

Chapter 6

Testing Automated Behavioural Response systems as a methodology to investigate behavioural hypotheses, using pygmy marmosets and the distracted prey hypothesis as a model system

6.0 Abstract

Every year approximately eight billion tourists visit terrestrial protected areas and diverse scientific studies have investigated the implications for wildlife. There are currently multiple hypotheses used to describe how humans change animal behaviour, including the distracted prey hypothesis, which postulates that prey species are distracted by any sound they can perceive increasing their predation risk. One problem in testing these behavioural hypotheses is the time required to field-test them and another is observer effects. Technological advances can overcome these problems and increase field efficiency. For example, the Automated Behavioural Response system (ABR), combines an audio playback with a camera trap, capturing a focal individual's response to an audio stimulus. ABRs can quickly generate large sample sizes, allowing statistical evaluation of hypothesis that would be untestable using other methods. In this study I demonstrate this by generating 1,268 video recordings and 147 successful playback trials in a 5-week pilot study. My application of the ABR system with wild primates demonstrates how this system can be applied to future behavioural ecology studies. Using the recommendations given in this chapter, the ABR has the potential to transform how playback experiments are conducted.

6.1 Introduction

Human visitation to natural areas has been found to have significant effects on the environment and the wildlife found in those landscapes (Shannon *et al.*, 2017). The ever-expanding tourism market has seen approximately eight billion visits per annum to terrestrial protected areas globally and generates approximately 600 billion US dollars for local economies (Balmford *et al.*, 2015). The ecotourism sector is already a significant portion of the global tourism market and is continuing to expand rapidly (Moorhouse *et al.*, 2015). Wildlife tourist attractions are a trade-off between visitor fulfilment, animal welfare and conservation (Reynolds and Braithwaite, 2001). As ecotourism has increased in popularity so have scientific studies looking at the implications of these activities on wildlife (Blumstein *et al.*, 2017).

Understanding the effect of human presence on wildlife and the disturbance to the ecosystem humans can bring is pertinent to ensuring continued effective conservation management of wildlife, especially in the context of increasing ecotourism. As their environment changes, animals will shift their behaviour in reaction to this change (Møller, 2017). Over time antipredator behaviours have been found to completely disappear in environments that are considered “predator-free”, through relaxed selection pressure these changes can occur on a more rapid timescale through a relaxed selection pressure (Blumstein *et al.*, 2004). These longer lasting effects are documented both the urbanisation and domestication processes, however, ecotourism can still have long lasting effects even if the tourist’s presence is only temporary (Geffroy *et al.*, 2015). The long-lasting effects of the temporal aspect of tourism is largely understudied, especially in regards to antipredator behaviours.

There are three main hypotheses that link risks of predation with the interactions found between humans and wildlife, and can be applied to wildlife viewing ecotourists: the human shield effect, the distracted prey hypothesis and the risk disturbance hypothesis. The human shield effect is thought to cause prey species to relax in the presence of humans and to cause them to decrease their antipredator behaviours (Geffroy *et al.*, 2015). It may also result in prey species moving towards humans due to this protective theoretical shield where they experience less predation (Muhly *et al.*, 2011). The second is the distracted prey hypothesis, which postulates that animals are able to become distracted by any stimulus it can perceive which can cause it become more susceptible to predation (Chan *et al.*, 2010). Hermit crabs (*Coenobita clypeatus*) are distracted when played the sound of a motor boat which allows simulated predators to get closer than they would otherwise (Chan *et al.*, 2010). The third is the risk disturbance hypothesis which postulates that animals perceive human disturbance similarly to predation risk (Walter, 1969). Nubian ibex (*Capra nubiana*) have been found to show similar behaviours in response to the tourists as they would to natural predators (Tadesse and Kotler, 2012)

These three hypotheses have been tested in various contexts, however, our understanding of which of these hypotheses is applicable in different wildlife-human interactions is difficult as most existing approaches have an observer confounding the results (Suraci *et al.*, 2017).

Additionally, studying the impacts of human activity on animal behaviour is particularly challenging in tropical rainforest environments as most of the wildlife found in these habitats occur at low densities and are naturally cryptic (Linkie *et al.*, 2008). Audio playback experiments allow a researcher to create a naturally rare event and are becoming a vital instrument when testing anti-predator responses (Suraci *et al.*, 2017). They are normally conducted by placing a speaker out of view and then a researcher records the focal individual's reaction to the audio stimuli (Fischer *et al.*, 2013). Researchers being present while recording data can be the cause for observer effects as the study organism shifts their behaviours due their presence (McDougall, 2012) and their presence has been found to bias behavioural studies (Jack *et al.*, 2008). Camera trap methodologies have allowed researchers to study predator-prey ecology in the wild without the need for habituation (Smith *et al.*, 2020). In a review conducted by Smith *et al.* (2020) they found that only 9% of predator-prey studies on wild animal populations used camera traps as a part of their methodology, they did however find an uptake in their use in the available literature.

The combination of playbacks and camera trap methodologies create a novel non-invasive approach to study predator-prey interactions and anti-predator responses in wild animals (Suraci *et al.*, 2017). The Automated Behavioural Response (ABR) system is the combination of auditory playback methodologies combined with a camera trap, it works by deploying an audio cue when the camera trap is triggered, so the camera trap then captures the focal individual's response to the audio stimuli (Suraci *et al.*, 2017). The ABR is a powerful experimental tool which allows researchers to capture the responses of focal species to a variety of audio cues. It also has the unique ability of generating sufficient sample sizes to be able to statistically evaluate ecological and behavioural hypothesis that were previously untestable using the former standard methods (Suraci *et al.*, 2017). This methodology has already been used successfully to explore wildlife responses to different anthropogenic disturbances (Smith *et al.*, 2017) and specifically ecotourists (Mugerwa, 2018). The wildlife in Mugerwa's study (2018) do not perceive ecotourists as threats by displaying the same reactions to ecotourists that they did for insects, they did show fear responses for local predator species. It has also been used to showcase the presence of fear-induced trophic cascades in multi-predator-prey systems (Rigoudy *et al.*, 2022). It has even been used in wildlife management, showing promise as an instrument to reduce crop damage by ungulates (Widén *et al.*, 2022) and suggested for potential use for primates as well

(Findlay *et al.*, 2022). Palmer *et al.* (2022) developed the ‘BoomBox’ which is an open-source Arduino-compatible board which one can attach to any commercially available camera trap to form an ABR system, making sourcing an ABR more cost and time efficient.

In this chapter I am conducting a pilot study to test the ABR system as a potential solution to overcome observer biases found when using more traditional playback experiments, using pygmy marmosets as the study species to see how the anthropogenic noise created by ecotourism in the Amazon influences their behaviour. I will test whether the ABR would be an appropriate choice to study and test behavioural hypotheses. Having a clearer understanding as to how ecotourists are affecting an animal’s perception of their environment is important as ecotourism continues to become more dominant in the tourism sector. If we can understand how these primates are interacting with ecotourists it will allow for more targeted management changes. For instance, if I find that motor boats are distracting prey species I could suggest that tour operators switch off the engine when viewing the monkeys, or use canoes to interact with the wildlife. Having concrete evidence to help guide these practices will help garner support for their widespread use in the ecotourism industry.

This study is comprised of two playback experiments, the first using anthropogenic noise, predator calls and control audios to create a baseline understanding of the marmoset’s reactions to these sounds. Then the second experiment explores the distracted prey hypothesis by testing if the anthropogenic noise that comes with tourism can distract the marmosets from potential predation events, by playing them audio of motor boats and human speech that have been spliced with the calls of birds of prey. After being played the audio of a predator call I expect the marmosets to either flee or spend the majority of their time being vigilant and calling more frequently than in the other conditions. For the anthropogenic noise audios, I expect an increase in vigilance but not as much as with predator calls. For controls I expect eating and self-grooming behaviours to dominate their activity budget. For the second experiment if the distracted prey hypothesis is applicable in this system then I suspect that motor boat audios will be more distracting and therefore the marmosets will display less frequent anti-predator behaviours (i.e. calling and fleeing) for the audios with the bird of prey call, but when played the audios with human speech they will be more vigilant in both spliced stimuli due to presence of

the human speech audio.

6.2 Methodology

6.2.1 Study Site

This research was conducted in the Área de Conservación Regional Comunal Tamshiyacu Tahuayo (ACRCTT) a communal reserve located in north-eastern Peru. It was established in 1991 by the local community, researchers and conservationists to protect the endangered red uakaris (*Cacojao calvus ucayalii*) and to try to limit activities by hunters and loggers from outside of the region (Newing and Bodmer, 2003). In 2009 the reserve was upgraded to the status of state reserve and its size was increased to 420,080 ha (Penn, 2009).

The only manmade structure inside the reserve is the Tahuayo River Amazon Research Centre (TRARC) on the black-water Tahuayo River. It is run by the tour operator, Amazonia Expeditions. Amazonia Expedition's main accommodation site opened in 1995, and is situated just out of the reserve limits. Their peak tourist season is July-August, during the dry low river season. Close to the main lodge and the reserve boundary is the local community, El Chino. There are a number of groups of pygmy marmoset whose main feeding trees are dispersed throughout the community. The main source of anthropogenic noise in the area comes from motor boats (Chapter 3), as this is the main transportation system for the area. Chapter 3 showed that across the different areas in this site (outside and inside the reserve) there is a significant difference in the amount and variety of anthropogenic noise, the majority of the anthropogenic noise coming from El Chino and the main lodge.

6.2.2 Study Species

The eastern pygmy marmoset, *Cebuella niveiventris*, is a Neotropical primate species found in the Amazon rainforests of Bolivia, Brazil and Peru (de la Torre *et al.*, 2021). Group size ranges from 2-9 individuals (de la Torre *et al.*, 2000) and they are habitat specialists found in the forests along river-edges. They have small home ranges, ranging from 0.1-0.5 ha which feature 1-6 central feeding trees (Soini, 1988). See Chapter 2 for an in-depth species description.

As pygmy marmosets are a specialist species, they are incredibly vulnerable to changes in habitat and human activity (de la Torre *et al.*, 2000). Their small home ranges and repeated visits to a

small number of trees where they extract sap and gum, make it relatively easy to predict their daily movements (de la Torre *et al.*, 2000), and makes them popular with tour operators. For these reasons they are the ideal primate species to conduct a camera trapping study. de la Torre *et al.* (2000) has provided evidence that this primate is sensitive to capture and human traffic which causes behavioural change and has been found to cause a decrease in group size and their reproductive rate.

6.2.3 Experimental stimuli

Predator Calls

Playback experiments have long been used to look at antipredator responses in primates in the field (Seyfarth *et al.*, 1980; Zuberbühler, 2014). With raptors being one of the main predators of primates (Mcgrae and Berger, 2013) their calls have been used across the available literature to test anti-predator behaviour and alarm call vocalisations (Fichtel and Kappeler, 2002; Cäsar *et al.*, 2012). Raptor calls were chosen for this study as little has been published on actual predation events on the pygmy marmoset, with the only main report being that when pygmy marmosets saw large raptors flying overhead (species not listed) that the marmosets displayed freezing behaviours and had a higher alarm call rate (Snowdon and Hodun, 1981). Using this knowledge raptors were chosen to be the predator vocal stimuli for this experiment.

Based on the results from the raptor survey conducted in Chapter 5 a pilot study was conducted with one group where they were played the calls of a great black hawk (*Buteogallus urubitinga*), roadside hawk (*Rupornis magnirostris*), ornate hawk eagle (*Spizaetus ornatus*), and a harpy eagle (*Harpia harpyja*). These audio clips were all obtained from the Macaulay Library. These four species were chosen as even though they were not encountered often in the study, they have all been found to consume primates, which means they could elicit anti-predator responses from the pygmy marmoset. Even if raptors are not heavily present or completely absent in an area they can still elicit anti-predator responses in primates, this was shown in Gil-da-Costa *et al.*'s (2003) study. Their study found that mantled howler monkeys (*Alouatta palliate*) reacted to harpy eagle calls even though harpies have been extinct in that area for 50-100 years, meaning that primates can detect and identify predators based on only audio cues. Based on the reactions observed in the pilot study group and knowledge about which of the birds encountered in the survey predate

upon smaller monkeys, the ornate hawk eagle and roadside hawk were chosen as the predator conditions for the pilot study.

Anthropogenic Noise

For the anthropogenic noise conditions there were two audios of motor boats (collected in the field) and two of human speech. The two audios of human speech were both in English and included one of a conversation between two people and the other of one person speaking (collected in the field).

Control Audios

The control audios consisted of a cicada (*Quesada gigas*) and one of blue-and-yellow macaws (*Ara ararauna*) both of which are found in the area, both audios were taken from YouTube videos and converted to mp3 files (Ambiance – Topic, 2019; Black Crow, 2021).

6.2.4 Experimental procedure

The custom ABR units used in this study were based off the specifications outlined in Suraci *et al.* (2017) and altered to fit the parameters of this study (see the appendix for Figures 6.8 and 6.9 with the diagrams of the circuitry of the ABR unit). The ABR was placed near an active feeding tree of 9 pygmy marmoset groups in the dry season over a 5-week period that spanned from September to October 2021. Before placing the ABR a preliminary site visit was conducted to the groups home range to see which sap holes were currently active and to map out where the ABR would be placed. Of these 9 groups, 5 had enough successful playbacks from both experiments and were included in the final analysis (Figure 6.1). The camera traps were mounted on trees adjacent to the main feeding tree, focusing on an active feeding hole of the individual pygmy marmoset group (see the appendix for Figure 6.10). The ABRs were between 1-3 meters away from the feeding tree, and 0-1.5 meters from the speaker. The speaker was concealed and camouflaged with leaves or other greenery to help disguise its presence from the marmosets. After two days the equipment was removed, if there were not enough successful playbacks for each condition then after a two day break the ABR was redeployed with the missing conditions.

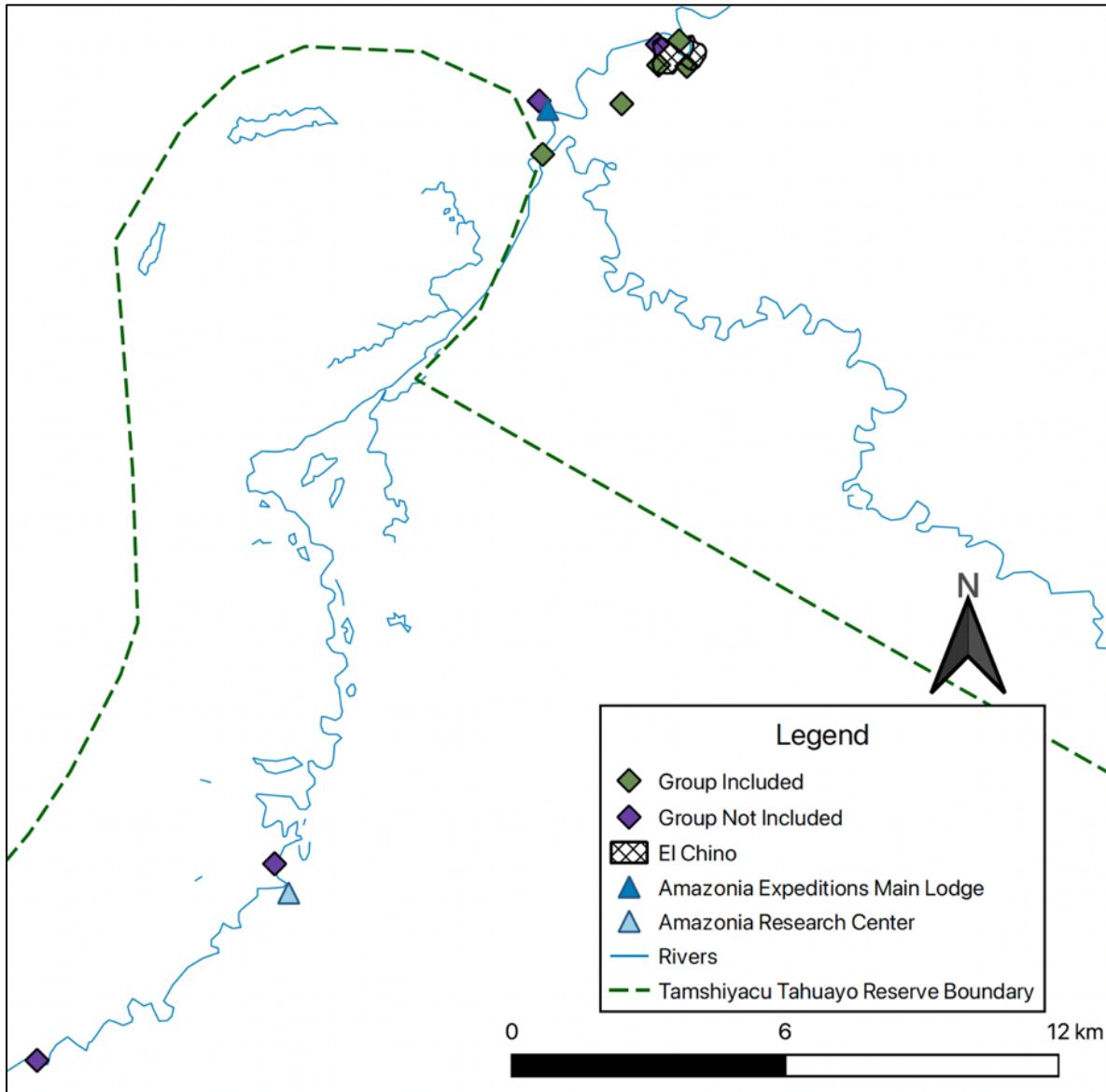


Figure 6.1 A map of the locations of the marmoset groups where the ABRs were placed in September to October 2021 with green diamonds denoting a group that underwent successful trials and was included in the final analysis and the purple diamonds denoting a group that did not complete enough playbacks to be included in the final analysis.

Experiment 1: Baseline Reactions

The first experiment was a simple playback with anthropogenic noise, predator calls and controls, to establish the marmosets' baseline behaviours when exposed to these audios. The audios were all 30 seconds in length and played 10 seconds after the camera trap was triggered and began recording. The ABR was set to record two-minute-long videos after being triggered

and was set to be in a holdoff period where the MP3 player could not be triggered for 12 minutes after a successful trial. If it is activated again after that 12 minutes then the next file on the MP3 player's playlist would play. The ABR was also set to hibernate after being active for 12 hours so the speaker would not continue playing while the marmosets were no longer active, due to this the ABRs were set up in the morning before 08:00.

Experiment 2: Testing the distracted prey hypothesis

This experiment was conducted to see if the ABR system could be used to test behavioural hypotheses. I investigated the distracted prey hypothesis by looking at the anthropogenic noise that comes with tourism in the Amazon, which I found and catalogued in Chapter 3. This playback experiment used the same anthropogenic noise audios, extending the audio length to one minute and spliced predator calls and controls into the anthropogenic noise conditions. The spliced control and predator calls played 20 seconds into the anthropogenic noise audio and lasted 15 seconds. The camera traps used the same triggering and hibernation rules as in the previous experiment.

6.2.4 Video Analysis

All the videos from successful playback experiments with the 5 groups were analysed in the software BORIS (version 7.13; Friard and Gamba, 2016), an event logging software for video coding. A successful playback was defined as videos where a pygmy marmoset was in frame when the audio was played. This individual became the focal individual for the extraction of the behavioural data. Within BORIS an ethogram was created (Table 6.1) which was used to code the videos and the data was exported into Excel datasheets and collated. From every group, two randomly selected videos without a playback were also analysed and included in the experiment 1 dataset. The video coding for this experiment was not carried out by an independent coder who was blind to the experimental conditions, so that needs to be taken into account when interpreting the results.

Table 6.1 The ethogram created and used in BORIS to code the playback videos.

Behaviour code	Description	Key	Excluded behaviours
audio	audio is played/stopped	a	
call	focal individual has called	c	eat,groom
look	focal individual looks at the camera trap	l	speaker,vigilance,eat,hunt,interact
speaker	focal individual looks at speaker	s	look,vigilance,eat,hunt,interact
vigilance	focal individual is surveying for threats	v	look,speaker,eat,hunt,interact
eat	focal individual is eating sap and or gauging sap holes	e	look,speaker,vigilance,hunt,interact
flee	focal individual flees (raid movement out of frame)	f	look,speaker,vigilance,eat,groom,hunt,interact
move	focal individual moves position in frame	m	eat,groom,hunt,interact
out	focal individual moves out of frame	o	look,speaker,vigilance,eat,groom,hunt
groom	focal individual is grooming itself	g	hunt
hunt	focal individual is hunting an insect	h	look,speaker,vigilance,eat,groom,interact
interact	focal individual is interacting with another pygmy marmoset on frame	i	look,speaker,vigilance,eat,hunt

6.3 Results

6.3.1 Experiment 1

A total of 65 videos were analysed for the first experiment (Table 6.2)

Table 6.2 A breakdown of the successful trials each group underwent in the first experiment.

Group ID	Total number of videos recorded	Total number of deployments	Total number of successful trials	Controls	Anthropogenic noise	Predator Calls	Included in final analysis
CV2	104	1	14	5	4	5	Yes
CV3	105	1	0	0	0	0	No
CV4	112	1	11	4	4	3	Yes
CV7	51	2	21	7	8	6	Yes
RC2	41	2	3	1	0	2	No
RC7	23	2	2	0	2	0	No
TL7	15	1	8	3	3	2	Yes
TL11	19	3	2	0	1	1	No
TL14	26	1	11	4	4	3	Yes
Total included				23	23	19	5

The conditions with predator call audios had the highest percentage of trials where an individual called, 74%, which also had the highest mean number of calls by an individual in a video ($M=6$, $SD=6$) (Figure 6.2). The next highest condition with the highest percentage of trials where calls occurred was motorboats, 50%, with a mean of 1 call ($SD=0.45$). However, the condition with the next highest mean number of calls a video was the condition where no sound was played ($M=4$, $SD=4$).

The focal individual fled the most in trials with predator calls (fleeing in 32% of trials) and human speech (fleeing in 31% of trials) (Figure 6.3). They fled in 11% of cicada and 10% of motor boat playbacks, and did not flee in any of the trials where the macaw and no audio were played (Figure 6.3).

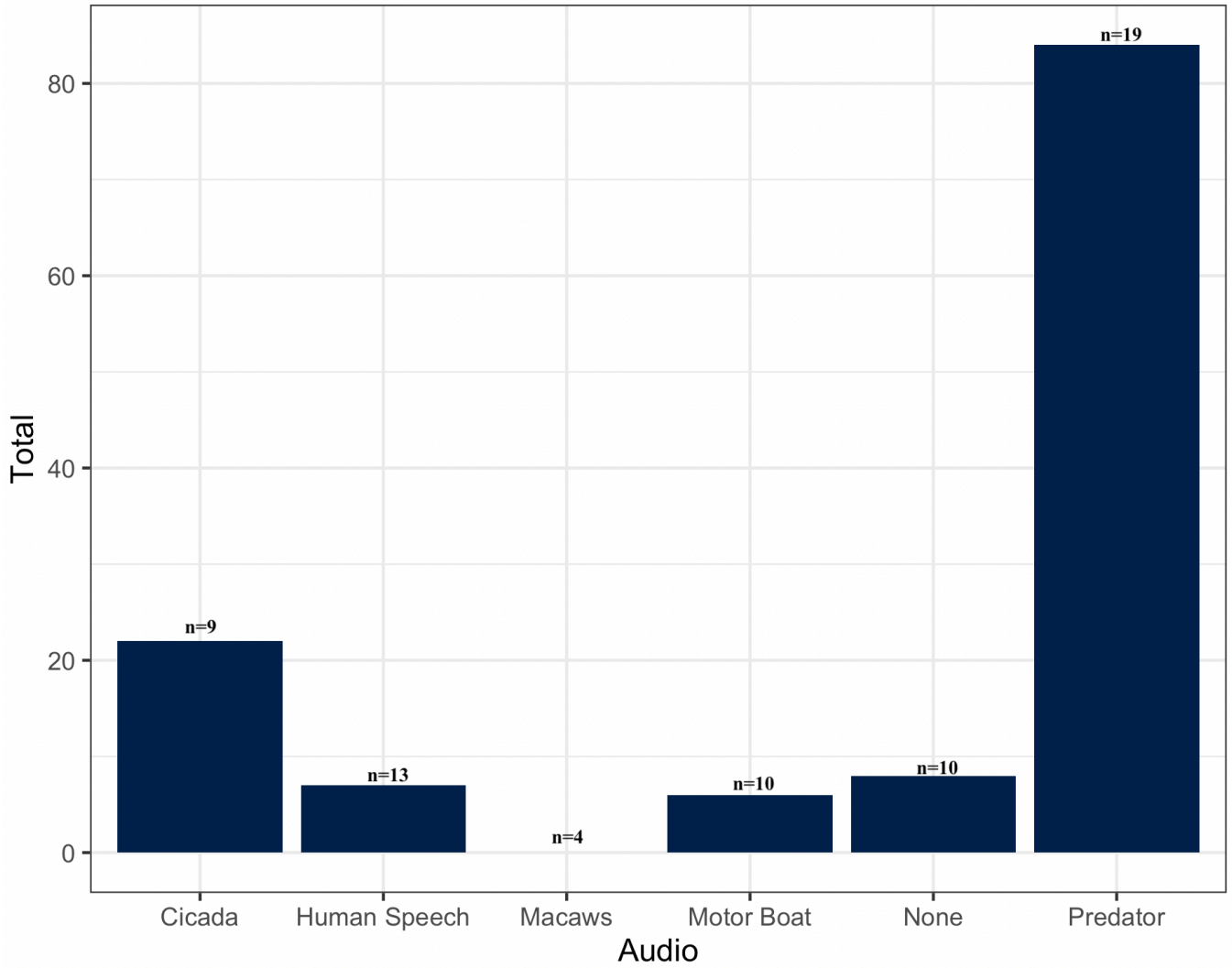


Figure 6.2. The total number of calls emitted by the focal individual across all the trials of each condition type. With n indicating the number of successful trials with that sound that the marmosets reacted to.

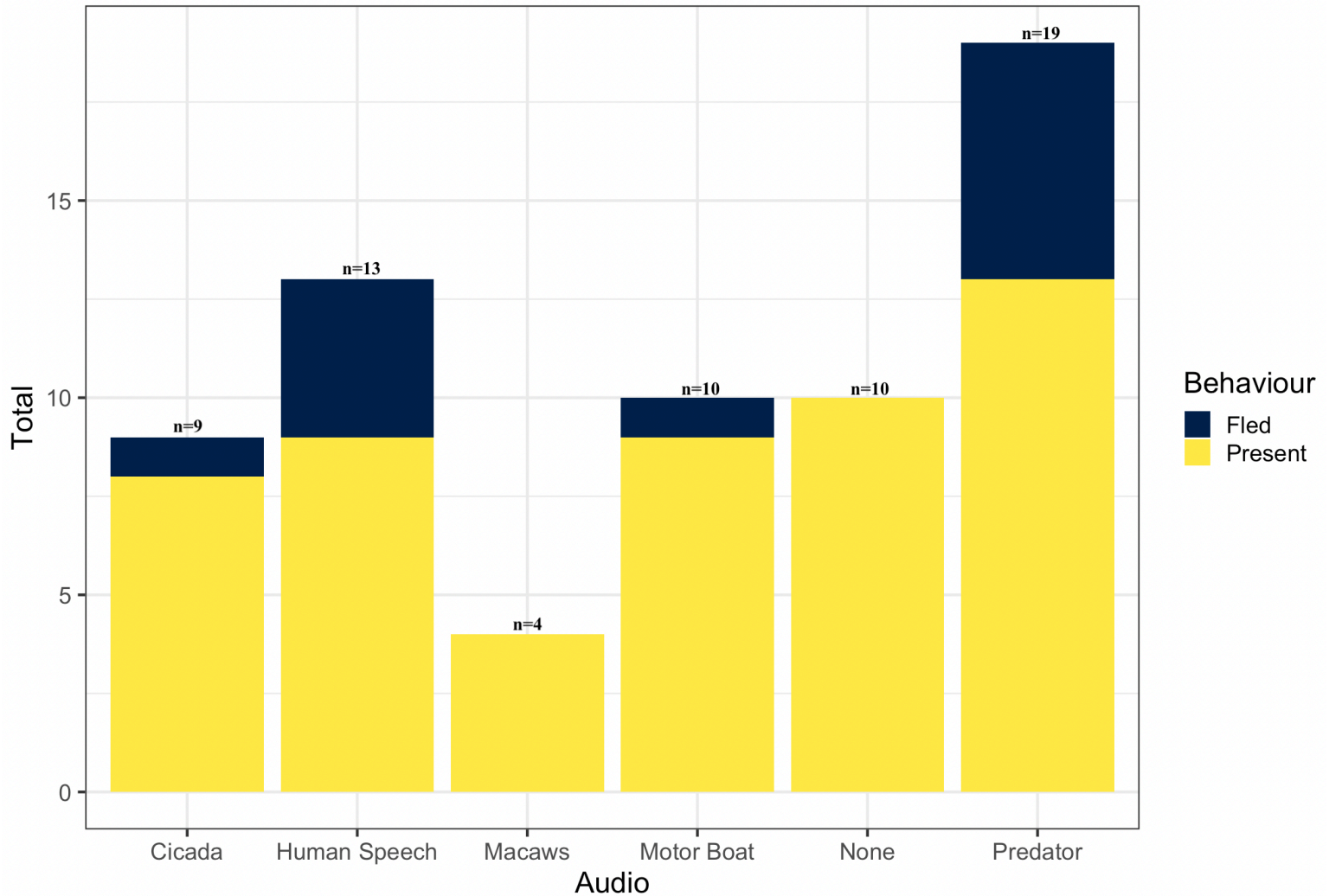


Figure 6.3. The total number of trials where the focal individual fled during a playback condition and trials where they were present during the duration of the trial (does include trials where the focal individual did move out of the video frame but not because they were fleeing). With n indicating the number of successful trials with that sound that the marmosets reacted to.

In playbacks with anthropogenic noise the focal individual spent the most time on average being vigilant, followed by looking at the camera, eating, and looking at the speaker, they also had one long instance of hunting for insects (Figure 6.4; Table 6.4 in appendix). Playbacks with control audios followed similar behavioural patterns with the focal individual spending the most time on average being vigilant, followed closely by eating, looking at the camera, looking at the speaker and grooming itself (Figure 6.4; Table 6.4 in appendix). Focal individuals in playbacks with predator calls spent the most time on average being vigilant (which was the highest average

behaviour across all conditions), followed by eating, looking at the speaker, looking at the camera and grooming themselves (Figure 6.4; Table 6.4 in appendix).

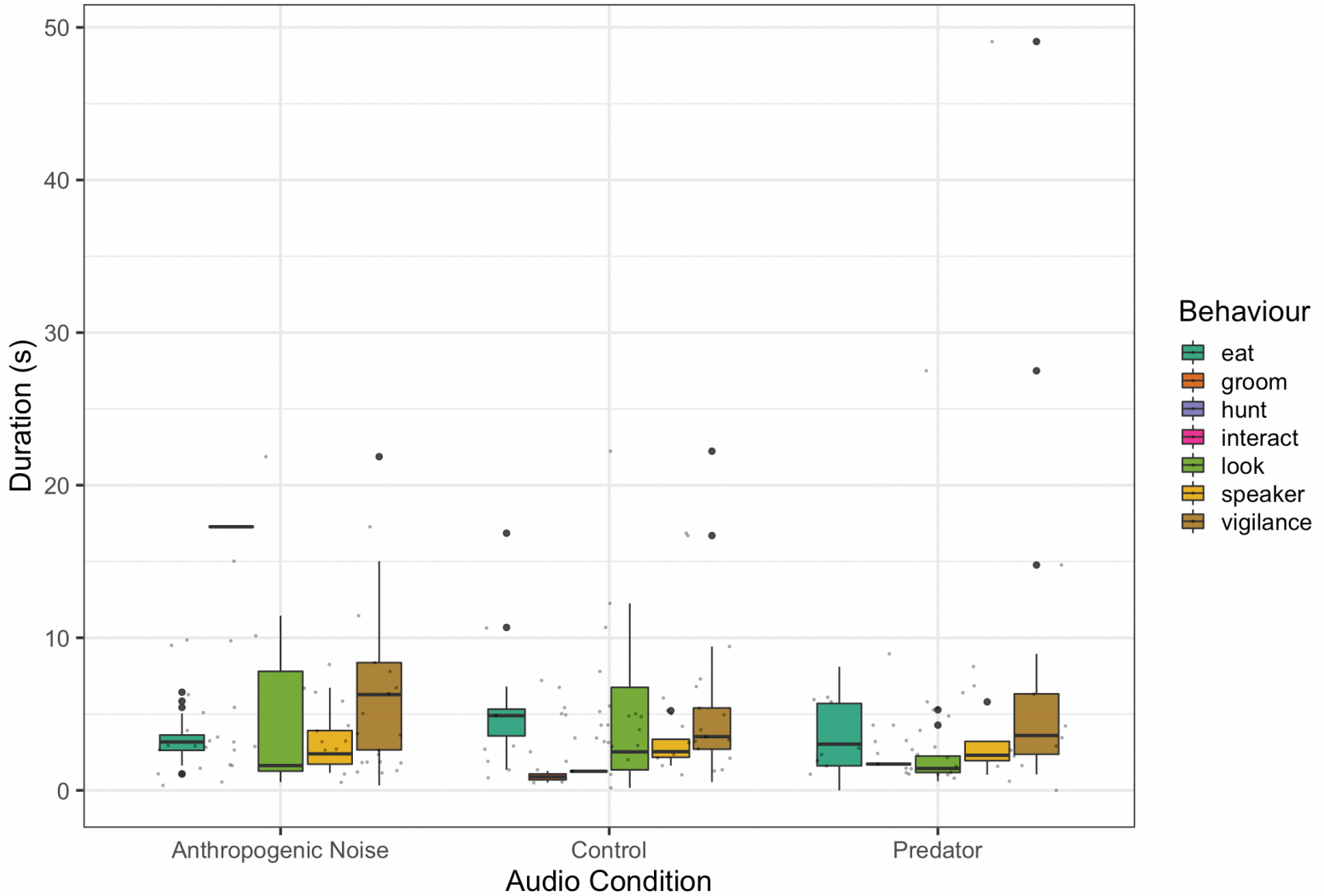


Figure 6.4 A breakdown of the duration in seconds the focal individual spent eating, grooming themselves, hunting for insects, interacting with other individuals, looking at the camera, looking at the speaker, and being vigilant. Displaying the mean duration of these behaviours across the three audio conditions; anthropogenic noise (motor boats and human speech), control (no audio, macaws, and cicadas) and predator call (the roadside hawk and the ornate hawk eagle).

6.3.2 Experiment 2

A total of 75 videos were analysed for the second experiment (Table 6.3).

Table 6.3 The breakdown of the trials each group underwent in the second experiment.

Group ID	Total number of videos recorded	Total number of deployments	Total number of successful trials	Motor Boat + Controls	Motor Boat + Predator	Human Speech + Controls	Human Speech + Predator Calls
CV2	93	2	17	3	3	2	9
CV4	201	3	12	1	4	3	4
CV7	16	1	10	3	2	1	4
TL7	196	3	14	4	4	1	6
TL14	266	2	22	3	7	5	7
Total included				14	20	12	30

The focal individual called in 25% of motorboat playbacks spliced with predator calls and 21% with controls (Figure 6.5) and calling more on average in a trial with the predator spliced condition ($M=2$, $SD=2$). The focal individual called in the same percentage of playbacks for both spliced conditions with human speech audios, 33%, also having the same average number of calls in a trial (control $M=3$, $SD=2$; predator $M=3$, $SD=3$) (Figure 6.5).

The focal individual fled in 15% of motorboat playbacks spliced with predator calls, but in none of the ones with controls (Figure 6.6). Fleeing occurred in roughly the same percentage for the two human speech spliced conditions; 25% in trials with controls and 23% with predator calls. (Figure 6.6).

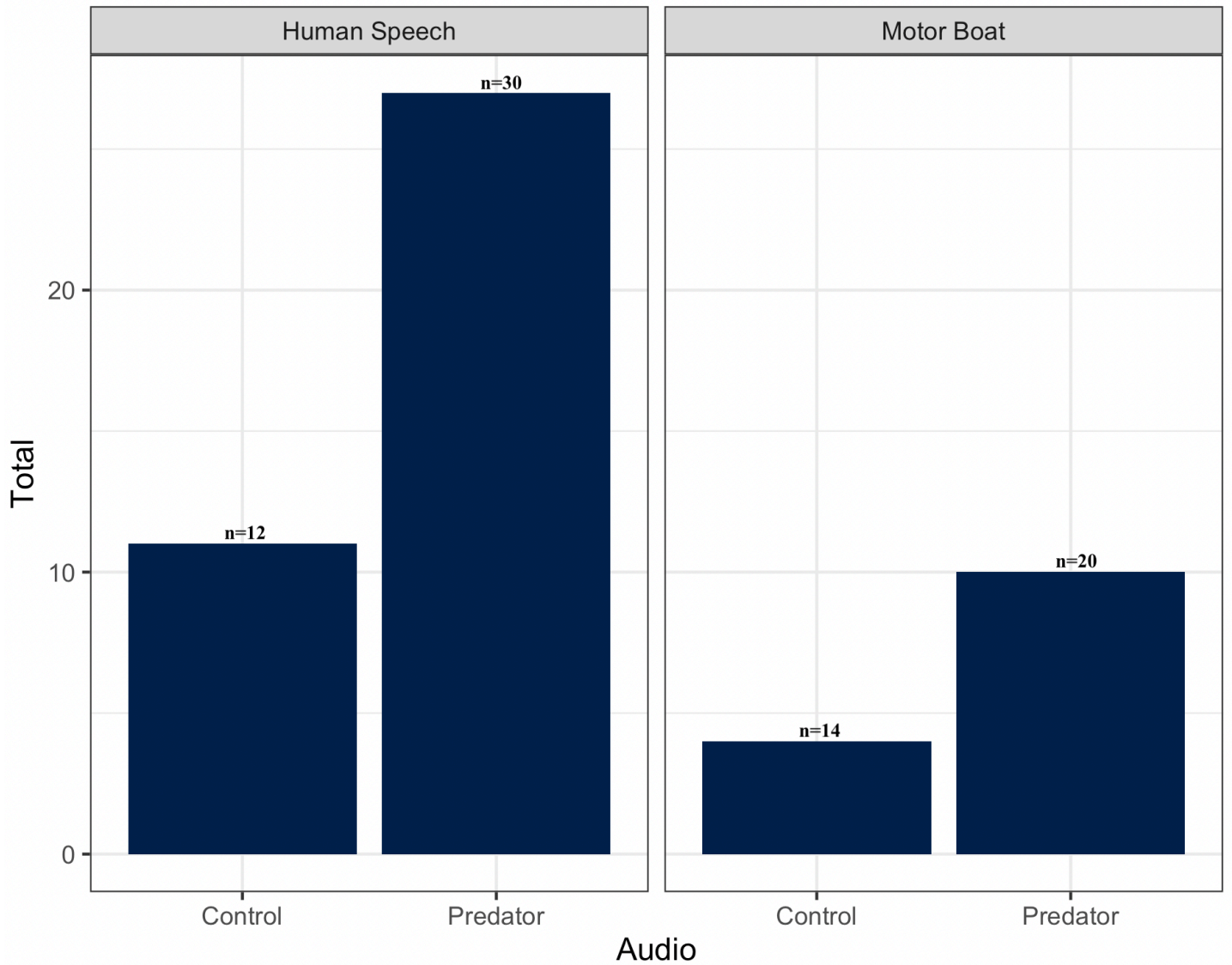


Figure 6.5. The total number of calls emitted by the focal individual across all the trials of each condition spliced with the two anthropogenic noise audios. With n indicating the number of successful trials with that sound that the marmosets reacted to.

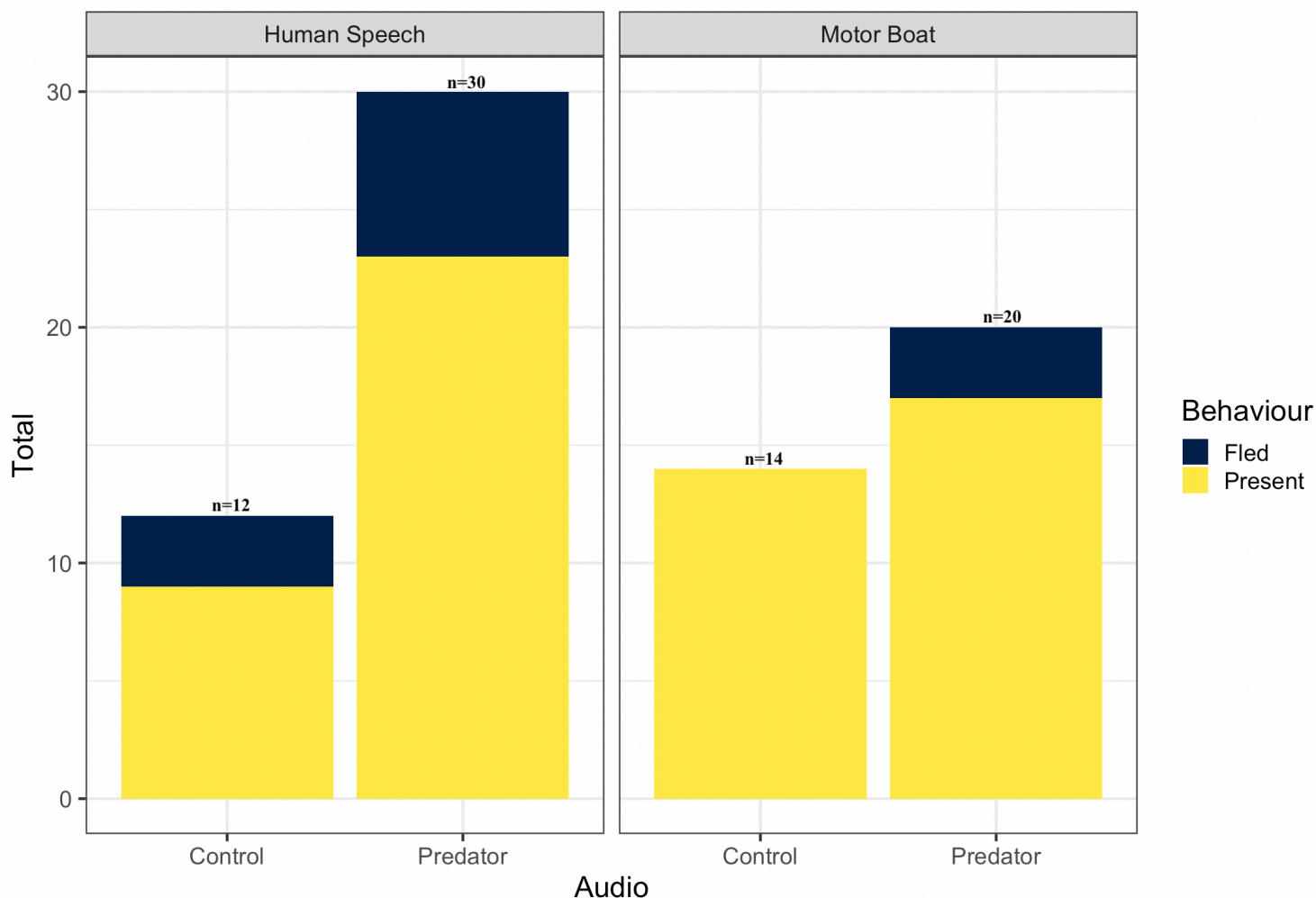


Figure 6.6. The total number of trials, for each condition spliced with the two anthropogenic noise audios, where the focal individual fled during a playback condition and trials where they were present during the duration of the trial (includes trials where the focal individual did move out of the video frame but not because they were fleeing). With n indicating the number of successful trials with that sound that the marmosets reacted to.

In the playbacks of the motor boat spliced with control audios the focal individual spent the most time on average eating, followed by hunting insects, being vigilant and looking at the camera and one instance of grooming themselves ((Figure 6.7; Table 6.5 in appendix). For the playbacks of the motor boat spliced with predator call audios the focal individual spent the most time on average eating as well, followed by being vigilant, looking at the speaker, looking at the camera and one instance of interacting with another individual ((Figure 6.7; Table 6.5 in appendix).

In playbacks where the stimuli was human speech spliced with control audios the focal individual spent the most time on average hunting for insects, followed by eating, being vigilant, looking at the speaker, looking at the camera and one instance of interacting with another pygmy marmoset (Figure 6.7; Table 6.5 in appendix). When exposed to the playbacks spliced with predator call audios the focal individual spent the most time on average being vigilant, followed by eating, looking at the speaker, looking at the camera and one instance of hunting for insects and one grooming themselves (Figure 6.7; Table 6.5 in appendix).

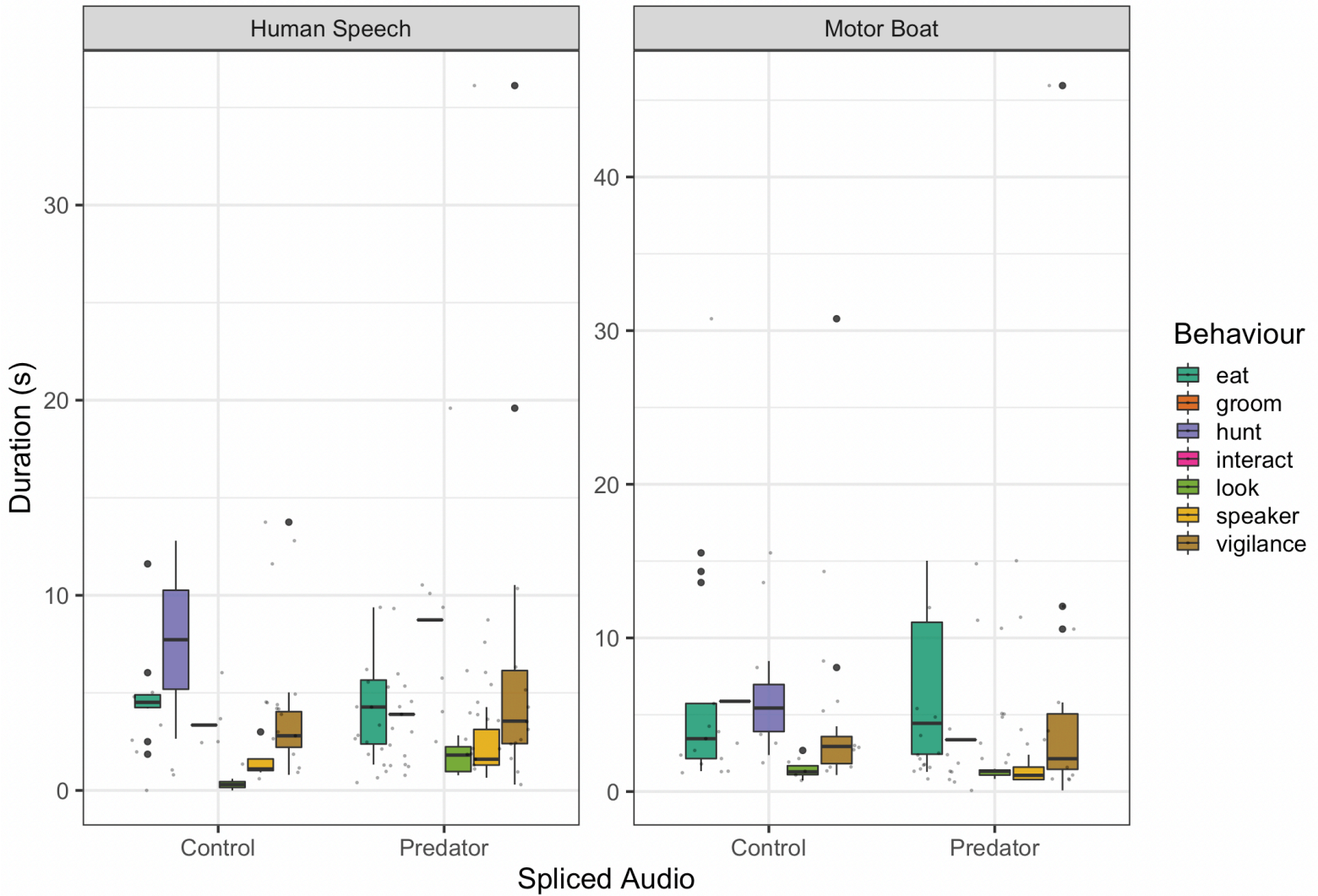


Figure 6.7. A breakdown of the duration in seconds the focal individual spent eating, grooming themselves, hunting for insects, interacting with other individuals, looking at the camera, looking at the speaker, and being vigilant. Displaying the mean duration of these behaviours across the across the two anthropogenic noise audios (motor boat and human speech) with the two spliced conditions (controls and predator calls).

6.4 Discussion

As I predicted playbacks with predator calls resulted in the most fleeing events and contained the most calls. Human speech playbacks having the second highest percentage of fleeing events aligns with what we know about pygmy marmosets' reactions to human audio stimuli. With pygmy marmosets moving out of sight when played loud recording of human speech (Sheehan and Papworth, 2019). I thought there would be a higher frequency of calls in the playbacks with human speech audio however this was not the case. Also, as expected the focal individual spent the most time being vigilant during predator call and anthropogenic noise playbacks. This also supports the findings in Sheehan and Papworth (2019) where the marmosets in playbacks with human speech were more likely to spend time being alert and less feeding and resting. I thought that for controls the behaviour most catalogued would be eating, however, it was only 0.25 seconds shorter on its mean time spent than vigilance.

The results from the second experiment were quite illuminating and would be very interesting to explore in a more expanded dataset. It seems like the marmosets are still able to gage the “fake” predation risk by calling more on average and fleeing more in the predator call conditions. They spent the most time feeding in both motor boat conditions followed by hunting for insects in the control trials and being vigilant in the predator call trials, following the patterns in the first experiment. The playbacks using human speech produced interesting results, with calling and fleeing probabilities evenly spread across both conditions. Hunting followed by eating were the most prevalent behaviours in the trials with control stimuli and vigilance followed by eating for the trials with predator calls. If I was able to expand on the sample size of this project and it were to follow along this trend, I would argue that the marmosets are reacting to human speech audios in a similar pattern as seen with the predator calls. This means for the spliced audios the even spread of calls and fleeing may be due to the marmosets already being more vigilant due to the human speech. The preliminary data seems to suggest that the marmosets are not being distracted by the motor boat audio and are still having anti-predator responses to the bird of prey calls. In a fully-fledged experiment it would be useful to look at how these changes are occurring on a gradient of exposure to these baseline anthropogenic sounds. This would give a more in-depth review of the applicability of the distracted prey hypothesis in this system and expanding on the

knowledge gap of how habituation to these sounds potentially plays a role in their behavioural responses (Shannon *et al.*, 2016).

This small-scale project provided very valuable insights into what worked and what needs refinement for future deployments of similar systems. From what I have learned from conducting this pilot I recommend a longer fieldwork deployment. The 5 weeks used in this study restrained the number of groups that experienced all the treatments in the study, which limited sample size. For most groups it took two or even three deployments to ensure a successful playback for all the conditions. Running a small pilot, if possible, is recommended in order to establish a baseline of how many deployments it will take in order to have enough successful trials so that can be factored in when planning on the duration of experimental period to ensure that redeployments can be conducted if needed. Although it still produced a smaller sample size than needed for a proper analysis it was quite successful. This pilot was conducted over 5 weeks and ended with 147 successful trials, while in comparison to a traditional playback experiment also conducted with pygmy marmosets, which produced 67 successful trials in under 3 months (Sheehan and Papworth, 2019). This shows that if the set-up time is adapted for the specific application the ABR still might provide the ability to complete more successful trials in a shorter time span. Other studies using the ABR system have used a wide variation in experimental duration, as it depends on your study species as well as the number of conditions, with Mugerwa (2018) running for just under two months only using 1 ABR system and 4 audio conditions. While Smith *et al.* (2017) had 14 conditions and ran for around 4 months and used 2 ABR systems.

Two out of the four groups that were not included in the final analysis of experiment one (and therefore were not included in experiment two) were located inside of the reserve boundary. These groups were very skittish of the camera and especially the speaker, which is why I was not able to get enough successful playbacks. At one of these groups the marmosets attacked the speaker, which was partially recorded by the camera trap. Therefore, I recommend leaving the equipment up (but with no battery supply) for a 2-3 days to ensure that the animals become more used to the presence on the system in their environment. Acclimatising these groups to the equipment is paramount since this would allow for a comparison of less habituated and more habituated groups. Where camera traps are more passive and eliminate the need for a human

observer, some animals still find its presence intrusive (Meek *et al.*, 2016) so acclimatising them to the equipment could help overcome this barrier.

Of the other two groups not included one was behind the main tourist lodge, this group did not frequent the holes where I had set up the camera as often as they did in the preliminary follow. When the follow was conducted there were no tourists staying at the lodge and in all three of the attempted deployments the tourists had once again returned to the lodge, so they were frequently visiting the feeding trees to see the marmosets and hiking along the trails by their tree. The increase in visitation making it more difficult to conduct the playbacks as pygmy marmosets are known to use higher levels of the strata when in the presence of tourists (de la Torre *et al.*, 2000). The last group not included was located on the outskirts of the community of El Chino, where the 105 videos produced were all of ants feeding on the running sap. While the group was present at this location I had chosen to place the ABR at this location as the holes seemed active however it had rained the previous day and therefore the holes all seemed active due to the increase in water. It is also important to do a preliminary site visit to the groups home range, this is in order to see which sap holes are currently active and to prepare the set-up (picking a location for where the camera and speaker can be placed). For pygmy marmosets specifically, it is also important to note that rain can make the sap holes seem more active as sap flow has been found to accelerate significantly after a tree absorbs water after rainfall (Schwinning and Sala, 2004) so it would be best to do this in dry conditions if possible.

Other studies who utilised the ABR system have mostly used it in the context of species interaction (Suraci *et al.*, 2017; Epperly *et al.*, 2021, Rigoudy *et al.*, 2022). The ABR has also been utilised to understand wildlife responses to anthropogenic noise and human speech (Smith *et al.*, 2017; Murgerwa, 2018). Recent developments have tested the ABRs use in reducing crop damage done by wildlife (Widén *et al.*, 2022). Before this study no one had directly tested behavioural hypotheses with this methodology, this study provides the crucial evidence to support the statement (Palmer *et al.*, 2022) that the ABR can generate sufficient sample sizes to evaluate ecological and even broader behavioural hypotheses. The evidence provided in the study proves that in years to come the ABR will drastically alter the landscape of field biology,

by allowing for the sound statistical evaluation of hypotheses that would be untestable using other methods due to the robust sample sizes created.

While this project shows the feasibility of using ABRs to test animal behavioural hypotheses in the field, it is a time-consuming analysis project. Across the two experiments, 1,268 videos were produced that then needed to be scanned in order to see if there was a successful playback. Of these 1,268 videos, 147 were then analysed in BORIS. This is one of the pitfalls of the use of camera trap technology, it does expand sample sizes but generates comprehensive audio-visual datasets that are often so large and data-rich that a timely categorisation and analysis by human researchers is no longer possible (Bain *et al.*, 2021). A potential future avenue to bypass this limitation would be to integrate Machine learning analytical methods. Machine learning, Artificial intelligence and Deep learning have all seen a recent spike in popularity (Pitchler and Hartig, 2022) and are revolutionising a wide scope of scientific studies (Jordan and Mitchell, 2015). The integration of these automated data processing algorithms is at the forefront of ethology and ecological studies. Showing early success with Bain *et al.* (2021) producing the first automated audio-visual behavioural action recognition using wild primates and camera trapping technology. Further developing these Machine and Deep learning algorithms would allow for a widespread application of the ABR systems to test a variety of behavioural hypotheses in this and other systems.

Our application of the ABR system with wild primates demonstrates how this system can be applied to future behavioural ecology studies. Using the recommendations discussed above, the ABR has the potential to transform how playback experiments are conducted. The ABR system allows researchers to test behavioural hypotheses (those often dealing with the impacts of human disturbance) without the bias created by human observers. By eliminating this bias, it allows researchers to fully understand the impacts of human presence especially ecotourists which can lead to practical mitigation efforts.

6.5 References

- Ambiance - Topic. 2019. Amazon Cicada. [Online Video]. 22 April 2019. Available from: https://www.youtube.com/watch?v=_TwQr9utP6g&t=48s. [Accessed: 1 August 2021].
- Bain, M., Nagrani, A., Schofield, D., Berdugo, S., Bessa, J., Owen, J., Hockings, K.J., Matsuzawa, T., Hayashi, M., Biro, D. and Carvalho, S. 2021. Automated audiovisual behavior recognition in wild primates. *Science advances* 7(46), eabi4883.
- Balmford, A., Green, J.M., Anderson, M., Beresford, J., Huang, C., Naidoo, R., Walpole, M. and Manica, A. 2015. Walk on the wild side: estimating the global magnitude of visits to protected areas. *PLoS biology* 13(2), e1002074.
- Black Crow. 2021. Blue and yellow macaw sounds. [Online Video]. 13 January 2021. Available from: https://www.youtube.com/watch?v=w96W_SrjL8U&t=64s. [Accessed: 1 August 2021].
- Blumstein, D. T., Daniel, J. C., and Springett, B. P. 2004. A Test of the Multi-Predator Hypothesis: Rapid Loss of Antipredator Behaviour after 130 years of Isolation. *Ethology* 110, 919–934. https://doi.org/10.1300/J021v28n01_05
- Blumstein, D., Geffroy, B., Samia, D. and Bessa, E. 2017. *Ecotourism's Promise and Peril: A Biological Evaluation*. 1st ed. Cham, Switzerland: Springer International Publishing AG.
- Cäsar, C., Byrne, R.W., Hoppitt, W., Young, R.J. and Zuberbühler, K. 2012. Evidence for semantic communication in titi monkey alarm calls. *Animal Behaviour* 84(2), 405-411.
- Chan, A. A. Y. H., Giraldo-Perez, P., Smith, S., and Blumstein, D. T. 2010. Anthropogenic noise affects risk assessment and attention: The distracted prey hypothesis. *Biology Letters* 6(4), 458–461. <https://doi.org/10.1098/rsbl.2009.1081>
- de la Torre, S., Snowdon, C.T. and Bejarano, M. 2000. Effects of human activities on pygmy marmosets in Ecuadorian Amazon. *Biological Conservation* 94, 153-163.
- de la Torre, S., Calouro, A.M., Wallace, R.B., Mollinedo, J.M., Messias, M.R. and Valença-Montenegro, M.M. 2021. *Cebuella niveiventris* (amended version of 2020 assessment). *The IUCN Red List of Threatened Species 2021*: e.T136865A191707236. <https://dx.doi.org/10.2305/IUCN.UK.2021-1.RLTS.T136865A191707236.en>.

- Epperly, H.K., Clinchy, M., Zanette, L.Y. and McCleery, R.A. 2021. Fear of large carnivores is tied to ungulate habitat use: evidence from a bifactorial experiment. *Scientific reports* 11(1), 1-11.
- Fichtel, C. and Kappeler, P.M. 2002. Anti-predator behavior of group-living Malagasy primates: mixed evidence for a referential alarm call system. *Behavioral Ecology and Sociobiology* 51(3), 262-275.
- Findlay, L.J., Lucas, C., Walker, E.M., Evers, S. and Hill, R.A. 2022. Testing the short-term effectiveness of various deterrents for reducing crop foraging by primates. *African Journal of Wildlife Research* 52(1), 29-43.
- Fischer, J., Noser, R. and Hammerschmidt, K. 2013. Bioacoustic field research: a primer to acoustic analyses and playback experiments with primates. *American Journal of Primatology* 75(7), 643-663.
- Friard, O. and Gamba, M. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in ecology and evolution* 7(11), 1325–1330.
- Geffroy, B., Samia, D.S.M., Bessa, E. and Blumstein, D.T. 2015. How nature-based tourism might increase prey vulnerability to predators. *Trends in Ecology and Evolution* 30, 755–765.
- Gil-da-Costa, R., Palleroni, A., Hauser, M.D., Touchton, J. and Kelley, J.P. 2003. Rapid acquisition of an alarm response by a neotropical primate to a newly introduced avian predator. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1515), 605-610.
- Jack, K. M., Lenz, B. B., Healan, E., Rudman, S., Schoof, V. A. M., and Fedigan, L. 2008. The effects of observer presence on the behaviour of *Cebus capucinus* in Costa Rica. *American Journal of Primatology* 70(5), 490–494. <https://doi.org/10.1002/ajp.20512>
- Jordan, M.I. and Mitchell, T.M. 2015. Machine learning: Trends, perspectives, and prospects. *Science* 349(6245), 255-260.
- Linkie, M., Haidir, W.A., Nugroho, A. and Dinata, Y. 2008. Conserving tigers (*Panthera tigris*) in selectively logged Sumatran forests. *Biological Conservation* 141, 2410-2415.
- McDougall, P. 2012. Is passive observation of habituated animals truly passive?. *Journal of ethology* 30(2), 219-223.

- Mcgraw, W.S. and Berger, L.R. 2013. Raptors and primate evolution. *Evolutionary Anthropology: Issues, News, and Reviews* 22(6), 280-293.
- Meek, P., Ballard, G., Fleming, P. and Falzon, G. 2016. Are we getting the full picture? Animal responses to camera traps and implications for predator studies. *Ecology and evolution* 6(10), 3216-3225.
- Moorhouse, T. P., Dahlsjö, C. A. L., Baker, S. E., D’Cruze, N. C., and Macdonald, D. W. 2015. The customer isn’t always right - Conservation and animal welfare implications of the increasing demand for wildlife tourism. *PLoS ONE* 10(10), 1-15.
- Møller, A.P. 2017. Transgenerational consequences of human visitation. In: Blumstein, D.T., Geffroy, B., Samia, D.S.M. and Bessa, E. eds. *Ecotourism’s Promise and Peril: a biological evaluation*. Cham, Switzerland: Springer, 47-58.
- Muhly, T. B., Semeniuk, C., Massolo, A., Hickman, L., and Musiani, M. 2011. Human Activity Helps Prey Win the Predator-Prey Space Race. *PLoS ONE* 6(3), 1–8.
- Mugerwa, B. 2018. *Wildlife in a Premier African Protected Area do not Perceive Ecotourists as Predators*. Master’s thesis, The University of Western Ontario. <https://ir.lib.uwo.ca/etd/5282>
- Newing, H. and Bodmer, R. 2003. Collaborative Wildlife Management and Adaptation to Change: the Tamshiyacu Tahuayo Communal Reserve, Peru. *Nomadic Peoples* 7, 110–122.
- Palmer, M.S., Wang, C., Plucinski, J. and Pringle, R.M. 2022. BoomBox: An Automated Behavioural Response (ABR) camera trap module for wildlife playback experiments. *Methods in Ecology and Evolution* 13(3), 611-618.
- Penn, J. 2009. RCF Update. Rainforest Conservation Fund: July, 2009. <http://www.rainforestconservation.org/archives/1>
- Pichler, M. and Hartig, F. 2022. Machine Learning and Deep Learning--A review for Ecologists. *arXiv preprint arXiv:2204.05023*.
- Reynolds, P.C. and Braithwaite, D. 2001. Towards a conceptual framework for wildlife tourism. *Tourism management* 22(1), 31-42.
- Rigoudy, N.L., Clinchy, M., Peel, M.J., Huebner, S., Packer, C. and Zanette, L.Y. 2022. Hierarchy of fear: experimentally testing ungulate reactions to lion, African wild dog and cheetah. *Behavioral Ecology*, arac041. <https://doi.org/10.1093/beheco/arac041>
- Schwinning, S. and Sala, O.E. 2004. Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. *Oecologia* 141(2), 211-220.

- Seyfarth, R.M., Cheney, D.L. and Marler, P. 1980. Monkey responses to three different alarm calls: evidence of predator classification and semantic communication. *Science* 210 (4471), 801-803.
- Shannon, G., McKenna, M.F., Angeloni, L.M., Crooks, K.R., Fristrup, K.M., Brown, E., Warner, K.A., Nelson, M.D., White, C., Briggs, J. and McFarland, S. 2016. A synthesis of two decades of research documenting the effects of noise on wildlife. *Biological Reviews* 91(4), 982-1005.
- Shannon, G., Larson, C.L., Reed, S.E., Crooks, K.R. and Angeloni, L.M. 2017. Ecological consequences of ecotourism for wildlife populations and communities. In: Blumstein, D.T., Geffroy, B., Samia, D.S.M. and Bessa, E. eds. *Ecotourism's promise and peril: a biological evaluation*. Cham: Springer, 29-46.
- Sheehan, R.L. and Papworth, S. 2019. Human speech reduces pygmy marmoset (*Cebuella pygmaea*) feeding and resting at a Peruvian tourist site, with louder volumes decreasing visibility. *American Journal of Primatology* 81(4), e22967.
- Smith, J.A., Suraci, J.P., Clinchy, M., Crawford, A., Roberts, D., Zanette, L.Y. and Wilmers, C.C. 2017. Fear of the human 'super predator' reduces feeding time in large carnivores. *Proceedings of the Royal Society B: Biological Sciences* 284(1857), 20170433.
- Smith, J.A., Suraci, J.P., Hunter, J.S., Gaynor, K.M., Keller, C.B., Palmer, M.S., Atkins, J.L., Castañeda, I., Cherry, M.J., Garvey, P.M. and Huebner, S.E. 2020. Zooming in on mechanistic predator-prey ecology: Integrating camera traps with experimental methods to reveal the drivers of ecological interactions. *Journal of Animal Ecology* 89(9), 1997-2012.
- Soini, P. 1988. The pygmy marmoset, Genus *Cebuella*. In: Mittermeir, R.A., Rylands, A.B., Coimbra-Filho, A.F., da Fonseca, G.A.B. eds. *Ecology and Behaviour of Neotropical Primates. Vol. 2*. Washington DC: World Wildlife Fund, 79-129.
- Suraci, J.P., Clinchy, M., Mugerwa, B., Delsey, M., Macdonald, D.W., Smith, J.A., Wilmers, C., C. and Zanette, L., Y. 2017. A new Automated Behavioural Response system to integrate playback experiments into camera trap studies. *Methods in Ecology and Evolution* 8, 957-964.
- Tadesse, S. A., and Kotler, B. P. 2012. Impact of tourism on Nubian ibex (*Capra nubiana*) revealed through assessment of behavioural indicators. *Behavioral Ecology* 23(6), 1257-1262. <https://doi.org/10.1093/beheco/ars110>

- Walter, F.R. 1969. Flight behaviour and avoidance of predators in the Thomson's Gazelle (*Gazella thomsoni* Guenther 1884). *Behaviour* 34:184-221
- Widén, A., Clinchy, M., Felton, A.M., Hofmeester, T.R., Kuijper, D.P., Singh, N.J., Widemo, F., Zanette, L.Y. and Crowsigt, J.P. 2022. Playbacks of predator vocalizations reduce crop damage by ungulates. *Agriculture, Ecosystems & Environment* 328, 107853.
- Zuberbühler, K. 2014. Experimental field studies with non-human primates. *Current Opinion in Neurobiology* 28, 150-156.

6.6 Appendix

Table 6.4 A breakdown of the behaviour of the focal individual across the three audio conditions used in the first playback experiment. With the mean time in seconds in the two-minute video that the focal individual spent doing said activity and the number of videos where the activity occurred denoted as sample size.

Audio Condition	Behaviour	Sample Size	Mean Time (s) and SD
Anthropogenic noise	Eat	21	3.26 ± 1.45
	Groom	0	0
	Hunt	1	17.27 ± n.a.
	Interact with another marmoset	0	0
	Look at camera	6	4.37 ± 4.89
	Look at speaker	6	3.10 ± 2.09
	Vigilant	21	6.43 ± 5.16
Control	Eat	22	5.16 ± 3.24
	Groom	2	0.89 ± 0.55
	Hunt	0	0
	Interact with another marmoset	1	1.25 ± n.a.
	Look at camera	13	4.10 ± 3.90
	Look at speaker	4	2.98 ± 1.56
	Vigilant	21	5.41 ± 5.23
Predator	Eat	18	3.54 ± 2.38
	Groom	1	1.73 ± n.a.
	Hunt	0	0
	Interact with another marmoset	0	0
	Look at camera	8	2.12 ± 1.69
	Look at speaker	4	2.86 ± 2.05
	Vigilant	18	8.13 ± 12.03

Table 6.5. A breakdown of the behaviour of the focal individual across the two anthropogenic noise audios with the two spliced conditions used in the second playback experiment. With the mean time in seconds in the two-minute video that the focal individual spent doing said activity and the number of videos where the activity occurred denoted as sample size.

Audio Condition	Spliced Audio	Behaviour	Sample Size	Mean Time (s) and SD
Motor Boat	Control	Eat	13	5.64 ± 5.24
		Groom	1	5.88 ± n.a.
		Hunt	2	5.44 ± 4.33
		Interact with another individual	0	0
		Look at camera	4	1.49 ± 0.83
		Look at speaker	0	0
		Vigilant	14	4.88 ± 7.66
	Predator	Eat	18	6.29 ± 4.76
		Groom	0	0
		Hunt	0	0
		Interact with another marmoset	1	3.37 ± n.a.
		Look at camera	3	1.21 ± 0.34
		Look at speaker	4	1.32 ± 0.76
		Vigilant	20	5.49 ± 10.03
Human Speech	Control	Eat	10	4.94 ± 2.63
		Groom	0	0
		Hunt	2	7.72 ± 7.18
		Interact with another individual	1	3.35 ± n.a.
		Look at camera	2	0.30 ± 0.42
		Look at speaker	4	1.53 ± 0.98
		Vigilant	11	3.86 ± 3.51
	Predator	Eat	23	4.32 ± 2.24
		Groom	1	3.9 ± n.a.
		Hunt	1	8.74 ± n.a.
		Interact with another individual	0	0
		Look at camera	8	1.72 ± 0.76
		Look at speaker	5	2.19 ± 1.48
		Vigilant	30	5.81 ± 7.01

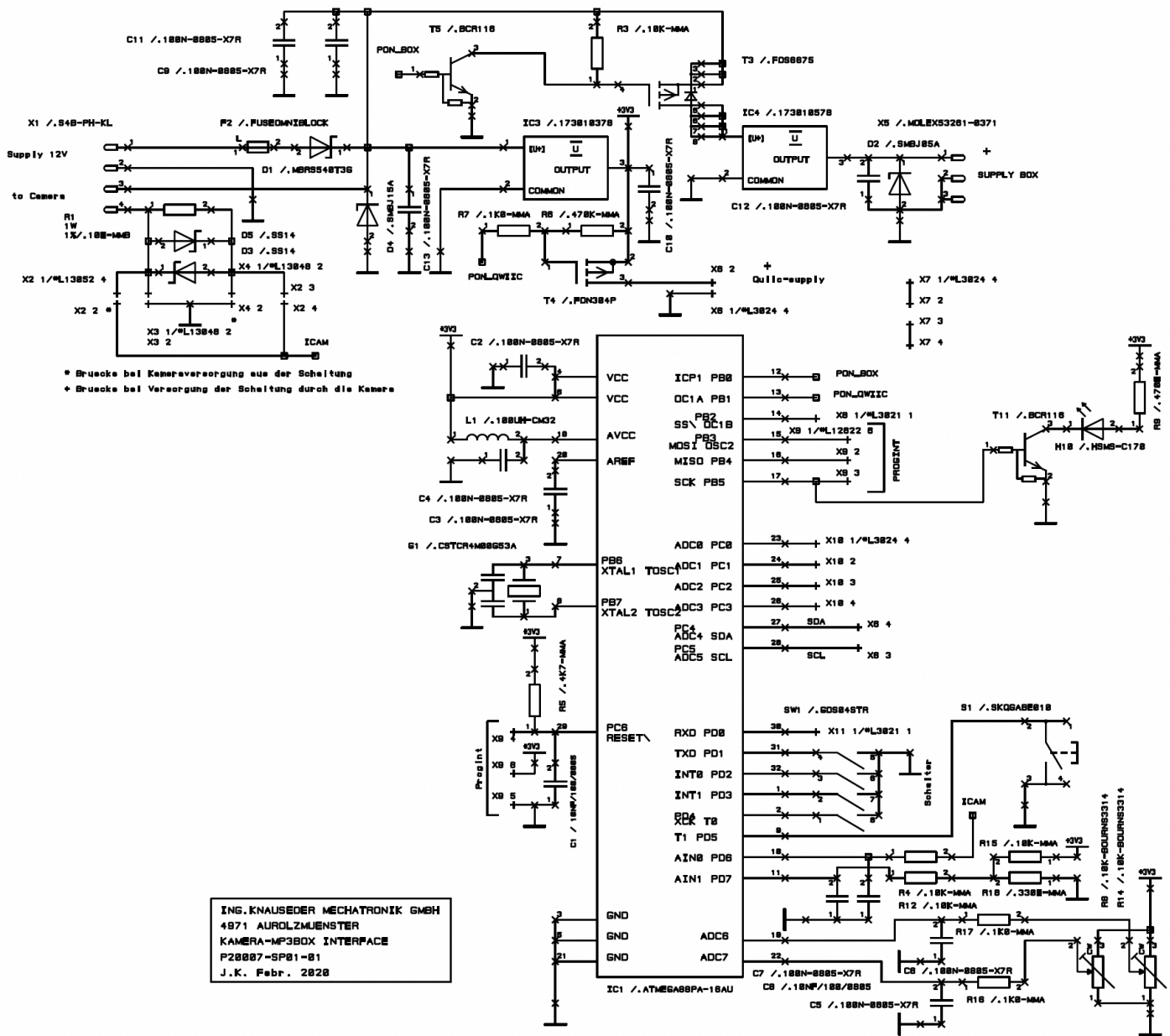


Figure 6.8 Circuit Diagram of the ABR Speaker Microcontroller and Camera Current Detector

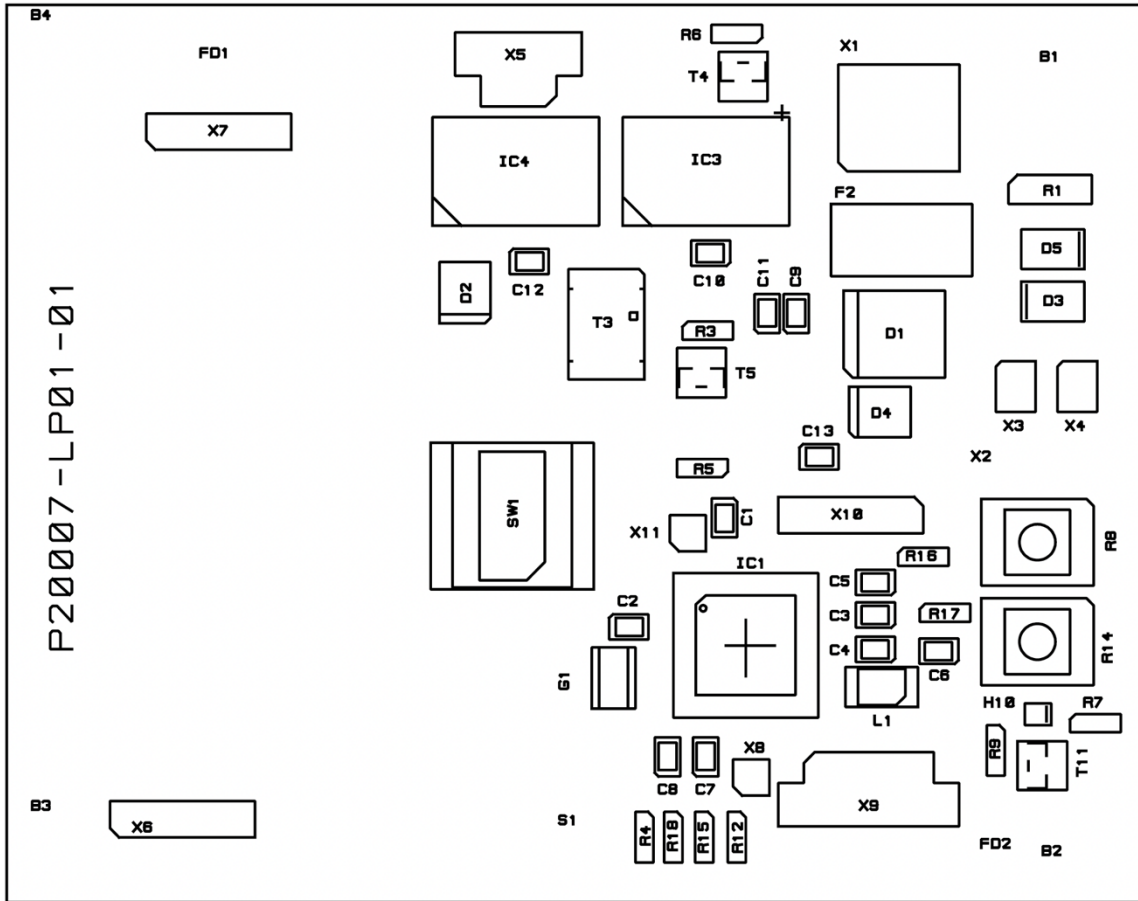


Figure 6.9 The layout of interface-board P20007-LP01-01



Figure 6.10 Experimental ABR setup. The maroon circle denotes the position of the camera trap, the grey circle the position of the battery pack, the blue circle the placement of the speaker and the yellow circle highlights the active sap holes. The image on the left was taken at the group TL14 and the right is the group RC2.