0	Weasel	0.00
2014	2004.2	2.00	0	Weasel	0.00
2014	2005.1	2.00	0	Weasel	0.00
2014	2008.1	1.96	0	Weasel	0.00
2014	2008.2	1.96	0	Weasel	0.00
2014 and 2015	1999.2	3.89	0	Weasel	0.00
2014 and 2015	2003.2	3.93	0	Weasel	0.00
2014 and 2015	2005.2	3.98	0	Weasel	0.00
2015	1998.1	1.83	0	Weasel	0.00
2014	1998.2	2.17	2	Weasel	0.00

Year	Transect	Observation period (d)	Distance (m)	Taxon	Activity (visits/day)
2014	1999.2	2.04	2	Weasel	0.00
2014	2003.1	2.00	2	Weasel	0.00
2014	2004.1	1.96	2	Weasel	0.00
2014	2004.2	2.00	2	Weasel	na
2014	2005.1	2.00	2	Weasel	na
2014	2008.1	1.96	2	Weasel	0.00
2014	2008.2	1.96	2	Weasel	0.00
2015	2003.2	1.97	2	Weasel	0.00
2014 and 2015	1998.1	3.83	2	Weasel	0.00
2014 and 2015	2005.2	3.98	2	Weasel	na
2015	1999.1	1.82	2	Weasel	0.00
2014	1999.2	2.04	4	Weasel	0.00
2014	2003.1	2.00	4	Weasel	0.00
2014	2004.1	1.96	4	Weasel	0.00
2014	2008.1	1.96	4	Weasel	0.00
2014 and 2015	1998.1	3.83	4	Weasel	0.00
2014 and 2015	1999.1	3.69	4	Weasel	0.00
2014 and 2015	2003.2	3.93	4	Weasel	0.00
2014 and 2015	2005.2	3.98	4	Weasel	0.00
2014	2003.1	2.00	8	Weasel	0.00
2014	2004.1	1.96	8	Weasel	0.00
2014	2004.2	2.00	8	Weasel	0.00
2014	2005.1	2.00	8	Weasel	na
2014	2008.1	1.96	8	Weasel	0.00
2014 and 2015	1998.1	3.83	8	Weasel	0.00
2014 and 2015	1999.2	3.89	8	Weasel	0.00
2014 and 2015	1999.1	3.69	8	Weasel	0.00
2014 and 2015	2003.2	3.93	8	Weasel	0.00
2014 and 2015	2005.2	3.98	8	Weasel	na
2014	1998.2	2.17	16	Weasel	0.00
2014	2003.1	2.00	16	Weasel	0.00
2014	2004.1	1.96	16	Weasel	na
2014	2004.2	2.00	16	Weasel	0.00
2014	2005.1	2.00	16	Weasel	0.00
2014	2008.1	1.96	16	Weasel	0.00
2014 and 2015	1999.2	3.89	16	Weasel	0.00
2014 and 2015	1999.1	3.69	16	Weasel	0.00
2014 and 2015	2003.2	3.93	16	Weasel	0.00
2014 and 2015	2005.2	3.98	16	Weasel	na
2015	1998.1	1.83	16	Weasel	0.00
2014	2003.1	2.00	64	Weasel	0.00
2014	2004.1	1.96	64	Weasel	na
2014	2004.2	2.00	64	Weasel	0.00
2014	2005.1	2.00	64	Weasel	0.00
2014	2008.1	1.96	64	Weasel	0.00
2014 and 2015	1998.1	3.83	64	Weasel	0.00
2014 and 2015	1999.2	3.89	64	Weasel	0.00
2014 and 2015	2003.2	3.93	64	Weasel	0.25
2014 and 2015	2005.2	3.98	64	Weasel	0.00

Table A2. Disaggregated mammal activity [visits/(d of observation)] at baited camera stations at varying distances from recreational trails (m) in Minnesota, USA, in 2014 and 2015. Each observation is listed independently in its respective year; see Table A1 for observations aggregated across repeated transects. A "visit" is defined as a motion-triggered set of 3 photos in which an animal appeared in at least one photo. The first four digits of transect codes refer to the restoration year of the prairie in that location, not the year in which data were collected. In 2014, the observation period was not recorded when no animal activity was observed, but in every case the observation period was about two days.

Year	Transect	Observation period (d)	Distance (m)	Ground squirrel	Vole	Mouse	Rabbit	Shrew	Opossum	Chipmunk	Weasel	Dog	Total Carnivora
2014	1998.1	2.00	2	0.00	0.50	0.00	0.00	na	na	na	na	na	0.00
2014	1998.1	2.00	4	0.00	7.50	0.50	0.00	na	na	na	na	na	0.00
2014	1998.1	2.00	8	0.00	4.00	0.00	0.00	na	na	na	na	na	0.00
2014	1998.1	2.00	64	0.00	4.50	0.00	0.00	na	na	na	na	na	0.00
2014	1998.2	2.17	2	0.00	17.54	1.38	0.00	na	na	na	na	na	0.00
2014	1998.2	2.17	16	0.00	11.08	0.00	0.00	na	na	na	na	na	0.00
2014	1999.1	unknown	0	0.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	1999.1	1.87	4	0.00	9.07	0.00	0.00	na	na	na	na	na	0.00
2014	1999.1	1.88	8	0.00	28.27	1.60	0.00	na	na	na	na	na	0.00
2014	1999.1	1.87	16	2.13	38.93	4.27	0.00	na	na	na	na	na	0.00
2014	1999.2	2.04	0	0.00	0.49	0.00	0.00	na	na	na	na	na	0.00
2014	1999.2	unknown	2	0.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	1999.2	2.04	4	0.00	8.33	0.00	0.00	na	na	na	na	na	0.00
2014	1999.2	2.04	8	0.00	3.92	0.00	0.00	na	na	na	na	na	0.00
2014	1999.2	2.04	16	0.49	2.45	0.00	0.00	na	na	na	na	na	0.00
2014	1999.2	2.04	64	4.41	45.55	0.98	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	0	8.50	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	2	57.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	4	29.50	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	8	42.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	16	28.50	21.50	0.00	0.00	na	na	na	na	na	0.00
2014	2003.1	2.00	64	8.50	1.00	0.00	0.00	na	na	na	na	na	0.00
2014	2003.2	1.96	0	0.00	0.51	0.00	0.00	na	na	na	na	na	0.00
2014	2003.2	1.96	4	0.00	4.60	0.00	0.00	na	na	na	na	na	0.00
2014	2003.2	1.96	8	0.00	9.70	0.00	0.00	na	na	na	na	na	0.00
2014	2003.2	1.96	16	0.00	59.23	0.51	0.00	na	na	na	na	na	0.00

Year	Transect	Observation period (d)	Distance (m)	Ground squirrel	Vole	Mouse	Rabbit	Shrew	Opossum	Chipmunk	Weasel	Dog	Total Carnivora
2014	2003.2	1.96	64	0.00	4.09	0.00	0.00	na	na	na	na	na	0.00
2014	2004.1	1.96	0	15.83	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2004.1	1.96	2	75.57	1.02	83.23	4.60	na	na	na	na	na	0.00
2014	2004.1	1.96	4	72.00	0.00	13.79	0.00	na	na	na	na	na	0.00
2014	2004.1	1.96	8	39.32	0.00	17.87	0.51	na	na	na	na	na	0.00
2014	2004.1	1.96	16	63.32	4.60	15.83	19.40	na	na	na	na	na	4.09
2014	2004.1	1.96	64	17.87	11.74	51.57	0.00	na	na	na	na	na	6.13
2014	2004.2	2.00	0	34.00	0.00	29.50	2.50	na	na	na	na	na	0.00
2014	2004.2	2.00	2	14.50	3.50	0.50	0.00	na	na	na	na	na	0.50
2014	2004.2	2.00	8	55.50	0.50	12.00	0.00	na	na	na	na	na	0.00
2014	2004.2	2.00	16	27.50	0.00	15.00	0.00	na	na	na	na	na	0.00
2014	2004.2	2.00	64	7.00	0.00	7.50	0.00	na	na	na	na	na	0.00
2014	2005.1	2.00	0	0.50	0.50	0.50	0.00	na	na	na	na	na	0.00
2014	2005.1	2.00	2	0.00	1.00	0.00	0.00	na	na	na	na	na	2.50
2014	2005.1	2.00	8	0.00	16.50	0.00	0.00	na	na	na	na	na	2.00
2014	2005.1	2.00	16	0.50	7.50	0.50	0.00	na	na	na	na	na	0.00
2014	2005.1	2.00	64	0.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2005.2	2.13	0	30.12	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2005.2	2.13	2	8.00	18.35	0.00	0.00	na	na	na	na	na	1.88
2014	2005.2	2.13	4	64.47	3.76	0.00	0.00	na	na	na	na	na	0.00
2014	2005.2	2.13	8	37.65	7.06	0.47	0.00	na	na	na	na	na	1.41
2014	2005.2	2.12	16	32.00	1.88	0.94	0.00	na	na	na	na	na	0.94
2014	2005.2	2.13	64	0.00	1.41	0.00	0.00	na	na	na	na	na	0.00
2014	2008.1	unknown	0	0.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2014	2008.1	1.96	2	0.00	0.51	0.00	0.00	na	na	na	na	na	0.00
2014	2008.1	1.96	4	0.00	5.11	0.51	0.00	na	na	na	na	na	0.00
2014	2008.1	1.96	8	0.00	16.34	1.02	0.00	na	na	na	na	na	0.00
2014	2008.1	1.96	16	0.00	3.06	0.00	0.00	na	na	na	na	na	0.00
2014	2008.1	1.96	64	0.00	8.17	0.51	0.00	na	na	na	na	na	0.00
2014	2008.2	1.96	0	0.00	0.00	0.51	0.00	na	na	na	na	na	0.00
2014	2008.2	unknown	2	0.00	0.00	0.00	0.00	na	na	na	na	na	0.00
2015	2003.2	1.97	2	55.73	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1998	1.83	0	0.00	0.00	1.64	0.00	0.00	3.82	0.00	0.00	0.00	3.82

Year	Transect	Observation period (d)	Distance (m)	Ground squirrel	Vole	Mouse	Rabbit	Shrew	Opossum	Chipmunk	Weasel	Dog	Total Carnivora
2015	1998	1.83	2	0.00	22.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1998	1.83	4	0.00	33.30	1.09	0.00	2.73	0.00	0.55	0.00	0.00	0.00
2015	1998	1.83	8	0.00	9.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1998	1.83	16	0.00	0.55	0.55	0.00	4.37	0.00	0.00	0.00	0.00	0.00
2015	1998	1.83	64	0.00	34.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	0	37.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	2	38.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	4	50.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	8	40.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	16	17.26	0.00	1.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2005.2	1.85	64	51.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.1	1.82	2	0.00	0.00	2.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.1	1.82	4	9.90	1.10	13.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.1	1.82	8	0.55	6.05	1.10	0.00	0.55	0.00	0.00	0.00	0.00	0.00
2015	1999.1	1.82	16	19.81	9.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.2	1.85	0	0.00	4.32	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.2	1.85	8	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00
2015	1999.2	1.85	16	0.00	159.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	1999.2	1.85	64	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00
2015	2003.2	1.97	0	50.66	1.01	0.00	3.04	0.00	0.00	0.00	0.00	0.51	0.51
2015	2003.2	1.97	4	18.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2015	2003.2	1.97	8	27.87	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00
2015	2003.2	1.97	16	0.00	0.00	0.00	12.67	0.00	0.00	0.00	0.00	0.00	0.00
2015	2003.2	1.97	64	69.92	0.00	0.00	3.04	0.00	0.00	0.00	0.51	0.00	0.51

Supplementary material 1

Tables S1, S2

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Data type: occurences

Explanation note: **Table S1.** Average mammal activity level [visits/(d of observation)] at baited camera traps at varying distances from recreational trails (m) in Minnesota, USA, in 2014 and 2015. Where points in the same location were used in two years, activity is listed as the average activity of the two years, weighted by the observation period in each year. See Table S2 for disaggregated observations listed independently in each year. A "visit" is defined as a motion-triggered set of 3 photos in which an animal appeared in at least one photo. The first four digits of transect codes refer to the restoration year of the prairie in that location, not the year in which data were collected. **Table S2.** Disaggregated mammal activity [visits/(d of observation)] at baited camera traps at varying distances from recreational trails (m) in Minnesota, USA, in 2014 and 2015. Each observation is listed independently in its respective year; see Table S1 for observations aggregated across repeated transects. A "visit" is defined as a motion-triggered set of 3 photos in which an animal appeared in at least one photo. The first four digits of transect codes refer to the restoration year of the prairie in that location, not the year in which data were collected. In 2014, the observation period was not recorded when no animal activity was observed, but in every case the observation period was about two days.

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