1	Mining noise affects loud call structures and emission patterns
2	of wild black-fronted titi monkeys
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29 Abstract

30 Human activity has resulted in increased anthropogenic noise on soundscapes. Noise pollution can 31 constrain acoustic communication and prevent effective animal communication. Our aim was to 32 investigate how the black-fronted titi monkey (Callicebus nigrifrons) is affected by noise produced by 33 mining activity in a fragment of Atlantic forest in Brazil. We installed two passive acoustic monitoring 34 devices to record 24h/day, 7 days every two months, for a year, one unit close to an opencast mine and 35 the other 2.5km away. Both sites presented similar habitat structures and were inhabited by groups of C. 36 nigrifrons. Sound pressure levels measurements were undertaken six times for 20 minutes on different 37 days at both sites. The number of Callicebus loud calls was quantified at both sites by analyzing the 38 recorded files. The site close to the mine presented higher noise levels than the one further away. More 39 black-fronted titi loud calls were detected at the far site and many vocalisations (20.32%) from the site 40 close to the mine were masked by noise. Duration of loud calls was longer at the site far from the mine 41 and the diel pattern of vocalisations was different between the two sites. Our results indicate that mining 42 noise can constrain Callicebus long distance vocal activity, probably, because their loud calls occupy a 43 similar frequency band of the noise. Given that vocalisations are important regulators of social behavior 44 in primates, consideration should be given to the impact of mining noise on their behavior in impact 45 evaluations and mitigation recommendations.

46

47 Keywords: Animal communication, anthropogenic activity, primates, social behavior, sound masking

49 Introduction

50 Acoustic communication is essential in the lives of many species as they use such signals to 51 transmit biologically relevant information; for example, to find reproductive partners (Brumm et al. 2009), 52 to escape from predators (Chan et al. 2010) and defend resources (Zuberbuehler et al. 1997). However, 53 the sound produced by human activities (anthropogenic noise) has become a common impact on animal 54 communication systems (Slabbekoorn and Ripmeester 2008; Barber et al. 2009; Laiolo 2010). Noise can 55 interfere with the propagation and detection of signals by masking animal sounds and thus, preventing 56 effective species communication (Foote et al. 2004; Bee and Swanson 2007). Noise pollution can also 57 affect the behaviour of many species. Studies have shown that animals avoid foraging in noisy areas 58 (Schaub et al. 2008), increase their vigilance behaviour in presence of noise (Delaney et al. 1999; Karp 59 and Root 2009), select quiet areas to perform their daily activities (Sousa-Lima and Clark 2009, Duarte et 60 al. 2011) and can be distracted by noise, thereby increasing the risk of predation (Chan et al. 2010). Noise 61 can also cause physiological stress (Campo et al. 2005, Kight and Swaddle 2011) and impact on 62 ecological aspects of animals lives such as population distribution (Reijen et al. 1998; Bejder et al. 2006), 63 species abundance (Bayne et al. 2008) and diversity (Proppe et al. 2013).

64 Animal survival can be severely impaired by anthropogenic noise and many studies have 65 documented a range of adaptive responses to minimize the immediate impact of noise of communication 66 systems including: changes in frequency (Slabbekoorn and Peet 2003; Parks et al. 2007; Nemeth and 67 Brumm 2009), amplitude (Brumm 2004; Brumm et al. 2009; Hage et al. 2013), calling rate (Sun and 68 Narins 2005) number of notes (Slabbekoorn and Boer-Visser 2006), timing (Fuller et al. 2007) and 69 duration of the calls (Brumm et al. 2004). The direct impact of noise on animal behaviour and ecology, 70 and incidental costs of maintaining an efficient communication system through compensatory 71 mechanisms can impose fitness costs on affected individuals (senders and receivers) and consequently on 72 their survival and reproduction (Chan et al. 2010; Schroeder et al. 2012), and lead to population-level 73 changes.

Beyond the effect of deforestation caused by mining, one less obvious impact on wildlife is the noise produced by such activity. Mining noise, especially if produced at the same frequencies that animals use in their vocalisations can mask important calls and, consequently, greatly reduce the efficiency of animal communication (Bee and Swanson 2007, Alvarez-Berríos and Aide 2015; Duarte et al. 2015). However, the effects of mining noise on animals have been poorly documented, especially in the Neotropical region. Smith et al. (2005) showed that diamond mines affect tundra birds by lowering breeding bird densities. In India, stone mining and crushing affected bird species diversity and population density in the areas adjacent to crushers (Saha and Padhy 2011). Thus, studies involving mining noise impact in terrestrial mammals and their communication systems are still lacking, and the noise effects on this group must be better understood, in particular with respect to the primates that use acoustic communication for a variety of vital processes.

85 Species of titi monkeys (genus Callicebus) exchange loud calls (duets) to either defend territories 86 or food resources in their home-ranges; thus, these vocalisations are important regulators of their social 87 behaviour (Robinson 1979, 1981; Kinsey and Robinson 1983; Price and Piedade 2001; Caselli et al. 88 2014). Primates of the genus *Callicebus* live in monogamous family groups, consisting of a reproductive 89 pair and up to four generations of offspring (Kinzey and Becker 1983; Mendoza and Mason 1986; 90 Valeggia et al. 1999). Titi monkeys are morphologically cryptic primates, which hinders surveying them 91 using traditional methods such as linear transects (Aldrich et al. 2008). Mated pairs of Callicebus species 92 regularly emit loud and coordinated calls (duets), which permit researchers to use an alternative and 93 potentially more accurate method to monitor populations based in call surveys (Melo and Mendes 2000; 94 Aldrich et al. 2008). Duetting is commonly used by many bird and primate species for both within and 95 between group communication (Hall 2004; Oliveira and Ades 2004). Studies of Callicebus species show 96 that their duets have a role in group location and avoidance of intergroup aggressive encounters (C. 97 lucifer, previously C. torquatus, Kinzey and Robinson 1983; C. personatus, Kinzey and Becker 1983; 98 Price and Piedade 2001), in territory establishment and probably mate defense (C. ornatus, previously C. 99 moloch, Mason 1968; Robinson 1979, 1981). Black-fronted titi monkeys (C. nigrifrons) loud calls are 100 used during intergroup communication to regulate access to important food resources, such as fruits. 101 There is also some evidence that loud calls are used for mate defense (Caselli et al. 2014).

Typically, titi monkeys vocalise mostly at dawn, but also during the day when another group is sighted or heard (Kinzey et al. 1977; Kinzey and Robinson 1983; Melo and Mendes 2000). For *C. nigrifrons* mostly loud calls are emitted more often from their core area or near from important food resources in their home range that usually is around 8 ha in Atlantic forest (Santos 2008, Caselli 2008, Santos 2012). Large areas demand more time and energy to patrol (Schoener 1987), and black-fronted titi monkeys advertise the occupancy of its territory via loud call emissions and do not use patrol and mark range boundaries (Santos 2012; Caselli 2013). 109 Many of the forests in South America, where titi monkeys live suffer from large scale mining 110 (Estrada 2009). In the state of Minas Gerais, Brazil, mining is an important economic activity and is 111 commonly conducted close to Atlantic forest, one of the world's richest biodiversity hotspots (Myers et al. 112 2000). The Atlantic forest is one of the most impacted habitats of the world retaining only 7% of its 113 primary vegetation (Myers et al. 2000) and is home to the black-fronted titi monkey (Callicebus 114 nigrifrons); an endemic primate classified as Near Threatened on the IUCN's Red List (Veiga et al. 2008). 115 Black-fronted titi monkey loud calls are characterized by different syllables composed by 116 components of high frequencies that ranges from 3 to 12 kHz and of low frequency that is near to 1 kHz 117 (Caselli et al., 2014). Due to spectral characteristics of titi monkey loud calls such as high amplitude and 118 low frequency, these calls can be heard over long distances (Melo and Mendes 2000; Caselli et al. 2014). 119 Unfortunately, the same acoustic characteristics that were adaptive for long distance communication are now bringing these sounds into competition with mining noise. 120

In this study, we investigated how the noise produced by one of the largest opencast mines of the world affects acoustic communication of *C. nigrifrons* in an Atlantic forest fragment in Southeast Brazil. Here we tested the following hypotheses: (1) noise levels are different in the sites close and far from the mine; (2) emission rate, duration and diel pattern of titi monkey loud vocalisations would change between the areas due to noise exposure.

126 Methods

127 Study area

This study was conducted at Peti environmental station, which is located in an Atlantic forest fragment of approximately 605 hectares. The reserve is located in the upper Rio Doce Basin (altitude range: 630-806m) in the municipalities of São Gonçalo do Rio Abaixo and Santa Bárbara, Minas Gerais state, Brazil (19°53'57''S and 43°22'07''W), one of the most fragmented Atlantic forest regions of Brazil (Machado and Fonseca 2000). Peti environmental station harbors approximately 46 species of mammals (Paglia et al. 2005), 231 species of birds (Faria et al. 2006) and 29 species of anurans (Bertoluci et al. 2009).

A large part of the reserve is covered by secondary arboreal vegetation, and is surrounded by a matrix mainly composed by *Eucalyptus*, small farms and areas of exposed soil due to the activities of the Brucutu mine. Mining activity occupies an area of approximately 8km² and produces noise through road traffic, heavy machinery, sirens and explosions during the day and night (Roberto 2010). Brucutu's iron 139 ore extraction started in 1992 and to increase the capacity of iron production, expansion projects started in

140 2004 placing Brucutu among the largest opencast mines in the world (Roberto 2010).

141 Data collection

142 To record black-fronted titi monkey loud calls, two song meters (SM2, Wildlife Acoustics) were 143 installed into the home range of two groups of titi monkeys. One group inhabited a forest fragment close 144 to a mine site and the song meter was installed at a distance of 100m from the closest mining road (Fig.1). 145 Another group inhabited a forest fragment 2,500m far from the mine and the song meter was installed 146 100m away from a low traffic ('quiet') road (to control for a potential border effect at both sites in the 147 same Atlantic forest fragment). The positions of geographic barriers such as roads and a river that 148 surround close and far sites suggest that the group which inhabit the close site is isolated from the group 149 that inhabit the far site. Both sites were habitat matched; they presented similar floristic compositions and 150 habitat structures.

151 The passive acoustic monitoring devices were programmed to record 24h/day during seven days 152 every two months from October 2012 to August 2013, in a total of six sessions and 2,016 hours of 153 recordings. Each SM2 was fixed on a tree 1.5m above the ground, leaving the two lateral microphones 154 free from any surface that could be an obstacle to incoming sound waves. They were configured to record 155 in wave format at a sampling rate of 44,100Hz, at 16 bits, and with a 36% microphone gain. Pilot studies 156 had found that this configuration to be optimal for recording the soundscape of the Atlantic forest (Pieretti 157 et al. 2015). The loud calls of titi monkeys can be detected up to 500 m away, with a 'critical distance' of 158 about 250 m (Robinson, 1981).

The sound pressure levels at both sites were characterized by using B&K2270 (Denmark) sound level meter configured on the A curve to conduct six measurements of 20 minutes length in at both sites, from 0600 to 1800 hours on weekdays. This research adhered to the Brazilian legal requirements and to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non Human Primates.

164 Data analyses

To test for a difference in noise levels between the sites close and far from the mine we extracted data from the sound level pressure measurements and analyzed them using BZ 5503 software (Bruel and Kjaer). To avoid bias in the measured levels we excluded all recordings, which included loud animal sounds (i.e., animals close to the microphone). The rate of emission and the duration of black-fronted titi monkey loud calls were measured in both sites during seven days by session from 0500 to 1700 hours totalizing 1,092 analyzed hours. All sound files used for analyses had to be visually and aurally checked in Raven Pro 1.5, since we try to use the band limited energy detector but this resulted in a large number of false positives and misses. We also manually detected all the loud calls, which were partially masked by anthropogenic noise at the site close to the mine (Fig. 2).

To verify a possible association between the noise produced by mining truck traffic at the site close to the mine (the road of far site was not trafficked by mining trucks) and the occurrence of the loud calls, we quantified all trucks passing from 0500 to 1700 hours at the road in front of the sampling site. This procedure was done by audio and visual identification of the trucks' noise pattern in spectrograms. An FFT size of 1024 points was used for all analyses in Raven Pro 1.5.

We used a nonparametric statistical approach with our data analyses since data did not meet the
requirements for parametric statistics even after data transformations. All the statistical analyses were
performed in Statistica version.8.0.

183 Results

Sound pressure (noise) levels were significantly higher at the site close to the mine (MannWhitney U-test: U=1, Z=2.72, N=6, p<0.01), as expected (Table 1).

186 Black-fronted titi monkeys emitted more loud calls than expected at the site far from the mine 187 (Chi-squared test: X^2 = 339.96, df=1, P<0.001, N_{close}=187, N_{far}=752). A considerable part (20.32%) of the 188 vocalisations found in the site close to the mine was partially masked by noise from mining activity (Fig. 189 2). Duration of loud calls were also significantly longer at the site far from the mine (Mann-Whitney U 190 test: U= 29142.5, Z= 12.40, N_{close}=187, N_{far}=752, P<0.01; Median_{close}=1.77, Median_{far}=16.33). The 191 temporal distribution pattern of the vocalisations was also different between the two sites (Fig. 3). At the 192 site far from the mine, titi monkeys were more vocally active early in the morning (from 0600 to 1000 193 hours, with peak vocal activity around 0700 hours), while at the site close to the mine they presented a 194 constant but very low activity from 0700 to 1000 hours with peak vocal activity occurring around 1300 195 hours.

196 The time period of highest truck passing activity coincided with the time period of the lower 197 number of loud vocalisations at the site close to the mine and the peak of loud calls also occurred when

- 198 there was a decrease in trucks passing (Fig 4). Despite this, a Spearman rank test showed no significant
- 199 correlation between the number of trucks and number of vocalisations ($r_s = -0.21$, t = -0.71, P>0.05).
- 200 Discussion

Our results show that the emission rate, duration and diel pattern of loud calls emitted by blackfronted titi monkeys is different between sites close to and far from mining activity. These changes in vocal parameters of titi monkeys calls are similar to those exhibited by some animals to compensate the impact of anthropogenic noise or as a response to avoid noise interference on their communication (Brum et al. 2004; Sun and Narins, 2005; Egnor et al. 2007).

206 The higher rate of loud calls found at the far site could be explained by several non-exclusive 207 hypotheses, such as: (1) more titi monkey groups are present at the far site; (2) more encounters between 208 titi groups at the far site; (3) titi monkeys from the close site were reducing their emission of calls due to 209 masking caused by mining noise; (4) call emissions masked by noise decreasing detection of vocal 210 activity at the site close to the mine. However, field observations and habitat matching showed that there 211 should be a very similar numbers of groups at both sites. The area monitored by the passive acoustic 212 monitoring devices was the same at both sites. Thus, while there will be some differences between sites, 213 these are unlikely to be the major factors affecting differences in the rate of loud call emissions. In 214 addition, as observed for C. personatus, and C. torquatus, C. nigrifrons also do not call more often 215 from their home range boundaries and encounters with neighbouring groups are not frequent (Kinzey et al. 216 1977; Price and Piedade 2001, Santos 2012, Caselli 2013), since the regularly loud call emissions from 217 core area or from more valuable sites can be more economical strategy. Another noteworthy factor is the 218 longer duration of calls at the far site. This fact supports the third hypothesis: as it demonstrates the 219 probably impact of mining noise on titi monkey's loud vocalisations.

220 A decrease of animal call rate in presence of noise has already been established in other studies 221 and can be interpreted as a response to avoid interference from anthropogenic noise (Miksis-Olds and 222 Tyack 2009; Sun and Narins 2005; Parks et al. 2007; Sousa-Lima and Clark 2008). This pattern may 223 indicate that animals wait until it is quiet to vocalize, exhibiting only minimal vocalisation effort during 224 periods of masking noise (Miksis-Olds and Tyack 2009; Sousa-Lima and Clark 2008). In this study, at the 225 close site many loud calls (20%) were partially masked by noise, thereby potentially disturbing the 226 exchange of acoustic information and preventing titi monkeys from communicating effectively (Lohr et al. 227 2003; Foote et al. 2004; Bee and Swanson 2007). One particularly important factor driving vocalisation

effort is the range over which the signaller and receiver must effectively communicate (Miksis-Olds and
Tyack 2009). In this context, when noise masks the vocalisations there is a decrease in the acoustic space
over which the information can reach.

231 The longer duration of titi monkey loud calls at the far site is further evidence of the noise 232 impact from mining. Research has already documented that some species adjust their vocal behaviour to 233 compensate for anthropogenic noise by increasing or decreasing the duration of the calls. Studies with 234 Saguinus oedipus showed a decrease in the average call duration to avoid masking noise (Egnor et al. 235 2007). However, common marmosets *Callithrix jaccus* increase the duration of their calls in presence of 236 noise and they use higher vocal frequencies (Brumm et al. 2004). Our results, suggest that there is more 237 available acoustic space at the far site, especially in the lower frequencies, which are naturally used by titi 238 monkeys. At the close site, noise from the mine overlap the titi monkeys' loud calls and could be 239 excluding them from an acoustic niche. Thus, they probably are emitting calls with shorter duration to 240 communicate more effectively and/or to save energy since acoustic communication is an energetically 241 expensive behaviour and vocalisation effort is increased by increasing call duration (Miksis-Olds and 242 Tyack 2008).

243 The difference in the diel pattern of loud calls between the two sites can be also a consequence 244 of the mining noise disturbance on titi monkeys' vocal behaviour. As observed in other primate species 245 such as Indris (Geissmann and Mütschler, 2006) and gibbons (Mitani, 1985), C. nigrifrons are vocally 246 active mainly during the first hours of the day (outside encounters) (Melo and Mendes 2000, Caselli 247 2013). Because of the higher humidity and lower temperatures in the first hours of the morning, these 248 primates concentrate the emission of loud calls in this period of the day, when transmission of sound 249 presumed to be more efficient (Mitani 1985; Wiley and Richards 1978). In our study, this natural pattern 250 was observed only at the far site. At the site close to the mine, animals displayed very low vocal activity 251 in the first hours of the day and peak of activity at 1300 hours. Many mammals affected by anthropogenic 252 noise have limited developmental capacity to change the acoustic parameters of their calls to avoid the 253 masking by noise such as some birds can do (Weiss et al. 2014). On the other hand, mammals may avoid 254 noise with other behavioural modifications, such as vocalizing during periods of low noise (Rabin et al. 255 2003) or moving to quieter areas (Duarte et al. 2011).

Loud vocalisations are key factors involved in the regulation of titi monkey social behavior(Caselli et al 2014). One consequence of the masking of such calls can be increased territory invasion by

258 neighboring group and consequently increased rates of inter-group agonistic encounters. Such changes 259 could impact on the survival and reproductive success of the affected individuals, and result in disruptions 260 with potential population-level consequences. In addition, studies with birds show that species with lower 261 frequencies calls are more likely to avoid roads than those that emit calls at higher frequency, indicating 262 how noise may change the organization of avian communities (Rheindt 2003; Francis et al. 2009). Similar 263 effect could happen to titi monkeys communities that can prevent to use suitable habitats to avoid overlap 264 of noise. Considering that C. nigrifrons is a "Near Threatened" endemic primate (Veiga et al. 2008), this 265 effect is very concern for species conservation in long term.

Lastly, our results suggest that, apparently, there is no noise effect on titi monkeys loud calls at site that is 2,500m distant from the opencast mine. This information provide insight that can help into developing distance regulations for areas of environmental compensation and/or biologically important, and highlight the importance of considering noise pollution when determining reserve locations (Madliger 2012).

Finally, here, we have shown for the first time how a noise disturbance can affects black-frontedtiti monkey communication. Our results provide important information to be considered during the elaboration of conservation strategies in natural areas affected by mining activity. Furthermore, we suggest that noise monitoring plans for wildlife should be part of the process of licensing large scale anthropogenic activities such as mining.

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521 Table 1. Equivalent sound pressure levels (Leq) at sites close to and far from an opencast mine site near

522 Peti environmental station, southeast Brazil.

Measurement	Close	Far
	Leq dB(A)	Leq dB(A)
1	42.6	33.8
2	38.7	30.3
3	42.0	30.1
4	60.9	37.2
5	42.9	38.8
6	41.2	33.3

523

524 Fig. 1 Sites close to and far from the Brucutu mine at Peti Environmental station, southeast Brazil. Red

525 lines represent the geographic barriers between the sites

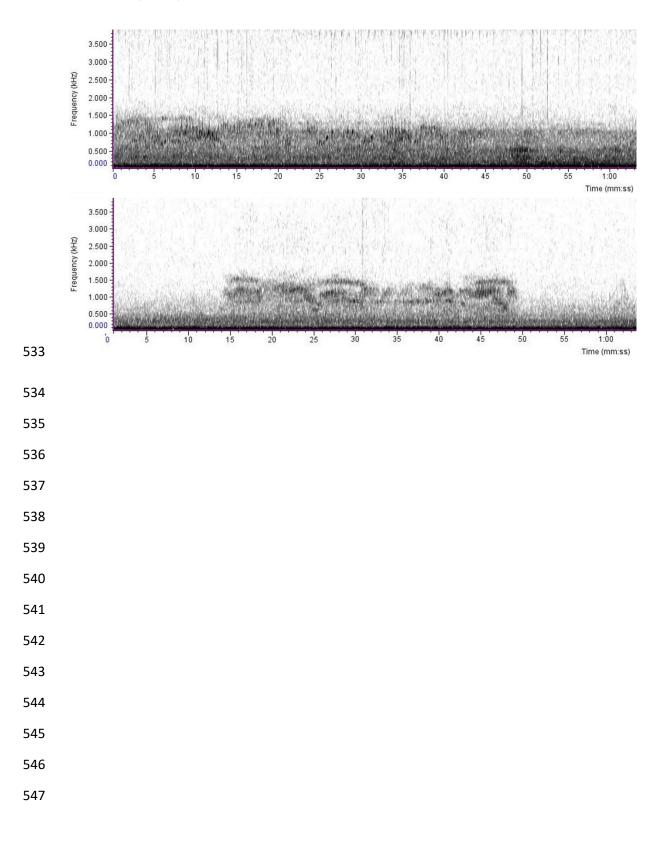




528

Fig. 2 Sound spectrograms of black-fronted titi monkey loud call, at Peti Environmental Station southeast
Brazil, showing masking by noise mining activities (top) at a location close to a mine site, and non

532 masked call (bottom) far from a mine site



548 Fig. 3 Daily distribution of the mean (±SD) number of loud calls emitted by black-fronted titi monkeys

at sites close to and far from an opencast mine site near Peti environmental station, southeast, Brazil

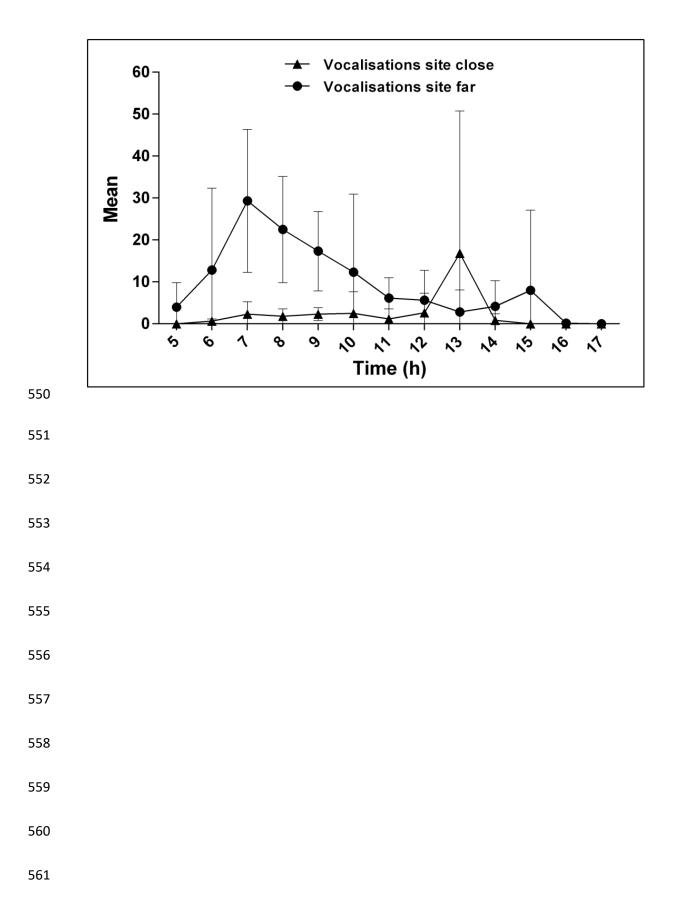


Fig. 4 Daily distribution of mean mining truck activity (number passing a fixed point) and mean
frequency of loud calls of black-fronted titi monkey close to an opencast mine site near Peti
Environmental station, southeast Brazil

