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Effects of a paved road on mortality and mobility of red bellied toads (*Melanophryniscus* sp.) in Argentinean grasslands

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Abstract. *Melanophryniscus* sp. is an endemic toad restricted to Sierra de la Ventana, in the Argentine Pampas. Part of its reproductive habitat is crossed by a paved road. We studied the effects of the road on the mortality and mobility of the toads. During the days following rain, we surveyed transects on both sides of the road, capturing, photographing and immediately releasing each toad. Population size in the area was estimated in 1074 individuals (871 to 1363, 95% CI) by means of mark-recapture analysis. We found fifteen individuals killed by vehicles in the 2003-04 and ten in the 2004-05 reproductive seasons. This mortality represents from 2.5 to 5.9% of the population annually, considering that 73% of the mortality period was sampled and a detection rate of dead frogs of 40%. Other factors associated to roadside habitat, such as rapid drying of roadside ditches, maintenance work, increased frequency of fire, pollution by gas, oil and fuel emissions and losses from vehicles, and poaching as pets by tourists, may also reduce the survival of the toads. We recorded capture sites and calculated the average distance between captures as 33.04 m for males and 22.50 m for females. Only two of the 76 observations of recaptured toads were made on the opposite side of the road. Roads can be considered as having a significant impact on this species by augmenting mortality, hindering the mobility of the species and increasing habitat isolation.

Introduction

Fragmentation is the process of habitat subdivision followed by the isolation of the resulting relicts of natural habitats (Wilcox, 1980). There are two main causes of the isolation of natural terrestrial habitats: (1) intensive agriculture, forestry and urban development, and (2) linear constructions, such as roads, canals, power lines and railways (Mader, 1984; Saunders, Hobbs and Margules, 1991). Roads affect ecosystems in different ways by increasing mortality from road construction and from collision with vehicles, changing animal behaviour, altering the physical and chemical environment, favouring the spread of exotic species, and promoting further alterations and uses of the landscape by humans (Forman and Alexander, 1998; Trombulak and Frissell, 2000). Road edges also promote microclimatic changes in temperature and humidity, wind speed, and levels of solar radiation, that can affect wildlife directly or through changes in the composition of roadside vegetation (Matlack, 1994). On one hand interior species that avoid edge areas may find roadsides unsuitable as breeding habitats (Ferris, 1979; Reijnen et al., 1995), while others that use borders for breeding may suffer from increased predation and parasitism or roadkill mortality (Paton, 1994; Githiru, Lens and Cresswell, 2005).

The process of fragmentation has been extensively studied in forest ecosystems throughout the world (Wilcove, 1985; Martin, 1988; Saunders, Hobbs and Margules, 1991; Reed, Johnson-Barnard and Baker, 1996; deMaynadier and Hunter, 1999) however much less is known about the effects of habitat subdivision on the biodiversity of shrub and grass steppes. This issue is even less developed in countries like Argentina, with a younger tradition in conservation studies. Fiori and Zalba (2003) studying an oil field in Patagonian shrublands, associated seismic prospecting lines and roads with the invasion of alien plants, the expansion of native leaf-cutting ants, the movement of predators and an increase in poaching activities. In the Argentine Pampas, Collado and Delafiore (2002) pointed to the reduction and fragmentation of natural grasslands by agriculture as one of the

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main causes for the decline of Pampas deer. However in both cases data were mostly observational and no analytical studies have been undertaken.

The Pampas grassland is probably the ecosystem that has undergone the greatest number of human induced changes in Argentina (León, Rusch and Oesterheld, 1984; Soriano et al., 1991; Dinerstein et al., 1995; Bertonati and Corcuera, 2000). Almost all of the grassland was historically incorporated into agricultural production and the last relicts of pristine habitats are still at risk (e.g. between 1988 and 2002, 924 000 ha, representing 3.6% of the grassland region, were converted to agriculture) (Bilenca and Miñarro, 2004). This is also the most densely populated region in the country, and so the one with the most extensive and heavily transited road network.

It has been stressed that amphibians are among the groups that are most intensely affected by habitat fragmentation (Blaustein, Wake and Sousa, 1994; Fisher and Shaffer, 1996; Gillespie and Hollis, 1996; Hecnar and M'Closkey, 1996; Wind, 1996; deMaynadier and Hunter, 1998; Young et al., 2004), mainly because of their low vagility and high philopatry, and also due to physiological constraints such as susceptibility to desiccation (Wind, 1999; Weyrauch, 2004). A paved road can represent a significant difficulty for amphibian movements, posing an important threat to their survival (Merriam et al., 1989; deMaynadier and Hunter, 1995; Fahrig et al., 1995; Gibbs, 1998).

In this work we explore the effects of a paved road separating breeding habitats of *Melanophryniscus* sp., a toad species endemic from Buenos Aires mountains in Argentina. We propose that the road poses a significant threat to the species, both as a factor of direct mortality by vehicles collisions and as an obstacle for toads' movements. If this assumption is true, we expect to confirm the following predictions: (1) The number of individuals found dead over the road will represent a significant proportion of the population; (2) Mean distances traveled by toads at each side of the road will exceed the distance across the road, and there will be a greater possibility to detect the same individuals at the same side of the road in successive observations.

Materials and methods

Study area

The Ernesto Tornquist Provincial Park, in Sierra de la Ventana, is one of the few protected areas in the whole of the Pampas. The reserve is located in the central zone of Ventania mountains (38°03′S, 62°02′W), in the province of Buenos Aires, Argentina. It covers 6718 ha ranging from 250 to 1100 meters above sea level (Bilenca and Miñarro, 2004). Climate is temperate, with a mean annual temperature of 14.6°C. Rainfall ranges from 600 to 800 mm annually, and is specially concentrated during the spring and summer, with a dry period between November and April and abundant precipitation from July to November (Frangi and Bottino, 1995). A paved road crosses a portion of the park where soil movements associated with the construction of the road, combined with the natural topography of the site, result in depressions on both sides which hold rain water. Depressions on each side of the road join together with heavy rains defining temporary streams that become small ponds and finally dry off as rain stops (fig. 1). Toads use this habitat for breeding, with pairs in amplexus, egg masses and tadpoles frequently observed on both sides of the road (pers. obs.) The width of the road is 6.8 meters and road verges are 3 meters wide each. Habitats at both sides of the road are similar except for reduced vegetation cover on the south side (pers. obs.). Traffic intensity on this road reaches 1200 vehicles daily, and is especially concentrated form October to January and in April (Buenos Aires Roads Department in lit.).

Species description

The genus *Melanophryniscus* (Anura: Bufonidae) is a putative monophyletic taxon (Graybeal and Cannatella, 1995) currently represented by 19 species distributed in three phenetic groups (Caramaschi and Cruz, 2002; Cruz and Caramaschi, 2003; Baldo and Basso, 2004). The taxonomic status of the *Melanophryniscus* populations from Sierra de la Ventana is still uncertain. These populations have been variably assigned to *M. motevidensis*, *M. atroluteus* y *M. stelzneri* (Cei, 1980; Klappenbach and Langone, 1992; Lavilla et al., 2000; Cabrera, 2001; Céspedez and Motte, 2001; Lavilla and Cei, 2001), species all pertaining to the *stelzneri* group.

They are small toads, with males and females differing in size and shape, the former being smaller (males: 22.7 mm and females: 26.3 mm in length) (Cairo, 2002). Adults are black with red and yellow dots, colour patterns are constant and individual-specific, allowing individual identification

Figure 1. Roadside following a rainfall exceeding 80 mm. Ernesto Tornquist Provincial Park, Buenos Aires, Argentina.

(Cairo and di Tada, 2005). They are mostly diurnal, and the males call in or near water. Reproduction occurs after rain, in streams and temporary ponds, during spring and summer. Larval development is rapid, completing metamorphosis about twenty days after hatching (S. Cairo, unpubl. data).

Sampling and data analysis

This study was carried out from February 2003 to February 2005. We selected two segments 1000 m long along Route 76, one at each road verge. We surveyed each side of the road during the two days following rains above 20 mm. Surveys started at noon and extended until dusk.

We searched for individuals, by means of visual and sound detection, captured each toad found and determined its sex. We photographed them on both dorsal and ventral sides using a FD88 Sony Digital Mavica camera with 640×480 pixels of resolution. We immediately released each animal at the same site where it was captured. The location of every individual along the verges was recorded at a one meter resolution by means of marks previously made on the road.

The species' spot pattern was used for individual identification (Heyer et al., 1994; Cairo and di Tada, 2005; Pellet, Helfer and Yannic, 2007) and photographs were compared to find matching entries (i.e., successive photographs belonging to the same individual). In order to avoid wrong matching the identifications were repeated by another researcher. Population size was estimated by means of mark-recapture data from ten rains during the 2003-2004 and 2004-2005 breeding seasons and using a joint hypergeometric maximum likelihood estimator (JHE) with the NOREMARK software (White, 1996). This model assumes that each animal in the population has the same sighting probability on an occasion and that the population is closed. After each rain we also surveyed the road and both roadside verges looking for individuals killed by vehicles. The number of dead toads found each year was corrected considering the proportion of the mortality period sampled (proportion of rains surveyed, rains being the triggering factor for the species movements) and an estimation of the detection rate of dead toads (Slater, 2002). The proportion of rains considered was 73%. Detection rate was estimated in 40% considering the small size of the toads, the low contrast between their colour and the pavement, their possible removal by scavengers and the fact that squashed corpses are usually flowed away by strong winds (pers. obs.). Total road mortality was then estimated as: total mortality = observed mortality/(proportion of mortality period sampled∗detection rate of dead toads). In this way we were able to calculate a 95% confidence interval of the proportion of the population killed by vehicles annually.

Data on the location of each captured individual was used to calculate the mean distance moved between successive observations, this mean distance was then compared with the combined width of the road and the road verges in order to discuss possible restrictions to individual movements related with the presence of the road. We also used a t-test to compare mean distances travelled by individuals at both sides of the road as an indicator of habitat similarity between both sites.

Results

Three hundred and thirteen toads (251 males and 62 females) were photographed from 27 February 2003 to 8 February 2005. All captured individuals were reproductive adults. We found 60 pairs in amplexus involving 92% and 25% of the captured females and males, respectively.

Fifty-eight individuals were recaptured: 44 (42 males and two females) were recaptured only once, twelve males were recaptured twice and two males were recaptured on four occasions, totalling 76 recaptures. Both females were captured and recaptured in amplexus, the times elapsed between capture and recapture being ten and fourteen days. Only one male was captured and recaptured in amplexus, with a time interval of two months between capture and recapture.

Population size was estimated in 1074 individuals (871 to 1363, 95% CI) considering data corresponding to the whole sampling period.

Fifteen and ten dead toads were found on the road with unequivocal signs of traffic derived mortality in each reproductive season, respectively. This leads to an estimated total mortality of 34.25 and 51.37 individuals, representing from 2.5 to 5.9% of the population annually. Unfortunately collision with vehicles prevents the identification of individuals based on spots patterns and so we were not able to determine if any of the toads found killed on the road corresponded to those previously photographed.

The mean distance between consecutive observations was 33.04 ± 15.71 m (95% CI; $n = 74$) for the males, and 22.50 m ($n = 2$) for the females. The maximum distance recorded between captures was 155 m, and corresponded to a male. Fourty-nine percent of the recaptures were made at least one reproductive season after the initial capture. In 41% of the cases the time elapsed between successive captures was between 15 days and eight months, and only nine percent of the recaptures corresponded to the day after the first register. Only two of the 76 observations of recaptured individuals were made on the opposite side of the road with respect to the point of previous capture (fig. 2). We found no significant differences between the mean distance travelled by males on either side of the road ($P = 0.97$).

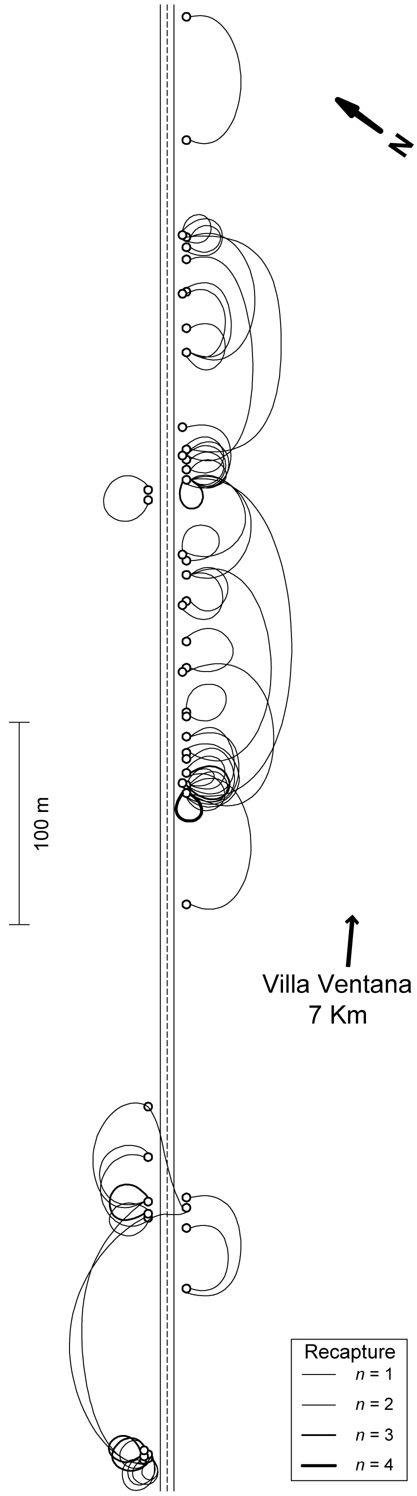

Figure 2. Mobility diagram showing displacements of *Melanophryniscus* toads along and across the road. Curved lines represent the movement of re-captured animals.

Discussion

Despite previous discussions about the effects of roads as factors threatening native biodiversity in the Argentine Pampas (Bertonatti and Corcuera, 2000), this is the first empirical approach pointing out their importance in relation to conservation. Habitat subdivision has been internationally cited as a significant factor hindering the continued persistence of amphibian populations mainly in forest habitats and our results support this idea for grassland toads.

At the international level, many efforts have addressed the issue of survival of amphibian eggs and tadpoles (Hayes and Jennings, 1986; Bradford, Tabatabai and Graber, 1993; Blaustein et al., 1996; Kiesecker and Blaustein, 1997), however, changes in the survival of juveniles or adults have been shown to have the strongest effect on the population dynamics of some declining pond-breeding amphibians (Biek et al., 2002). Many papers stress the specific effect of roads on amphibians mortality, but usually without an estimation of the importance of this factor relative to population size (Ashley and Robinson, 1996; Fahrig et al., 1995; Vijayakumar, Vasudevan and Ishwar, 2001; Seburn and Seburn, 2000; but see Hels and Buchwald, 2001 and Orłowski, 2007, both reporting a 10% annual mortality). In the case of this study, the estimated number of individuals dead due to collisions with vehicles represent up to an annual mortality of 5.9% of the total estimated population size. This figure could be an underestimation of the real casualties by vehicles in our study site, as far as we used an optimistic 40% detection rate (see Slater, 2002 for a discussion about differences between number of corpses found and number of individuals killed). The fact of finding many more dead toads on the road than those that proved to successfully disperse across it, despite the great effort done to capture live individuals, could suggest that a high proportion of the toads that tried to cross the road were killed by vehicles. This assumption is reinforced by the intensity of traffic in the road, combined with the fact that the species is diurnal in coincidence with the higher daily concentration of vehicles. According to Puky (2005) traffic mortality in amphibians is usually independent of density. This means that traffic is most detrimental to rare species and so it should be considered a matter of concern for the species focused in this work.

In addition to the cited mortality caused by vehicles collision, other factors may reduce survival in these roadside habitats, including rapid drying, high reproductive failure, sudden habitat changes caused by maintenance works, increased frequency of fires, pollution by gas, oil and fuel losses from vehicles, and poaching as pets by tourist or collectors, as has been cited for other species of amphibians in different ecosystems (Puky, 2005). In particular, roadside maintenance in the study area implies the removal of vegetation and soil, resulting in the complete destruction of breeding ponds (pers. obs.). A more appropriate timing of maintenance work could significantly reduce the negative impact on the species. As mentioned above, the species' breeding activities are concentrated in spring and summer and metamorphosis is completed in about twenty days. Therefore by avoiding soil movements in these seasons, or at least in the 20 days following major precipitations, the impact of road maintenance on this population could be greatly reduced. Fire, in turn, has been reported as a key factor affecting amphibian populations both directly, killing larvae, juveniles and adults (Spencer and Hauer, 1991; Driscoll and Roberts, 1997) and indirectly through habitat changes (Pilliod et al., 2003). In the Argentine Pampas, wildfires are more frequent during summer time, which coincides with the species reproductive activities. Moreover fire in the study area is more frequent along roadsides than in natural environments, mainly due to lit cigarettes butts thrown from vehicles and from accidents with power lines (Ernesto Tornquist Provincial Park Rangers Service, pers. comm.). An interesting feature related to the mortality associated to roads is the possibility of roadsides acting as ecological traps for the species. An 'ecological trap' is an area that appears to be a good breeding habitat, but which is affected by local conditions resulting in high mortality (Ratti and Reese, 1988; Kokko and Sutherland, 2001). Small ponds associated with road verges could result in an apparently adequate breeding habitat for the species. Although these ponds and ditches may be initially considered a beneficial consequence of road building for adult and juvenile amphibians, they may act as population sinks, as suggested for other amphibians by deMaynadier and Hunter (1995).

Apart from an effect on the survival of the toads, the road could act as a barrier reducing the willingness of individuals to move from one side to the other. The mean distance travelled by toads was significantly longer than the width of the road and the road verges combined (ca. 13 m). However, in 97% of the recaptures, the individuals moved along the road and only two out of 76 recaptures were made on opposite sides of the road. This could be considered as an indication of possible restrictions to the movement of the toads associated to the presence of the road. Nevertheless it is important to note that these conclusions about the effects of the road on toads' mobility must be treated with caution due to the restricted distribution of the species which did not allow the use of a control area without a road. Movement patterns of anurans are known to be influenced by environmental conditions, such as temperature and moisture availability (Sinsch, 1988; Bayliss, 1995; Bartelt, 2000) and there is a possibility that the reduced crossings are a consequence of a tendency of this toad to remain close to the streams. In previous observations made by the authors in the study area, adults of *Melanophryniscus* were recorded at distances from the nearest stream that greatly exceeded the distance between the ditches on either side of the road, which reinforces the idea that the reduction in mobility detected in the present study could be associated with the presence of the road. In the same direction, the interval between captures and recaptures (more than eight months in fifty percent of the cases) allowed time for significant individual movements and so recaptures on the same side of the road can not be blamed on a reduced time between observations. So, in spite of the cited restrictions in sampling design derived from the limited distribution of the species, we think that these results should be taken as an indication that paved roads could hinder the mobility of this species.

Finally, our results also showed some new interesting features about the species' biology in the area that in turn might affect the impact of the road. The sex ratio calculated in our study is 4:1, but for the proportion of re-captures it is 28:1, favouring the males in both cases. Males are much more conspicuous and easier to locate than females due to their vocalizations, and so the method used is not appropriate for determining an absolute sex-ratio, but a considerable effort was made to detect both sexes during the surveys and so the reported difference between captures and re-captures may be indicative of a real bias in the proportion of active individuals of each sex. This could result in a larger number of males being impacted by direct mortality caused by vehicle collisions what in turn could affect the sex ratio of the population, being this effect probably hidden by the cited higher mobility of males.

Our study area is undergoing a rapid increase in tourism and so road traffic is expected to rise in the near future. It has been shown that the proportion of dead frogs and toads increases with growing traffic intensity (Fahrig et al., 1995, but see Mazerolle, 2004). In this context, a significant impact on this species and others with similar habits should be considered a concrete threat and it is important to develop mitigation and or compensation measures, like underpasses, in order to avoid irreversible changes in their populations.

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