

The rusty-margined guan (*Penelope superciliaris*) in the Brazilian Atlantic rain forest: density, population size, activity and habitat use

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Abstract

Context. Population estimation and monitoring are important tools for the support of programs and actions of conservation for most wildlife species, including birds. For validation of such support, a set of reliable, consistent and comparable parameter estimates is essential (through systematic monitoring over time), in addition to studies on ecological aspects of the species.

Aims. In this study, our aim was to analyse the population of *Penelope superciliaris* in the Vale Natural Reserve (VNR), located in north-eastern Espírito Santo, Brazil, providing estimates of density, population size and activity. We also analysed occupancy and detectability of the species, relating them to six covariates (distance from forest edge, water and road, as well as poaching intensity, canopy cover and understorey cover) based on previous knowledge of the ecology of rusty-margined guan.

Methods. We used line-transect surveys to estimate density and abundance, and camera traps to estimate habitat use by *P. superciliaris*, using the occupancy modelling approach.

Key results. Estimated density for *P. superciliaris* was 2.5 ± 1.0 groups per km² with a group size of 6.0 ± 2.0 individuals, and estimated population size for the entire VNR was 3544 ± 495 individuals. Occupancy by *P. superciliaris* was best described by three covariates: (1) distance to road; (2) distance to water; and (3) poaching intensity. Detectability was affected by two covariates: (1) understorey cover; and (2) poaching intensity.

Conclusions. Our results provide the first information on density, habitat use and activity of *P. superciliaris* in the VNR. We concluded that *P. superciliaris* is active diurnally, especially during morning and late afternoon, prefers areas near water sources in denser forests and tends to avoid areas with habitat modification and intense poaching activity.

Implications. Our data highlight the impact of poaching on the species and can be useful as a baseline for future studies and Cracid conservation plans.

Additional keywords: Cracidae, detectability, distance sampling, guans, occupancy modelling, population abundance.

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Introduction

Population estimation and monitoring are important tools for the support of wildlife programs and conservation action, including birds (Buckland *et al.* 2008; Taylor and Pollard 2008). For validation of such support, a set of reliable, consistent and comparable parameter estimates (e.g. density and abundance) is essential through systematic monitoring over time, in addition to studies on ecological aspects of the species (Sutherland 2000). Among the general methods used for avian ecological studies, camera traps have been used for studying activity period (e.g. O'Brien and Kinnaird 2008), habitat use (e.g. O'Brien and Kinnaird 2008),

reproduction (e.g. Leite *et al.* 2017) and behaviour (e.g. Pietz and Granfors 2005), especially for large terrestrial birds such as Cracids.

The avian family Cracidae (curassows, guans and chachalacas), commonly known as Cracids, are large frugivorous birds that play a key role in the maintenance and regeneration of forests through seed dispersal, and in population control through seed predation (Brooks and Strahl 2000; Mikich 2002; Zaca *et al.* 2006). Cracids represent one of the most endangered bird families in the neotropics, because they are affected by continuous hunting and are sensitive to habitat disturbance (Sick 2001; Brooks and Fuller 2006).

Within the family Cracidae, the genus *Penelope* is the most diversified, with a total of 15 species (Sick 2001). The rusty-margined guan (*Penelope superciliaris* Temminck, 1815) has the widest distribution, occurring from south of the Amazon and Madeira rivers, through central Brazil, to Paraguay and Rio Grande do Sul, Brazil (Sick 2001). *Penelope superciliaris* is primarily an arboreal species, occupying lowland forests, although it may also occur in open areas and small forest fragments (Delacour and Amadon 1973; Sick 2001).

Penelope superciliaris is a common species, but despite its world population showing a declining trend (BirdLife International 2016) and its ecological importance in seed dispersal (Mikich 2002; Zaca *et al.* 2006), few studies are available on its biology and ecology (e.g. Guix 1997; Mikich 2002; Zaca *et al.* 2006; Thel *et al.* 2015). *Penelope superciliaris* is a widely hunted species (Chiarello 2000; Cullen *et al.* 2000; Thel *et al.* 2015), and together with other cracids affected by human disturbance, can play an important role as a bioindicator of habitat quality (Brooks and Strahl 2000).

The Vale Natural Reserve (VNR) is part of the largest forest block in the state of Espírito Santo. This region is characterised by intensive hunting activity, affecting birds such as *Penelope superciliaris*, red-billed curassow (*Crax blumenbachi*) and solitary tinamou (*Tinamus solitarius*; Ferregueti *et al.* 2018), as well as mammals and reptiles (Chiarello 2000; Ferregueti *et al.* 2015, 2016, 2017; Sousa and Srbek-Araujo 2017).

To evaluate factors affecting its occurrence in the VNR, we studied the local population of *P. superciliaris* to estimate density of groups, group and population size, and analyse occupancy and detectability of the species, relating six covariates of habitat selection and human threats based on previous knowledge of *P. superciliaris* ecology (e.g. Brooks and Strahl 2000; Sick 2001; Thel *et al.* 2015). We also estimated activity period of the species in the VNR.

Methods

Study area

This study was conducted in the VNR, a protected area of 23 500 ha belonging to the Vale Co. The reserve is located in the neighbouring municipalities of Linhares and Jaguaré (19°06'–19°18'S and 39°45'–40°19'W), in north-eastern Espírito Santo state, Brazil (Fig. 1).

The VNR is inserted within the Atlantic Rainforest biome, and composed of a mosaic of four main vegetation types (adapted from Jesus 1987; Kierulff *et al.* 2014): evergreen coastal plain; riparian; sandy soil forests; and natural grassland. The evergreen coastal plain forest has two or more upper strata and high densities of epiphytes, and covers ~68% of the total area of the VNR. The riparian forest, which covers some 4% of the reserve, is a mixed vegetation type associated with streams, and is characterised by widely spaced trees and a

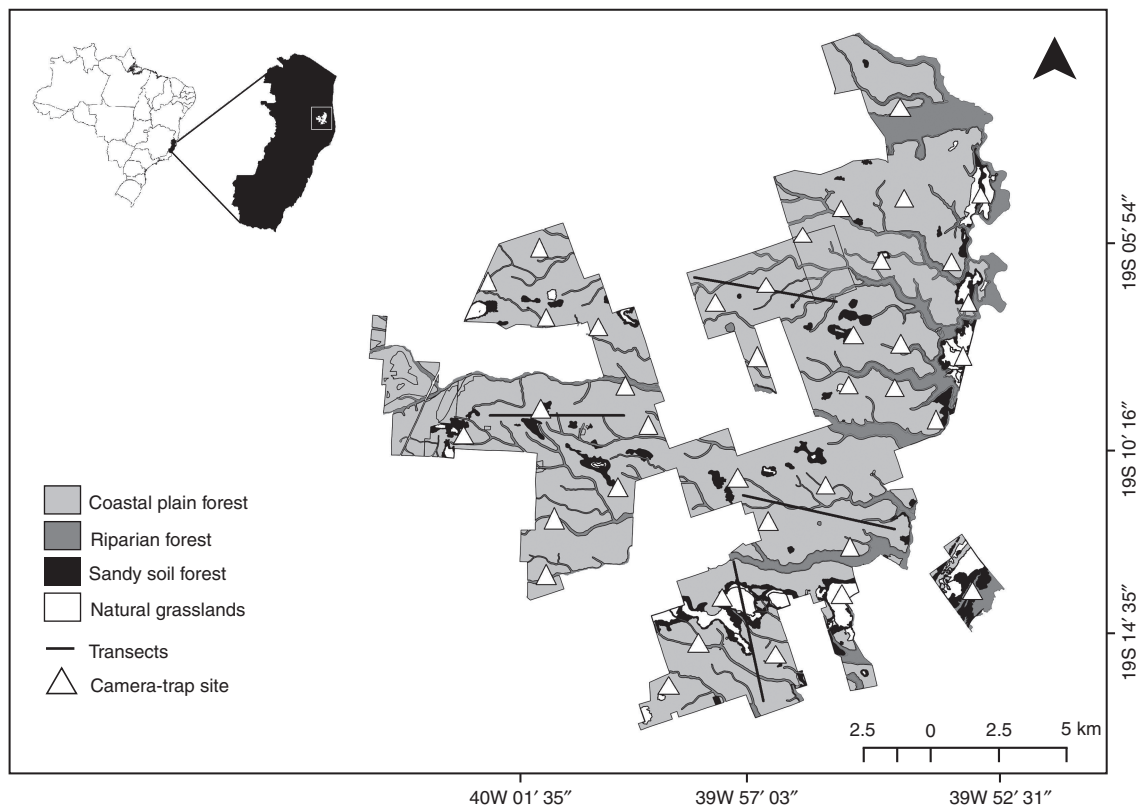


Fig. 1. Location and main vegetation types of the Vale Natural Reserve in Espírito Santo State, Brazil. Black triangles represent the location of each camera trap, and red lines represent survey transects.

predominance of palms. The sandy soil forest (covering ~8% of the VNR) is a type of woody vegetation on sandy soils, physiognomically similar to the coastal plain forest at an early or intermediate stage of regeneration. The natural grasslands occur as enclaves within the forest, which were once the sites of ponds in the geological past, and cover ~6% of the area of the reserve. Together with these vegetation types, wetlands (swamps) and streams cover nearly 8% of the VNR (Fig. 1), and the remaining 6% is composed of administrative facilities.

Line-transect surveys

To estimate density and abundance of *P. superciliaris*, we established four 5-km line transects, separated by >4 km between each (Buckland *et al.* 2001), using the RAPELD protocol (Magnusson *et al.* 2005). RAPELD is a technique that seeks to standardise the collection of biological data and aims to develop methods for long-term ecological research (PELD) and allow rapid inventories (RAP=Rapid Assessment Program).

During a 13-month period (April 2013–May 2014), transects were surveyed monthly using distance sampling techniques (Buckland *et al.* 2001). Transects were surveyed by a single observer starting at sunrise (between 0530 and 0630 hours) and waiting 3 h before starting the afternoon survey (between 1300 and 1400 hours). The observer walked transects at a speed of ~1 km h⁻¹.

We recorded perpendicular distance of every group detected along the transect line with a measuring tape, flock size (number of sighted individuals), date, time of day and transect number. We considered the first individual detected of the group to measure the perpendicular distance.

Camera trapping

We selected 39 sampling sites using a systematic random design, stratified by vegetation type, to ensure that all four main vegetation types in the VNR were represented. This scheme was designed to model occupancy probability of the VNR by *P. superciliaris*, as well as to register its activities (Fig. 1).

We placed a grid over a digital map of the reserve, with each cell of 2 km² receiving a number, and identified the sampling sites by randomly selecting grid cells through a numerical draw. This approach resulted in a relatively even distribution of points within the VNR, while maintaining independence among points, which were separated from one another by a distance of >1 km (Magnusson *et al.* 2005). At each site, we installed one passive infrared Bushnell camera trap (Bushnell Outdoor Products, Overland Park, Ka) in picture function, ~40–50 cm above the ground, for continuous surveying throughout the study. All stations were examined every 20–25 days to collect photographic records and to change batteries when necessary. Camera traps were programmed to operate for 24 h per day; bait was not used to attract birds.

Occupancy model covariates

We used six covariates to model the occupancy probability and detectability of *P. superciliaris*: (1) distance (m) to forest edge ('edge'); (2) distance (m) to the nearest source of water

(ponds, streams, small streams) ('water'); (3) poaching intensity (n per km²; 'poach' – see below for details of estimation); (4) canopy cover (%; 'canopy'); (5) understorey cover (%; 'under'); and distance (m) to nearest road ('road').

We established four plots (30 × 50 m) at each sampling point, arranged by cardinal compass points (north, south, east and west). Canopy cover was estimated with a convex spherical densitometer, using digital camera images in the centre of each plot for all plots (Lemmon 1957). In addition, ocular estimates were used in the comparison. We averaged the four measurements to obtain the final value for each sampling site. Understorey cover was measured along the central (longitudinal) line of each plot, considering 5 m on each side of the line (transect width of 10 m). Understorey cover was measured every 10 m using a 2.0 × 0.5 m sighting frame (each 0.5 m portion representing 25% visibility). Understorey cover within each plot was estimated by the mean visibility of the sighting frame in the five points sampled in the central line.

Three spatial covariates, (1) distances to forest edge, (2) water resource and (3) road, were measured for each of the 39 sampling sites using ArcGIS software (ESRI*ArcMap 10.1, Redlands, Ca).

Poaching intensity was calculated using the georeferenced database of a 10-year period, in which poaching events were recorded by the reserve's security guards (source: VNR). The density of poaching (records per km²) was calculated for each grid cell in which a camera trap had been installed.

Data analysis

Density and population size were estimated by total number of groups observed along each trail through the program 'Distance 7' (Buckland *et al.* 2001). Distance 7 uses the perpendicular distances (animal-track) to estimate effective strip width (ESW) in the study area and model the detection function that best suits the probability of detection of an animal at a given distance track (Laake *et al.* 1994; Buckland *et al.* 2001). We also estimated the size of the groups to multiply and obtain the total number of individuals for the VNR. We fitted the models using different key functions (half-normal, uniform and hazard rate) to the data as clusters, and selected the best model with AIC value <2 (Burnham and Anderson 2002). Burnham and Anderson (2002) also recommended using the AIC to select models only when the number of observations is greater than or equal to 60. This minimum number of observations allows for accurate estimates.

We classified camera trapping data using the 7-day occasions (28 occasions) based on the Mackenzie *et al.* (2006) approach to constructing a reliable detection history. We estimated site occupancy (Ψ) and detection probability (p) for the species, with three possible outcomes: (1) the site was occupied and the species was detected ($\Psi \times p$); (2) the species was present but not detected ($\Psi \times [1 - p]$); and (3) the species was not present and therefore was not detected ($1 - \Psi$).

We estimated detection probabilities based on detections obtained from each site on 28 occasions. The probability was the parameter projected by a maximum likelihood estimation of the proportion of sites occupied (Ψ) during the sample period.

In our occupancy analysis, we assessed the covariates that might affect occupancy and detectability, in an attempt to identify habitat use for *P. superciliaris*. We constructed a set of candidate models for the species, which were selected by *a priori* hypotheses based on three different approaches: (1) considering occupancy probability and detectability as constant across all sites; (2) considering the variation in occupancy as a function of covariates; and (3) considering both the variation in occupancy and detectability as a function of covariates. This allowed us to evaluate if the differences in habitat use were determined by a single covariate or a set of covariates, which would contribute to an improvement in the model's performance.

We generated all the occupancy models presented above (single-species, single-season) using the 'Unmarked' package in Program R (Fiske and Chandler 2011). Top models were selected using Akaike's Information Criterion adjusted for small sample size (AICc). All models with an $\Delta AICc$ value < 2 were considered equivalent. We also used the weight (AICwt) for each model, which corresponds to the amount of evidence in favour of a given model, to choose the best model to test our hypotheses. We used 2000 bootstraps to assess the adjustment fit (\hat{z}) and the over-dispersion parameter (\hat{c}).

We used the number of independent times that individuals of the species were photographed to assess species activity patterns. To avoid pseudo-repetition, more than 1 h had to pass before we could consider the records independent. For those individuals photographed more than once, we considered the first photo obtained of each individual in the analysis. We used a conditional circular kernel density function to estimate the activity patterns (Oliveira-Santos et al. 2013). This circular kernel had the same features as the home range kernel estimator, including a smoothing parameter and a conditional density isopleth. This is defined as the threshold of probability that specifies the section of the function that accounts for a given proportion of the entire probability function (Oliveira-Santos et al. 2013). Analyses were conducted in the 'Circular' package in R with associated analytic packages. Circular summaries (Lund and

Agostinelli 2007) were used to determine the mean overall timing of the activity as recorded by camera traps.

Results

Density and population size

We surveyed a total of 908 km of transects (196 samples), during which *P. superciliaris* was sighted 165 times.

Estimated density for *P. superciliaris* was 2.5 ± 1.0 groups per km², with a group size of 6.0 ± 2.0 individuals. Estimated population size for the entire VNR was 3544 ± 495 individuals. The data fitted best to a half-normal curve with cosine adjustment. Effective strip width (ESW) was 5.95 ± 1.29 m, with sightings being obtained at distances of between 0 and 24 m from the transect. The coefficient of variation for all parameters was 13.32%, and the detection probability and encounter rate were responsible for 61% and 39% of the total variation, respectively.

Occupancy and detectability models

A total of 7020 trap days was conducted during the study. We obtained 364 independent detections of *P. superciliaris* and observed the species in 25 of the 39 sites, resulting in a naïve occupancy of 0.64 and a detectability of 0.36. From the occupancy models produced (Model fit = 0.18 and c-hat = 1.23, obtained with 2000 bootstraps; Table 1 shows the 10 top models), the best model to predict occupancy and detectability was ' $\Psi(\text{road};\text{water};\text{poach});p(\text{poach};\text{under})$ '.

Occupancy was best described by three covariates: (1) distance to the road ('road'), which had a positive relationship in which occupancy increased with higher distances to the road ($\Psi = 0-0.95$; Fig. 2A); (2) distance to water ('water'), which had a negative relationship, in which occupancy by *P. superciliaris* decreased as the distance to water increased (dropping to $\Psi = 0.97-0.03$; Fig. 2B); and (3) poaching intensity ('poach'), which predicted a negative relationship in which occupancy decreased in sites with higher poaching intensity (dropping to $\Psi = 0.99-0$; Fig. 2C).

Table 1. Single-species, single-season occupancy and detectability models for the rusty-margined guan (*Penelope superciliaris*) in the Vale Natural Reserve, Brazil, estimated by camera-trapping during April 2013–May 2014, grouped in sampling intervals of 7 consecutive days

Covariates: distance (m) from water resource (water), distance (m) from road (road), understorey cover (under) and poaching intensity (poaching). Ψ = occupancy, P = detectability, AICw = Akaike weight

Model	AICc	$\Delta AICc$	AICcw	n°parameters
$\Psi(\text{water};\text{poach}) p(\text{under};\text{poach})$	453.43	0	0.234	6
$\Psi(\text{water};\text{poach};\text{road}) p(\text{under};\text{poach})$	454.23	0.8	0.225	7
$\Psi(\text{water};\text{road}) p(\text{under};\text{poach})$	455.02	1.59	0.225	6
$\Psi(\text{poach};\text{road}) p(\text{under};\text{poach})$	455.12	1.69	0.094	6
$\Psi(\cdot) p(\text{under};\text{poach})$	458.76	5.33	0.092	4
$\Psi(\text{water};\text{poach};\text{under}) p(\text{under};\text{poach})$	459.32	5.89	0.064	7
$\Psi(\text{water}) p(\text{under};\text{poach})$	459.45	6.02	0.031	5
$\Psi(\text{road}) p(\text{under};\text{poach})$	461.34	7.91	0.021	5
$\Psi(\text{poach}) p(\text{under};\text{poach})$	467.54	14.11	0.01	5
$\Psi(\text{poach};\text{road};\text{under}) p(\text{under};\text{poach})$	469.87	16.44	<0.01	7

Model fit = 0.18, and c-hat = 1.23 obtained with 2000 bootstraps

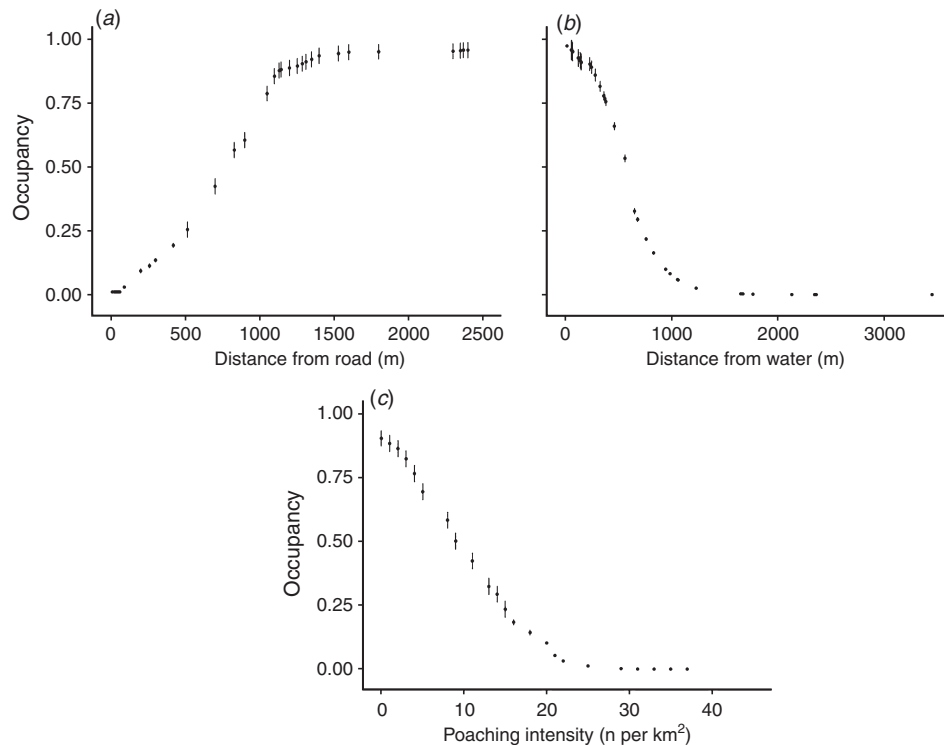


Fig. 2. Relationship between occupancy of *Penelope superciliaris* and (A) distance (m) to road, (B) distance (m) to water resources, and (C) poaching intensity (records km^{-2} ; number of events recorded at each particular area of the reserve), as estimated by camera trapping from April 2013 to May 2014, in Vale Natural Reserve, Espírito Santo State, south-eastern Brazil. Poaching intensity was estimated by a georeferenced database of a 10-year period in which poaching events were recorded by the reserve's security guards (source of the data: Vale Natural Reserve).

Detectability was affected by two covariates: (1) understorey cover ('under'), which predicted a negative relationship with detectability ($P=0.62-0.02$; Fig. 3A); and (2) poaching intensity ('poach'), which predicted a negative relationship with higher detectability in sites with lower poaching records ($P=0.64-0$; Fig. 3B).

Activity patterns

Penelope superciliaris showed two peaks of activity in the VNR, based on records from camera traps throughout the 24-h cycle (Fig. 4). The species was active early in the morning between 0500 and 1100 hours, and late afternoon to dusk between 1400 and 1800 hours.

Discussion

The population density of *P. superciliaris* in the study area (15 individuals per km^2) was similar to the results of other studies estimating densities for the species, as well as other members of the genus *Penelope* in other areas. For example, in the Araripe National Forest in Ceará, Brazil, the estimated density of *P. superciliaris* was 19.17 individuals per km^2 (Thel *et al.* 2015). In forest fragments in southern Ecuador, the estimated density for the bearded guan (*P. barbata*) ranged from 2.3 to 17.1 individuals per km^2 (Jacobs and Walker 1999). The average density estimated for species in the Penelopinae

subfamily (guans and chachalacas) was 17.7 ± 9.4 individuals km^2 (Kattan *et al.* 2016).

Our results showed that the occupancy and detectability of *P. superciliaris* were negatively affected by poaching intensity, reinforcing the assumption that this factor locally reduces the areas of use by the species. *P. superciliaris* is a target species for poaching in VNR and surrounding areas (Chiarello 2000), and has been considered critically endangered in Viçosa, Minas Gerais state, due to high poaching activity (Ribon *et al.* 2003). The lower abundance of the species in areas with higher poaching intensity is well documented. For example, in a study on the effects of poaching in Atlantic Forest fragments in south-eastern Brazil, *P. superciliaris* was more abundant in a state park area that had the lowest poaching intensity when compared with other areas studied in the park (Cullen *et al.* 2000).

Cracids are commonly hunted in several places in the Americas (Brooks *et al.* 1999; Kattan *et al.* 2016), and the impact of hunting on populations is well documented (e.g. Begazo and Bodmer 1998; Brooks *et al.* 1999; Barrio 2011; Barros *et al.* 2011; Kattan *et al.* 2016). In the state of Espírito Santo, other cracids besides *P. superciliaris* are affected by poaching, such as *Crax blumenbachii*, which is restricted to the north of the state, and *Pipile jacutinga*, which is considered extinct in the state due to high poaching intensity and extensive deforestation (Chiarello 2000; Pereira and Brooks 2006).

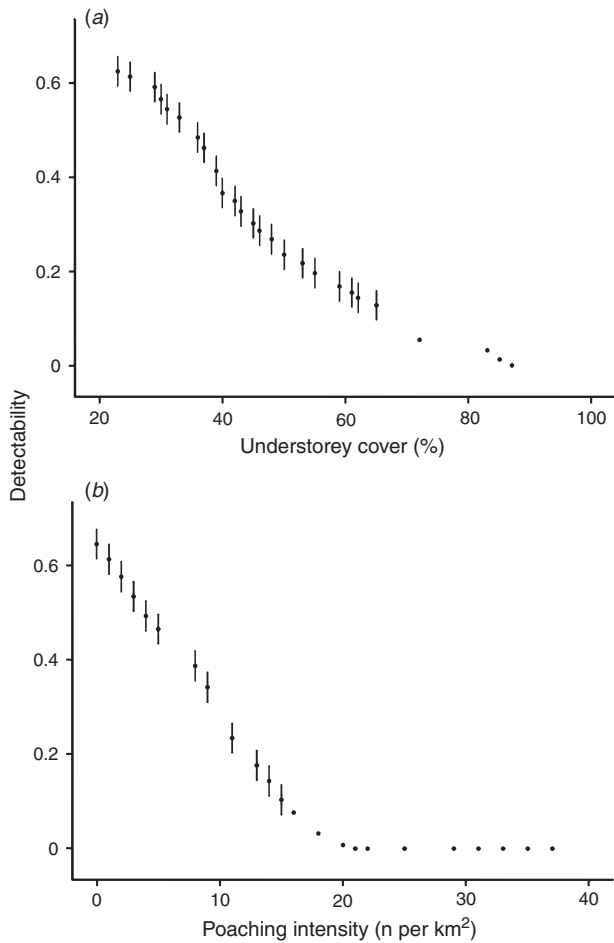


Fig. 3. Relationship during detectability of *Penelope superciliaris* and (A) understory cover and (B) poaching intensity as estimated by camera trapping between April 2013 and May 2014, in Vale Natural Reserve, Espírito Santo State, south-eastern Brazil.

Despite this, few studies have estimated population data (Alves *et al.* 2015) and no study has quantified the effects of poaching for Cracids in Espírito Santo. Thus, our study reinforces the importance of studies monitoring the poaching of Cracids in the state of Espírito Santo and other areas of occurrence.

In the present study, the occupancy rate of *P. superciliaris* was influenced by distance to the road, which increased considerably with an increase in road distance. This may be due to Cracids being susceptible to habitat modification and destruction (Brooks and Strahl 2000), although some species may also occur in less preserved areas (Borges 1999; Loures-Ribeiro *et al.* 2011). *Penelope superciliaris* poaching in the state of Espírito Santo and in other areas of occurrence was more frequent in primary forests, but can also occur in small fragments and disturbed areas (Sick 2001). The sensitivity to environmental disturbances of this species was already considered low (Loures-Ribeiro *et al.* 2011) to medium (Ribon *et al.* 2003), depending on the study site. In the present study, *P. superciliaris* occurred more frequently in the forest interior, far from disturbed habitats. The higher detectability of the species in areas with lower understory cover may also be related to this, because guan abundance increased in coastal plain forest vegetation (i.e. primary and dense forest with large trees), which has low understory cover when compared with sandy soil forest (i.e. forest with smaller trees and higher understory cover), as well as in natural grasslands (i.e. open areas with sandy soil) (Kierulff *et al.* 2014).

The distance to water resources had an important relationship with *P. superciliaris* occupancy, resulting in a higher rate in areas closer to water sources. Water sources seem to be important for other species of Cracids (Martinez-Morales 1999; Hill *et al.* 2008; Luna-Maira *et al.* 2013), although Alves *et al.* (2017) showed that distance to water was not related to detection of *Crax blumenbachii* in the VNR. Our result was similar to that found by Martinez-Morales (1999) for the Cozumel Curassow (*Crax rubra*

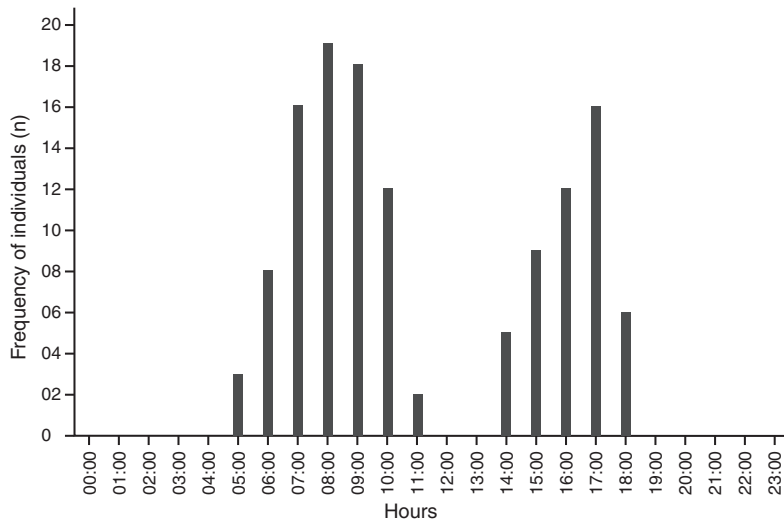


Fig. 4. Activity of *Penelope superciliaris* in the Vale Natural Reserve, Espírito Santo state, Brazil, estimated by camera-trapping during April 2013 to May 2014.

griscomi), where the highest densities of this species were recorded near water resources, decreasing considerably as the distance to water increased. The relationship of *P. superciliaris* with water resources is of fundamental importance because it may have implications for conservation of this species, such as impacts related to human activities (e.g. damming of streams, redirecting rivers and draining wetlands for crops) and climate (e.g. drought), which may increase the species' vulnerability in the reserve.

Penelope superciliaris exhibited diurnal and crepuscular activity in the VNR, with activity peaks at ~0800 and 1700 hours. In general, bird observations were higher early in the morning (e.g. Järvinen *et al.* 1977; Blake 1992), although activity of the guan may vary according to factors such as seasonality (Antunes 2008). In a study of diurnal variation in bird activity in a forest fragment in south-eastern Brazil, most *P. superciliaris* encounters occurred between dawn and 0900 hours. (Antunes 2008). The bimodal daily activity of *P. superciliaris* in the present study was also similar to that of the Trinidad piping-guan (*Aburria pipile*) in Trinidad, and the red-billed curassow (*Crax blumenbachii*) in VNR, which exhibited higher activity in the early morning and late afternoon ((Hayes *et al.* 2009; Srbeek-Araujo *et al.* 2012).

Conclusion

The present study presents consistent results and provides the first information on density, habitat use and activity patterns of *P. superciliaris* in the VNR. We conclude that this species is most active during diurnal and crepuscular periods, prefers areas near water sources within denser forests and tends to avoid areas that have undergone habitat alteration and more intense poaching activity. Our data highlight the impact of poaching on the species and can be useful as a baseline for future studies and conservation planning for Cracids.

Conflicts of interest

The authors declare no conflicts of interest.

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