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Who is Present? - Individuality in the Call Structure of the Eurasian Otter (*Lutra lutra*) Whistle

Master Thesis

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1 Abstract

In conservation management, knowledge about the presence, abundance, density and range of a species is crucial. However, the monitoring of elusive species is challenging. An approach to survey such species can be through acoustic means where calls can be extracted from passive, non-invasive recordings, and assigned to individuals, based on the condition of them having an individual acoustic signature. Surveys of the cryptically living Eurasian otter (*Lutra lutra*) currently relies on indirect observations, such as spraints and tracks. In this project I collected otter calls with the approach of passive acoustic monitoring to investigate and find differences in their call structure.

For this, the calls of eleven otters living in Swiss zoos and wildlife parks were recorded using stationary recording devices from January to September 2019. I collected additional recordings with directional microphones. With the help of playback experiments, I tried to trigger additional vocalizations of otters. The playback experiments produced mixed results but no increase in whistle call rate. The stationary recorders collected over 2000 calls, with whistles being the most common call type, a long-distance call carrying up to 100 meters. Furthermore, I got access to an acoustic database from a 30-year old study on Eurasian otters done in Germany. I investigated 20 different acoustic parameters of the whistle call and checked for their consistency over several days. The two individuals recorded repeatedly over six consecutive days revealed the calls to be very stable.

A discriminant function analysis on acoustic parameters revealed that the analysed eleven Eurasian otters showed individual differences in their call structure. Thereby, the two parameters ‘mean fundamental frequency’ and ‘sound duration’ were sufficient to correctly classify high-quality whistle calls at a higher correct classification percentage than expected by chance (96.4%) but also for low-quality whistle calls (79.3%). Additional research is needed on the transmission range of these whistle calls to make a better decision on how effective and economic this method may be to detect the Eurasian otter in their natural habitat. However, the distinct individual signatures identified for the eleven captive animals indicate a potential for passive acoustic monitoring as an additional tool for monitoring otters in the wild, thus complementing the existing survey methods.

2. Introduction

Biodiversity is declining at a rapid rate. Over the past centuries, many species went extinct and in most cases, the extinction of the species can be associated directly or indirectly with human activities (Johnson et al. 2017). In addition, many species are listed as vulnerable, endangered or critically endangered on the International Union for Conservation of Nature (IUCN) red list (IUCN 2018). Many of the listed species decreased in number over the last decades due to several factors, including loss of habitat, pollution or climate change (Bonebrake et al. 2010). However, some species have increased in numbers over the last years due to restoration, reintroduction or by protection through conventions and laws. But no matter if a species is increasing or decreasing in numbers, knowledge about abundance, density and range of any given species is crucial for successful conservation management.

Assessing population size can be challenging depending on factors such as ecology, behaviour or habitat. A commonly utilized method to calculate population size is the use of the matrix population model which is given as the basic BIDE equation (Cohen 1969):

$$N_{t+1} = N_t + B + I - D - E$$

where N_t is the number of individuals in a population at any given time t, *B* the number of individuals being born, *I* the number of immigrants, *D* the number of deaths and *E* the number of emigrants. The number of animals at time *t+1* can be calculated if the numbers of births, immigration, deaths and emigration are known (reviewed in Cohen, 1969). Even though the equation is very simple, the input variables can be difficult to calculate accurately. The most important variable is N_t, as it forms the basis of the equation. However, in the wild, it is almost impossible to count the total number of animals in an area. Therefore, the variable has to be estimated with the help of an additional equation:

$$\widehat{N} = \frac{C}{\hat{p}}$$

where $\widehat{N}$ equals the estimated population or sample size, C the counted animals and $\hat{p}$ the estimated detection probability. Both the counted number of animals and the detection probability depend entirely on the method of monitoring.

The most likely oldest method for monitoring is the visual observation of individuals including direct observation and indirect observation such as identification of tracks and faeces. However, this method quickly reaches its limits in nocturnal, elusive or very rare animals (Hoffmann et al. 2010). In the last decade, the use of camera traps has been established to estimate population size and density as an additional visual observation method (Silveira et al. 2003; Rowcliffe et al. 2018). This method allows to observe over a longer time period and reduces the disturbance caused by the presence of human beings. In addition, the use of infrared cameras enables to monitor nocturnal animal species (Trolliet et al. 2014). In animals with a sexual dimorphism the gender ratio of a population can be determined and species with unique fur patterns allow to identify individuals (Weingarth et al. 2012), although this can be very challenging in group living species.

To obtain information on sex ratio and individuality of a species without visual sex dimorphism, DNA analysis from faeces or hair samples is can be performed (Mills et al. 2000). However, the success rate of such analyses can be very modest due contamination of faeces samples with DNA of prey items, missing hair roots or fast degradation of the DNA (Zhang et al. 2009). DNA extraction from blood can improve the success rate for the analysis. However, this requires the capture and handling of animals, which is a major stress factor. In addition, capturing animals can be difficult, as it is the case for large animals (Hoffmann et al. 2010) or for aquatic species (Walker et al. 2012).

A different approach is passive acoustic monitoring (PAM). It has been used for decades in marine mammal studies (Watkins and Schevill 1972; Gillespie 2004; Mellinger et al. 2007; Abbot et al. 2010) and since a few years the use of PAM has increased in highly vocal species like bats (Adams et al. 2012; Russo and Voigt 2016) and birds (Sanders and Mennill 2014; Towsey et al. 2014). In comparison with other methods, PAM has many advantages such as the continuous recording over an extended time period, no need of human presence, no induced stress for the animals and the coverage of a large area by combining several recording devices (Spillmann et al. 2015). In contrast to camera traps, which record only locally and in a specific direction, the biggest advantage of PAM is the recording of the entire acoustic background. With the recorded soundscape it is possible to describe different biotic and abiotic elements of an ecosystem. On a species level, different populations or groups of animals can be identified based on the species-specific communication which is used to coordinate the social life of individuals or groups (Boinski 1993; Radford 2004; Braune et al. 2005). Even solitary species use intraspecific communication, for example to indicate the occupation of an area as their territories (Agren et al. 1989) or to signal readiness to mate (Richardson 1996). If an animal communicates with conspecifics, it typically reveals information about itself. This information can then be used by humans to detect differences between individuals, even if the information is not processed by the animals (Schibler and Manser 2007).

The use of this vocal individuality for monitoring has often been suggested (Terry et al. 2005). So far passive acoustic monitoring on an individual level has been successfully applied in highly vocal species, such as birds (e.g. corncrake [*Crex crex*], Peake and McGregor 2001; great bittern [*Botaurus stellaris*], Gilbert et al. 2002; African wood owl [*Strix woodfordii*], Delport et al. 2002), apes (e.g. orangutan [*Pongo pygmaeus*], Spillmann et al. 2010; chimpanzees [*Pan troglodytes verus*], Kalan et al. 2015) or in marine mammals (e.g. fin whales [*Balaenoptera physalus*], McDonald and Fox 1999; Blainville's beaked whales [*Mesoplodon densirostris*], Marques et al. 2009). These individuality studies mostly make use of the source-filter theory (Fant 1970) of vocal production. This theory predicts that in the vocal apparatus a call is produced in the source and then shaped by the filter and thus conveys information about the individual. In mammals, the call is formed in the source, the larynx. Air is forced through the vocal folds, causing the vocal folds to vibrate. The oscillation of the vocal folds produces a sound with a fundamental frequency (F0). The longer and heavier the vocal folds are, the lower is the fundamental frequency of the sound. Since the larynx is made of cartilaginous material and is controlled by skeletal muscles, the F0 does often not seem to be a good indicator for body size within species as the larynx can grow independently of the body size (reviewed in Taylor and Reby 2010). Nevertheless, many studies found a correlation between fundamental frequency and body size. This allowed then to discriminate between categories such as sex (or age class, dominance status). It has been shown, for example, in common toads (*Bufo bufo*, Davies and Halliday 1978), Asian small clawed otters (*Aonyx cinereus*, Lemasson et al. 2014), giant pandas (*Ailuropoda melanoleuca*, Charlton et al. 2009) and Subantarctic fur seals (*Arctocephalus tropicalis*, Charrier et al. 2003). However, within a specific category (e.g. males) the discrimination based on body size seems to be less reliable. (Altig 2010; Pisanski et al. 2014).

The filter consists of the entire space between the glottal opening in the larynx and the lips or nose (Fant 1970). The length and size of these cavities is strongly limited by the anatomy of the animal and usually does not grow independently. Therefore, the vocal tract is often positively correlated with the body size. Depending on shape and size, the tube emphasizes certain frequencies and attenuates others. The emphasized frequencies are seen as amplified frequency bands, which are called formants (reviewed in Taylor and Reby 2010). These formants seem to give reliable information within and between a biological or social category (e.g. sex, age). Correlations between formants and body size/ vocal tract length have been shown for several species like rhesus macaques (*Macaca mulatta*, Fitch 1997), fallow deer (*Dama dama*, Vannoni and McElligott 2008), red deer (*Cervus elaphus*, Reby and McComb 2003), koalas (*Phascolarctos cinereus*, Charlton et al. 2011) and domestic dogs (*Canis lupus familiaris*, Riede and Fitch 1999). However, there are no studies on formants with high fundamental frequencies, either because it is very difficult to identify formants in high pitched calls due to the distances between the amplified frequency bands or because there are no formants (Fitch and Fritz 2006). In summary, it can be said that both stages of the vocal tract convey information about individuality. The investigation of the call structure can therefore not only be used to specifically implement conservation, but it can also give an overview of the population structure and provide information about individuals. This has been shown in fin whales where population estimations were more accurate using PAM than by the previous applied method of visual observation (McDonald and Fox 1999). In elephants (*Loxodonta africana*), the implementation of PAM allowed not only estimations about group size and composition, but also reproductive status of the individuals (Payne et al. 2003).

For elusive species, PAM can be a great tool to estimate population size. For example, all 13 Otter species belong to such elusive animals. As top predators, otters are very vulnerable to changes in the ecosystem but also important for their sustainability (Reisewitz et al. 2006; Cianfrani et al. 2018). Out of all otter species, the Eurasian otter (*Lutra lutra*) has the largest distribution. In the last century, the populations declined in large parts of Europe, resulting in local extirpation. In recent years, the otter has started to recover. For example, in Switzerland the Eurasian otter has only recently returned after having been absent for almost 20 years (Weinberger 2017) but globally they have an IUCN status of 'Near Threatened' with declining tendencies (IUCN 2018). Eurasian otters are semiaquatic, nocturnal and quite elusive animals (Kruuk 2006). They live solitarily, with home ranges of several kilometres' river length (Pradel 1996). They are very territorial and physically defend their territories against conspecifics of the same sex, but patches of males and females overlap (Erlinge 1967; Durbin 1998a). The habitat of the Eurasian otter is very diverse, they live in fast-flowing rivers but they can also be found in lakes, rice field and at coastal areas. The standard method for monitoring otters proposed by the IUCN otter specialist group uses several 600 m transects along water bodies within a 10 km^2 grid (Dolch et al. 2000). Because this species has the potential of being in conflict with humans by preying on fish relevant for humans, it is important to monitor this species. Due to their habitat, their large home ranges and the nocturnal and solitary lifestyle, otters are difficult to visually observe and count. For this reason, Eurasian otters are being surveyed with the help of indirect signs, such as footprints and spraints (faeces of otters). This has become the standard method for monitoring otters as proposed by the IUCN otter specialist group (Kruuk et al. 1986; Mason and Macdonald 1987). However, the absence of spraints or footprints does not confirm the absence of otters. According to Parry et al. (2013) the probability of finding evidence with the proposed standard method of population monitoring is less than 30% on a 600 m transect. DNA analysis of faeces has become an additional tool to get information on population structure and size (Jansman et al. 2001; Somogyiensis 2005). However, the success rate of identifying individual otters based on DNA analysis can still be rather low and varies among studies between 14% and 48% (Prigioni et al. 2006; Lanszki et al. 2008; Hájková et al. 2009). By analysing only fresh spraint samples (Hung et al. 2004) or samples with anal jelly (Hájková et al. 2009; Lampa et al. 2015), the success rate of genetic identification can be improved up to 65%. In addition to the low succes rate of DNA analysis, studies have shown that the marking behaviour varies between sexes (Lampa et al. 2015) and seasons (Macdonald and Mason 1987).

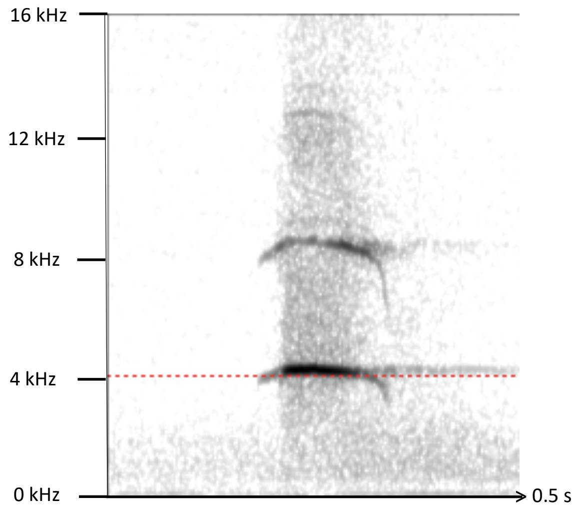

Figure 1 Spectrogram of a typical whistle call (sampling frequency 44’100 Hz, call duration 0.17s).

Depending on calling behaviour between sexes, the use of acoustic monitoring methods may help to correct the imbalance in detection probabilities between the sexes and seasons and help to provide information on population size and composition. So far, a total of six call types of the Eurasian otter have been described by different authors (reviewed in Gnoli and Prigioni 1995). The most frequently reported call type in nature is the whistle call (Fig. 1), as it is used as a long-distance call that can travel up to 100 metres (Kruuk 2006). Other call types described as close-contact calls are the murmur, the mewing and the cries, as well as the twitter, a vocalization associated with pups. In addition, the call repertoire is supplemented by a noisy vocalization described as a blow or a snarl. Besides the description of those different call types there are no other published studies on the Eurasian otter vocalizations.

The aim of this study was to investigate the individuality of the call structure of the Eurasian otter by comparing acoustic parameters of their vocalization. As shown in various studies with different mammal species (Davies and Halliday 1978; Charrier et al. 2003; Charlton et al. 2009; Lemasson et al. 2014), I predict to discriminate individuals of the Eurasian otter based on the acoustic parameters of their call structure. To answer this question, I investigated the calls of captive otters recorded in different zoos. This study tries to contribute knowledge to the almost unexplored field of Eurasian otter acoustics. Moreover, it investigates the potential of acoustic monitoring as a potential tool to monitor populations of the Eurasian otter.

3. Material and Methods

In this study I included data from two different sources, namely newly collected data in 2019 in several zoos in Switzerland, as well as historic data recorded by Barbara Rogoschik in a research centre in Oderhaus, Germany in 1987. The new data were collected using two different sampling methods: passive acoustic recording with stationary recorders and recordings with a directional microphone. In addition, I conducted playback experiments to elicit additional calls from the otters.

3.1 Dataset from zoos in Switzerland

3.1.1 Study population and study sites

The calls of otters were recorded in Swiss zoos and animal parks between January and September 2019. Six institutions in Switzerland that harbour the Eurasian otter agreed on participating in this study (Fig. 2, Table 1). The study population across all recording sites consisted of eleven captive individuals (five males, six females). Three of the parks harboured a single otter (Zoo La Garenne, Fischottergehege Männedorf and Tierpark Goldau). The Zoo Zürich kept the two individuals for most of the study in separated enclosures. These two otters were brought together towards the end of the study (12 August 2019). The Wildnispark Zürich harboured four otters in an enclosure with different compartments. The different compartments were connected to each other and the animals could move freely between the compartments. In the course of the study, an animal of the Wildnispark Zürich had to be euthanized. Only a few calls could be recorded from this animal with the directional microphone. In the same zoo, at least two pups

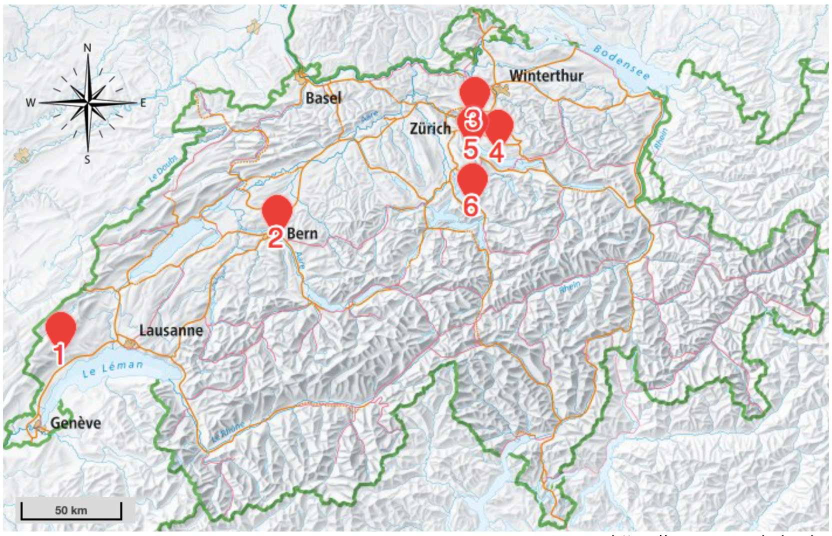

Figure 2: Map of Switzerland with the locations of the zoos and animal parks.

were born towards the end of the study. The Tierpark Dählhölzli kept two individuals in an enclosure to which a wild otter came to visit some nights. Most individuals of the study population were born in captivity. Two of the otters (males from Tierpark Dählhölzli and Zoo Zürich) were wild-caught and could not be released back into the wild due to injuries and their young age. All enclosures were species-appropriate as they provided structures that served as hiding places, a flowing stream and access to a water basin. At any time during the day, the animals were free to spend their time anywhere in the enclosure.

Table 1 Zoos and parks that harbour the Eurasian otter and animals that have been included in this study. The numbers refer to figure 2.

Name of Zoo	Number of animals	Canton
1. Zoo La Garenne	1 female	Vaud (VD)
2. Tierpark – Bern Dählhölzli	1 male, 1 female, 1wild	Bern (BE)
3. Zoo Zürich	1 male, 1 female	Zürich (ZH)
4. Fischottergehege Männedorf	1 male	Zürich (ZH)
5. Wildnispark Zürich	1 male, 3 females ≥ 2 pups	Zürich (ZH)
6. Tierpark Goldau	1 male	Schwyz (SZ)

The numbers in the first column refer to figure 2.

3.1.2 Sound recordings

The otter calls were recorded using two different recording methods: stationary recorders and directional microphones. Stationary recorders were installed in the enclosures for passive acoustic recordings and a directional microphone was used to record from outside the enclosure. Although the stationary machine recorded continuously for several days, recording success depended on the movement of the otters. I used the directional microphone less often but in a more targeted way thus increasing recording success. While recording with the directional microphone, I recorded all different behaviours in an otter's daily routine. The simultaneous use of stationary and directional recorders made it possible to cover a larger area and a longer time period.

3.1.2.1 Stationary microphone recordings

In each enclosure, I installed up to three Song Meter 3 (Wildlife Acoustics Inc., USA) stationary recorders depending on the size of the enclosure. The settings of the recorders are given in Table 2. An SMM-A1 Acoustic Microphone (Wildlife Acoustics Inc., USA, frequency response 20 – 20,000 Hz, +/- 4 dB) was added to the left channel, for higher quality recordings. I placed the recorders close to water or where the otter was expected to spend most of its time. The stationary recorder was mounted either on a tree, fixed on walls or secured to the fence with wire, screws and string (Fig. 3). I installed the recorders between 30 and 70 cm off the ground or a surface the otter could reach. The stationary recording devices were left inside each enclosure for at least 50 hours of continuous recordings. I extended the recording time up to two days if there was a bad signal-to-noise ratio. This was the case with rain or with running water, in particular when waterfalls within the enclosure could not be switched off for several days in a row or only half a day.

Table 2: Settings for the stationary Song Meter 3 recorders.

HPF	OFF
Gain	Auto
TS WAV	48,000
FRQMin	16K
FRQMax	192K
DMin	001.5
DMax	Off
TRGLVL	Auto
TRGMin	3.0
TRGMax	15.0
At Date	*
At Time	**
Record	00:30:00
UNTCOUNT	INF

* specific date when recording starts
** specific time when recording starts

The Song Meter 3 saved recordings as 30-minute files, which were stored on four 32 or 16 GB SD-cards. The recording units were each powered by 4 type-D alkaline batteries. The batteries and the storage capacity lasted for a whole recording session. To ensure that the recorder was still running, I checked them every other day.

Figure 3: Left: recorder mounted on a wall in the enclosure in Männedorf. Right: recorder on a tree in Tierpark Dählhölzli.

3.1.2.2 Directional microphone recordings

I recorded with a directional microphone between 09:00 – 22:00 hrs depending on how long I had access to the enclosure. For the recordings from outside of the enclosure I used a Sennheiser ME66 directional microphone with a K6 powering module (Sennheiser electronic GmbH & Co. KG, Germany, frequency response 40 – 20’000 Hz, +/- 2,5 dB) with a Reinhardt whisper microphone windshield (Wilkins Sound ApS, Denmark) connected to a Roland R-26 portable recorder (Roland Corporation, Japan) with the following settings: Rec Mode: 2 channels, Rec Source: analog, Sample Rate: 48.0 kHz, Rec Format: WAV 24-bit. On a second recording channel, annotations about the caller identity, behaviour and special events were recorded with the help of a hand-held Beyerdynamic TG V30d s Dynamic Vocal Microphone (Beyerdynamic GmbH & Co. KG, Germany, frequency response 30 – 15,000 Hz). I used the directional microphone especially in zoos where more than one animal lived (Table 1). In this way the calls could be clearly assigned to a specific individual (mean distance to caller 14 $\pm$ 6 m). In addition, I used the directional microphone within the playback experiments to record and comment on potential vocal and behavioural responses of the focal individual.

3.1.2.3 Camera trap

In June and August 2019, two motion triggered Browning Strike Force HD camera traps (Browning Arms Company, USA) were mounted next to the stationary recorder for the same duration as the recorder in order to be able to unambiguously assign calls to specific individuals in enclosures with several animals. The settings were selected as follows: Video Quality = ultra, Video Length = 20 seconds. The camera was triggered by motion using an infrared function. It therefore also recorded at night.

3.1.2.4 Playback experiment

I conducted playbacks between June and August 2019 to elicit whistles from otters, especially from those who had not been calling by then. I constructed the sound files for playbacks with the use of short natural sequences of whistle calls. The call sequences used were copied from the middle of a call sequence from a previously recorded file from an individual living in a different zoo with the sound program Audacity version 2.1.2.0 (Audacity Team, 2012). To make sure that playbacks had a normal call rate, neither the call interval nor the composition of the whistle calls were altered in the sequence. For the playback experiment, I created four such sequences from four different animals (two males, two females). The four sequences used for the playbacks were 30 seconds long. Due to the fact that there was no behavioural response by the focal individuals to the playbacks in the first trials, the sequences were extended to 90 seconds to increase exposure time. This was accomplished by tripling the original 30-second sequences resulting in 90-second sequences of straight calls. I constructed the playback sequence with reduced amplitude at the beginning. Then I increased the volume slowly (using the program Audacity) until the sequence reached its normal amplitude after 20 seconds. The reduction of the amplitude was necessary because some of the original call sequences had loud background noise. The natural sequences would have caused a sudden noise onset at the start of the playback that could potentially intimidate the focal individuals. The sequence used for the playback was randomly selected for any given trail.

To play back the calls to the individuals at a natural amplitude (not counting for the first 20 seconds) I measured the distance between a focal individual and the directional microphone with a Leica LRF 800 laser rangefinder (Leica Camera AG, Germany) while recording the individual’s calls. In a second step, the recorded calls were played back in a quiet environment with a JBL Charge 2+ loudspeaker to the Sennheiser directional microphone from the same distance with 16 different volume levels. For the playback, I selected the volume level at which the amplitude matched that of the original sequence best. I played the sequence back with the help of a JBL Charge 2+ speaker (Harman International Industries, USA, frequency response 55 - 20,000 Hz). I placed the loudspeaker outside the enclosure, not to give the impression that a foreign animal had entered the enclosure, when the playback was running. The 90-second file was played back up to 3 times in a row with 5 seconds of silence between the repetitions, depending on what the animal was doing. Since the animals close their ears while diving and the same animals kept diving in the water, I ran the sequence often enough to make sure the focal individual was hearing to the sequence.

The following conditions had to be fulfilled in order to conduct a playback experiment:

- The stationary recorder had to be installed one day before the playback so that the animals had time to get used to the new equipment.
- The last playback had to be passed/done at least 1.5 hours ago.
- The focal individual had to be outside of the den and not be eating.

I established the following restrictions in order to avoid habituation to the playback.

- A maximum of 3 playbacks were played per day.
- Only calls from one sex were played per day.
- There was at least a one-day break between two playback days.
- A maximum of 9 playbacks were conducted per individual within a week.

If all these conditions were met, I played back a random sequence. I pointed the directional microphone towards the individual for the playback and I continued recording with the directional microphone after the playback as long as the focal animal showed any behavioural interest towards the place the playback was conducted. Then, the animal was visually monitored for 5 minutes after the playbacks. Often the visual monitoring was aborted earlier because the focal animal swam into a remote corner of the enclosure or withdrew into its den.

3.2 Historical datasets

3.2.1 Germany

Additional data were provided by Barbara Rogoschik, who recorded otters between 1985 and 1987 in Oderhaus, Germany, to study the vocal development of newborn Eurasian otters. The otters were accommodated in six adjacent enclosures in which several microphones were mounted. In her study two different microphones were used, homemade microphones (frequency response 45 – 18,000 Hz) as well as Sennheiser ME 88 microphones (Sennheiser electronic GmbH & Co. KG, Germany, frequency response 50 – 15,000 Hz). (personal communication, Barbara Rogoschik 1987, unpublished PhD thesis).

3.2.2 Footage

Kaspar Klopfenstein provided a short footage of a wild otter that is calling. The camera trap was set at the river Aare, in the Area of Münsingen, Bern. The wild otter was filmed in March 2017 with an Ultrafire XR6 camera trap (Reconyx, USA). The animal ran calling in front of the camera, while a car was heard in the background.

3.3 Data processing

3.3.1 Labelling and cutting of calls with Audacity

I labelled the calls in the .wav files with the program Audacity. The following spectrogram settings were used: scale: linear, viewing range: 0 - 16,000 Hz, algorithm: frequencies, window size: 1024, window type: Hanning. The label contained the type of vocalization, animal location, name of the individual, date, time, number of the used recorder and number of the call within the file to enable a specific identification of the call. I cut the labelled calls in Audacity with the option Export Multiple and saved them as separate .wav files (sampling frequency 44,100 Hz, 16 bit) in a new folder, using the label as the file name.

3.3.2 Standardizing and analysing with Praat

I used the program Praat (Boersma & Weenink, 2018) to standardize the sound files and extract the acoustic parameters of the calls (sampling rate 44,100 Hz) with the following settings: spectrogram settings – window length (s): 0.01; advanced spectrogram settings – method: Fourier, window shape; Gaussian; pitch settings- pitch range (Hz); 500 – 5000, analysis method: cross-correlation; advanced pitch settings –silence threshold: 0.03, voicing threshold: 0.6, octave cost: 0.1, octave-jump cost: 0.8, voiced / unvoiced cost: 0.14.

I processed the data with the help of two Praat scripts. The first script standardized the files by reducing them to one-channel files and resampling with 44,100 Hz. To reduce low-frequency background noise, the script filtered out everything below 200 Hz by using a stop Hann band filter. In addition, the script added 0.3 seconds of silence at the start and at the end of the files and saved them as 16 bit .wav files in a separated folder. I adjusted and modified the second script originally written by Elodie Briefer. It corrected the added silence at the beginning and end of the call and extracted and saved the parameters, such as sound_duration, Mean F0, StDev F0, F0 start, F0 end, Max F0, Time Max F0, Min F0, F0 abs slope, F0 CV, infl_asc, infl_desc, inflex, sumvar, variationtot, Time of max intensity, %Time of max intensity, Fpeak_all, Fpeak_maxAMP and jitter and in a excel file (for explanations see Table 3).

In addition, I extracted the parameters sound_duration, pitch_mean, pitch_standard deviation, pitch_min, pitch_max and jitter_local manually with the the voice report function in Praat using same settings as for the Praat scripts (Table 3). The manual extraction of the parameters in Praat was done for 50 calls and then compared to the values extracted with the Praat script of the same 50 calls. All the parameter showed identical values, only sound_duration differed between the two methods by a few milliseconds. Since both methods generated the same values, the parameters of the remaining calls were extracted using the Praat script. Only values extracted with the Praat script were used for the statistical analysis.

3.3.3 Camera trap videos analysed with QuickTime Player

I analysed the 20 seconds videos from the camera trap with QuickTime Player version 10.4 and I marked when an otter was seen on the footage. In addition, I marked when the animal was visibly vocalizing by noting the time code. I synchronized date and time to link the footage to the audio recordings.

Table 3 Description of the 20 investigated acoustic parameters inclusive matching manually extracted parameter.

Praat script extracted parameter	Description	Measurement	Manually extracted parameter
Mean.F0	Mean fundamental frequency	Fundamental frequency	Pitch_mean
Max.F0	Maximum fundamental frequency	Fundamental frequency	Pitch_max
Min.F0	Minimum fundamental frequency	Fundamental frequency	Pitch_mean
StDev.F0	Standard deviation of fundamental frequency	Fundamental frequency	Pitch_SD
F0.start	Fundamental frequency at start of call	Fundamental frequency	
F0.end	Fundamental frequency at end of call	Fundamental frequency	
F0.CV	Coefficient of variation of fundamental frequency	Fundamental frequency	
F0.abs.slope	Mean absolute slope of fundamental frequency	Fundamental frequency	
Infl_asc	Number of ascending inflection points in fundamental frequency	Frequency variation	
Infl_desc	Number of descending inflection points in fundamental frequency	Frequency variation	
Inflex	Sum of Infl_asc and Infl_desc divided by call duration	Frequency variation	
Variationtot	Absolute value of the sum of the difference between consecutive pitch points	Frequency variation	
Sumvar	Absolute value of the sum of the difference between consecutive pitch points divided by call duration	Frequency variation	
Jitter	Frequency variation from cycle to cycle	Frequency variation	Jitter_local
Fpeak_all	Frequency with the highest intensity measured from the power spectrum of whole call	Spectral Energy	
Fpeak_maxAMP	Frequency with the highest intensity measured from the power spectrum of a 10 ms slice taken at the point of maximum intensity in the call	Spectral Energy	
Sound_duration	Total call duration	Temporal	Sound_duration
Time.Max.F0	Time at which maximum fundamental frequency occurs	Temporal	
Time.of.max.intensity	Time at which maximum intensity occurs	Temporal	
%Time.of.max.intensity	Percentage of time through the call that maximum intensity occurs	Temporal	

3.4 Statistical Analysis

All statistical analyses were done using the statistical computing program R version 3.6.1 (2019) with the help of RStudio version 1.1.423 (2016).

3.4.1 Consistency over time

For most individuals, high quality calls from only a single day could be found within the files. In order to show that the investigated parameters of the calls remain constant over several days, I established the potential of consistency coding (PCC). The PCC is derived from the potential of identity coding (PIC, Robisson 1993).

$$\frac{CV_{inter}}{\overline{\overline{X\ (CV_{intra})}}} = PCC$$

The mean value of the coefficients of variation for each parameter per day (CV_{intra}) is compared with the coefficient of variation over all days (CV_{inter}). The coefficient of variation was calculated by dividing the sample standard deviation by the sample mean. The ratio of the two coefficients of variation indicates if a parameter is consistent over time. Whereby the ideal value is 1, values between 0.5 and 1.5 suggest a consistency of the parameter. If the PCC is greater than 1.5, this indicates a difference between the days. A value of ≥2 shows that the parameter clearly differentiates between the days and therefore there is no consistency over time. I calculated the PCC for two individuals, from whom whistle calls had been recorded for six consecutive days.

3.4.2 Individual distinctiveness

3.4.2.1 High quality-calls of few individuals

To analyse individuality in the Eurasian otter whistle calls I performed a discriminant function analysis (DFA). The data set included ten random calls per individual. Due to the fact that a DFA is very susceptible to outliers (Poulsen and French 2008), I tested the data for outliers. As a result, for one individual with few calls, I could only include nine calls. Thus, the final data set consisted of 59 calls from six individuals. Beecher's information statistic (H_S, Beecher 1989) was used to reduce the number of parameters. All parameters with a value higher than H_S = 0.5 were integrated into further analysis.

An assumption for a discriminant function analysis is that there is no multicollinearity. My data set violated this assumption (kappa = 1356.35). To counter this issue, a principal component analysis (PCA) was performed with the help of the prcomp() function from the stats package. The loadings for the first principal component were all fundamental frequency measurements and they weight of their loadings were almost identical. Therefore, I removed all but one fundamental frequency parameter. This procedure allowed to continue without the need of a PCA. With the help of model selection, I removed also the temporal parameter jitter. The final analysis was run with only two parameters, sound duration and mean fundamental frequency.

A cross-validation was performed using the "leave-one-out" method, in which the discriminant functions are derived from all objects but one, which is then classified. The analysis in R was done using the lda function from the MASS package (Venables & Ripley 2002). According to Mundry and Sommer (2007) a linear discriminant function analysis is likely to overestimate the correct classification due to the assumption of independence. They suggest to run a permuted discriminant function analysis (pDFA), which combines the DFA with the permutation of the data. The pDFA was conducted with the pDFA.nested using the function written in R by Roger Mundry. The function is based on the function lda of the R package MASS.

3.4.2.1 Low-quality calls of more individuals

For some individuals it was not possible to extract enough calls from the recordings or they were of low quality. I included these calls in a second analysis, where the procedure was conducted the same way as with the robust data. The data set consisted of 85 calls for eleven individuals, with a range of four to ten calls per animal.

3.5 Ethical note

The passive acoustic recording with the stationary recorder and the directional microphone was conducted with the permission of the cantonal veterinary offices and the corresponding zoos/parks. Recording the animals did not harm them or expose them to stress. The instalment of the recording devices can be seen as enrichment and the recording devices had been extensively inspected and examined by the animals. After a short habituation (few hours) the otters were used to the new equipment: they ignored the recording devices and showed their normal behaviour. To conduct the playback experiments, an animal experiment permit was received from the respective cantonal veterinary offices (ZH077/2019).

4 Results

4.1 Identified call types

All known Eurasian otter call types were identified in the recordings: whistles, cries, murmurs, mewings, twitters, blows (Table 4). The most abundant call types emitted over the recording period were the whistle (Fig. 1), the cry (Fig. 6-C) and the mewing (Fig. 6-D). I recorded the murmur (Fig. 4) only when two adult individuals were close to each other. Particularly often, I collected this type of call in the zoo of Zurich after the two otters had been brought together for the first time. The blow (Fig. 6-A) was recorded only very rarely, and it was difficult to distinguish the blow from an exhalation close to a microphone. A call only recorded from newborns (few days old) was the twitter (Fig. 6-B).

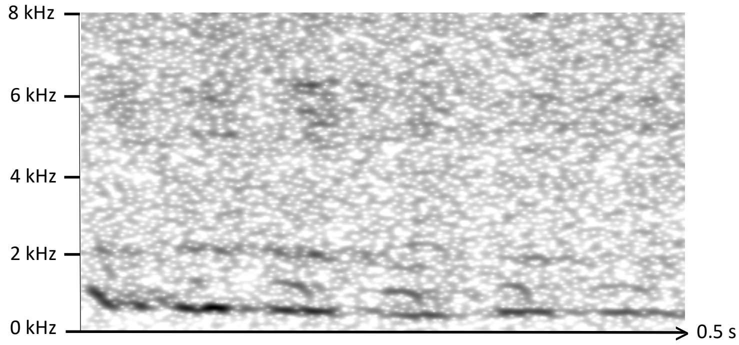

Figure 4 Spectrogram of the murmur, a short close contact call with the lowest fundamental frequency of all the recorded calls.

In addition, I found various intermediate forms of different call types, where the vocalization started with one call type and then graded into another. The most common gradation I recorded was the merging from cries and mewings (Fig. 7). The gradation between the two call types occurred in both directions. In a single case I found a whistle at the end of a gradation from mewing to cry. Gradations were also found between murmurs and cries with the call usually starting as a murmur merging into a cry. Furthermore, I detected a vocalization that was not previously described. I recorded the call 29 times in ten different events (between 1 – 8 calls in one event). The call (Fig. 5) was emitted by a single individual in the zoo of La Garenne. It has a mean fundamental frequency of 756 Hz ($\pm$ 90 Hz) and a mean duration of 145 ms ($\pm$ 45 ms). It was given as a single call with an inter-call interval of at least two seconds. The call was produced while eating or shortly before or after a feeding event.

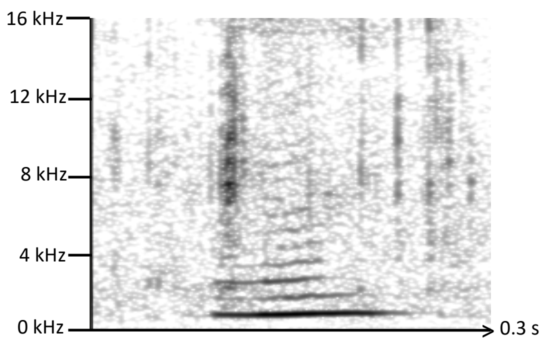

Figure 5 Spectrogram of a call emitted by an Eurasian otter in association with food.

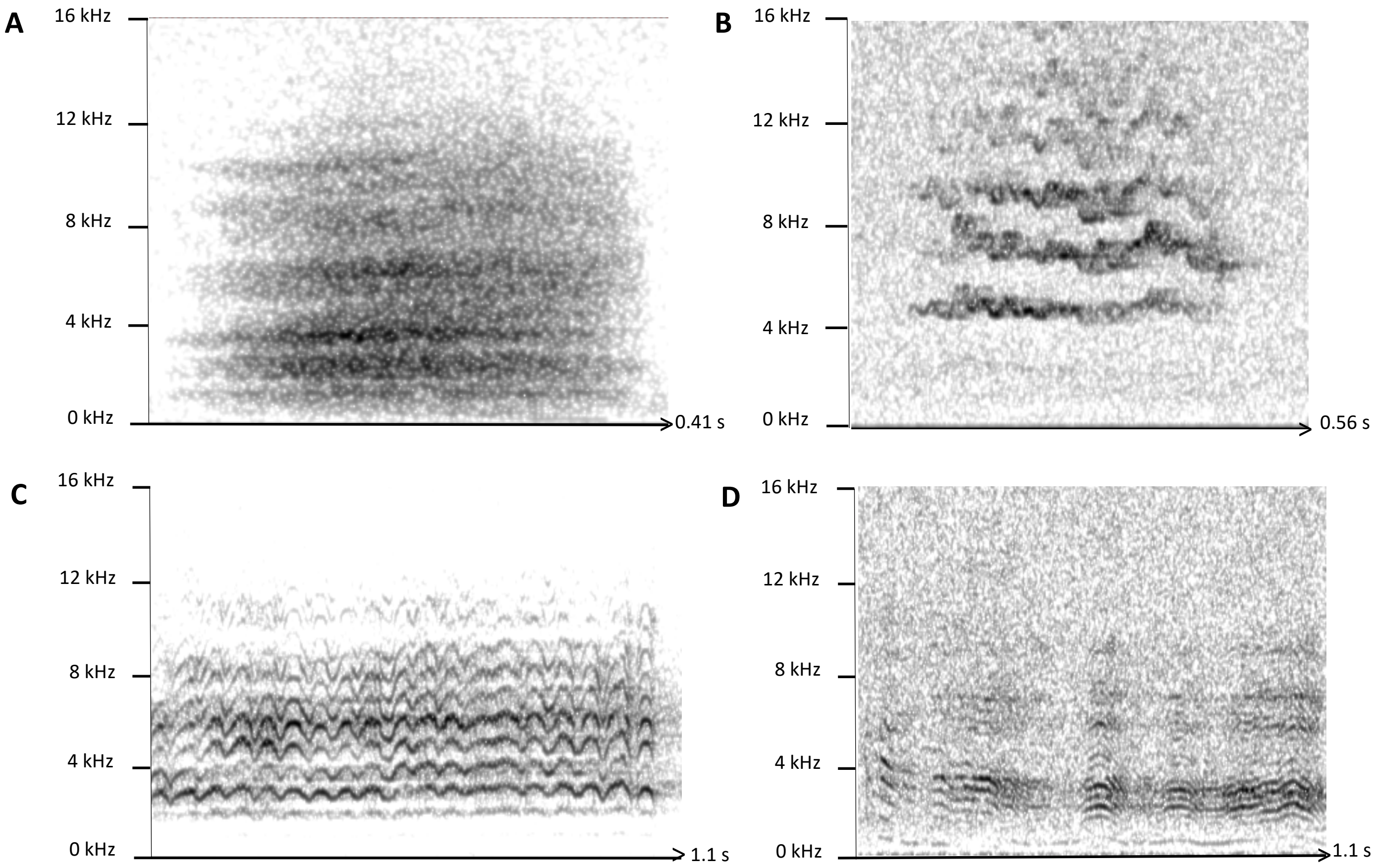

Figure 6 Spectrograms of the different call types recorded on the captive animals in Switzerland. **A** Blow, a short and noisy non-tonal call. **B** Twitter, a call frequently produced by newborn pups. **C** Cry, aggressive vocalization in context with conspecifics with flexible call duration. **D** Mewing, a prolonged whiny sound with flexible call duration.

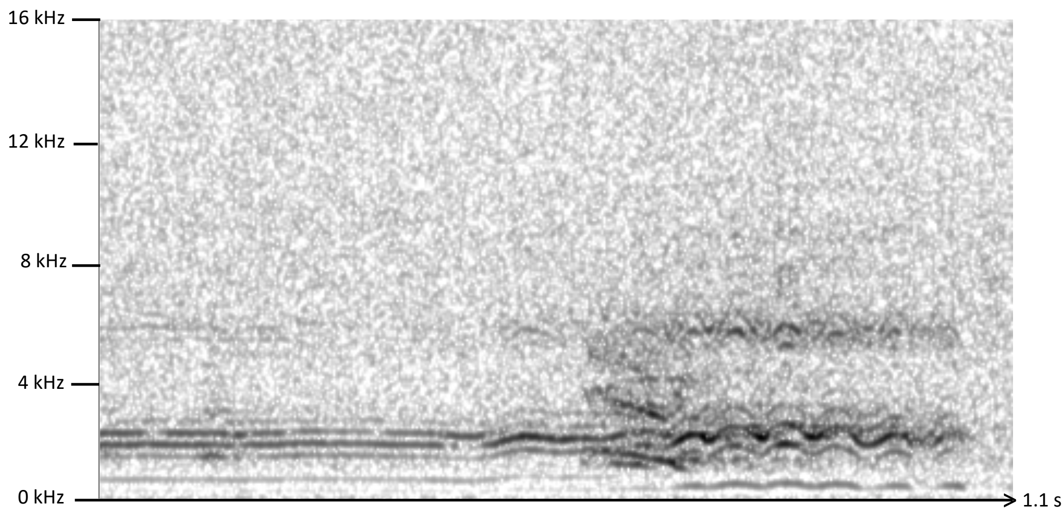

Figure 7 Spectrogram of a graded call transitioning from a mewing into a cry.

Eurasian otter vocalizations were recorded at five of the six zoos included in this study (Table 4). I recorded the close contact calls cries, murmurs, mewings and the graded calls exclusively in zoos, where the otters were kept in pairs or in groups. In addition, I recorded the whistle in the Tierpark Dählhölzli, Zoo Zürich, Fischottergehege Männedorf and Wildnispark Zürich. In the zoo La Garenne I recorded only undescribed calls. Also, I did not record a single otter vocalization in the Tierpark Goldau. In summary, I recorded long distance calls (whistles) in enclosures with solitary and group living otters, while the close contact calls were only recorded in enclosures with more than one individual.

Eurasian otters called much more frequently during the night-time than during the day-time, though this also depended on the call type. Calls were most likely recorded between 02:00 and 04:00 hrs in the morning or at 23:00 hrs in the evening. The whistle (Fig. 8) followed this pattern, more whistles were recorded in the night-time, with peaks between 02:00 and 03:00 hrs in the morning and between 22:00 and 23:00 hrs in the evening. The same pattern was observed with the murmur (Appendix, Fig. 14) and mewing call types (Appendix, Fig. 15). This was different to the cry which was more evenly emitted throughout the whole day and the most frequent call produced during daylight hours (Appendix Fig. 16). I excluded all graded calls, as they could not be reliably assigned to a specific single call type category as well as the blow and the twitter.

Table 4 Overview of the different zoos and call types that animals emitted. The category 'undef calls' contains all the graded calls as well as vocalizations emitted by otters that do not belong to the six described call types. Twitter and murmur are underestimated due to grouping several calls to sequences and not labelling each single call.

Name of Zoo	**Number of animals**	**Hours** recorded	**Hours** analyzed	**Whistle**	**Cry**	**Murmur**	**Mewing**	**Twitter**	**Undef calls**
Zoo La Garenne	1 female	148	66	0	0	0	0	0	Yes
Tierpark – Bern Dählhölzli	1 male, 1 female	532	67.5	1334	259	5	58	0	Yes
Zoo Zürich	1 male, 1 female	649	372.5	12	371	61	0	0	Yes
Fischottergehege Männedorf	1 male	124.5	4.5	534	0	0	0	0	Yes
Wildnispark Zürich	1 male, 3 females, ≥ 2 pups	384.5	228	Adult: 83 Pup: 128	88	4	3	195	Yes
Tierpark Goldau	1 male	154.5	101.5	0	0	0	0	0	No

The results (Fig. 8) give an overview at what time of the day a call was recorded, but they do not indicate the quantity of calls within these periods. For example, the whistles were sometimes recorded more than 200 times in half an hour around 02:00, while during the day only up to 25 whistle calls were recorded during the same period.

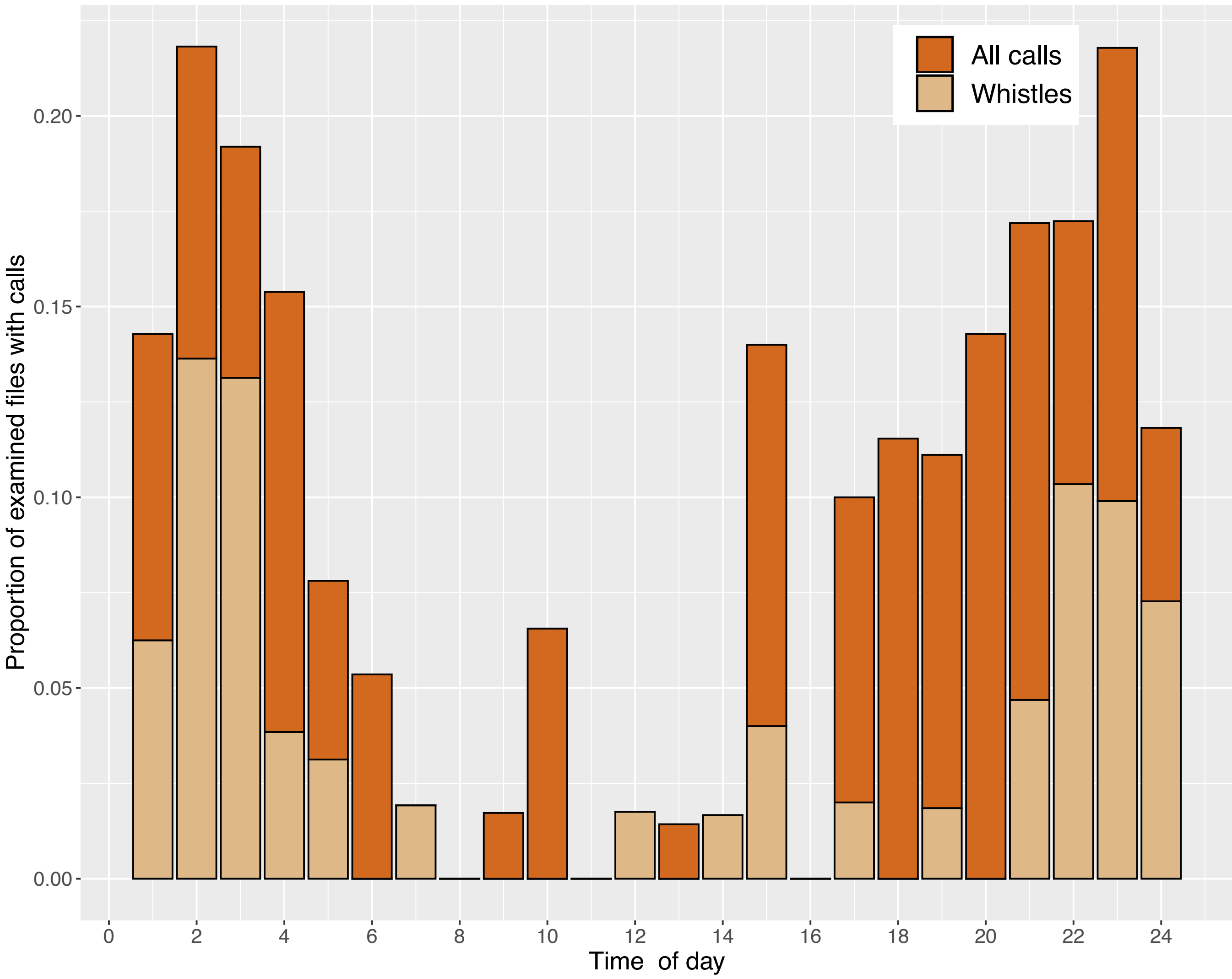

Figure 8 Overview at which time of day audio files with at least one call/whistle were recorded. The x-axis indicates the time of day, while the y-axis shows the proportion of audio files containing a call. The orange bars indicate the percent of the examined 30 min audio files that contained at least one call of any type. The beige bars show what percent of the examined files contained at least one whistle call. (N = 80.5 record days of effort in 6 zoos with 11 individuals)

4.2 Consistency over time

I investigated the acoustic parameters for their consistency over time. The potential of consistency over time of all 20 measured acoustic parameters extracted from whistle calls indicated consistency over the six-day period for the two individuals (males) where high-quality recordings on six consecutive days were available (Table 5). The parameters closest to one indicating the highest similarity were Fpeak_maxAmp, time.Max.F0 and Mean.F0. The most differing parameters were F0.abs.slope and Jitter. The variation within most parameters was similar for both individuals, but the difference between the two animals was clear in the fundamental frequency (Fig. 9-A).

Table 5 The potential of consistency over time coding (PCC) of two individuals (Ind.1 and Ind.2) calculated for acoustic parameters extracted from whistle calls. Values between 1 and 1.5 indicate a potential of consistency over time. Values above 1.5 indicate large variation of the parameter between the days.

	PCC Ind.1	PCC Ind.2		PCC Ind.1	PCC Ind.2
Sound_duration	1.15	1.29	Infl_asc	1.21	1.34
Mean.F0	1.10	1.05	Infl_desc	1.19	1.36
StDev.F0	1.25	1.16	Inflex	1.31	1.18
F0.start	1.10	1.33	Sumvar	1.06	1.10
F0.end	1.17	1.08	Variationtot	1.32	1.29
Max.F0	1.29	1.09	Time.of.max.intensity	1.02	1.14
Time.Max.F0	1.02	0.96	%Time.of.max.intensity	1.02	1.12
Min.F0	1.19	1.09	Fpeak_all	1.10	1.30
F0.abs.slope	1.47	1.29	Fpeak_maxAMP	1.01	0.96
F0.CV	1.22	1.14	Jitter	1.25	1.39

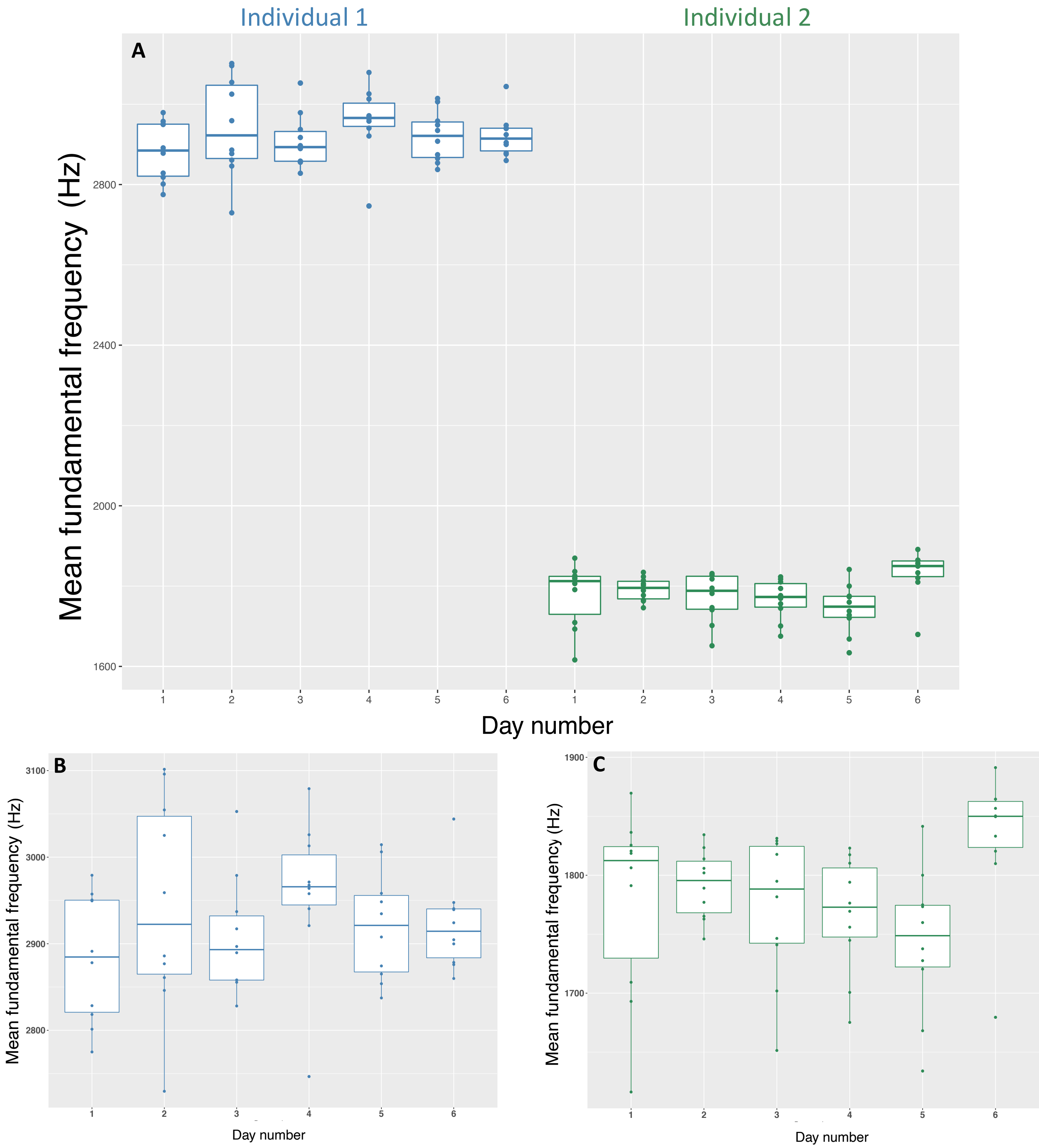

Figure 9 **A** The mean fundamental frequency of two individuals differed substantially. Within the individuals, the mean fundamental frequency was highly consistent over the six consecutive days. Each day contains ten high quality calls (for each individual N=60 calls). **B** Detailed view of individual 1 (Frequency range = 2750 Hz – 3100 Hz). **C** Detailed view of individual 2 (Frequency range = 1630 Hz – 1880 Hz).

4.3 Individual distinctiveness

4.3.1 Principal component analysis

A principal component analysis was conducted on eight acoustic parameters with orthogonal rotation. The first and second principal component explained together 89.9% of the variation (Fig. 10). The loadings of the first principal component consisted mainly of fundamental frequency measurements, all having the same weight for the calculation of the component (arrows have the same length pointing into the same direction). The second principal component was loaded with mainly temporal parameters (sound_duration, jitter). The six individuals group nicely together.

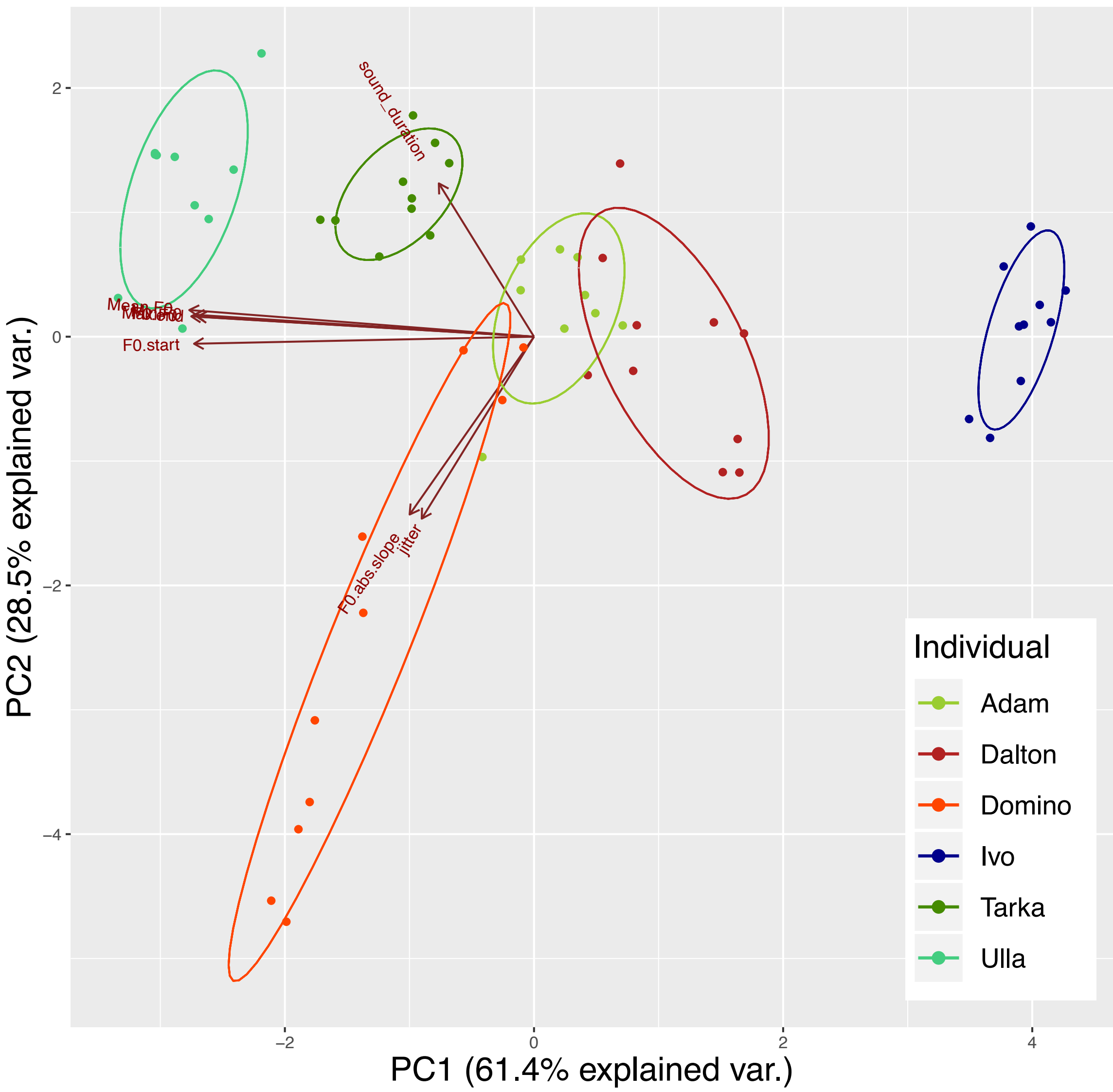

Figure 10 The first two principal components (PC) for eight acoustic parameters. PC1 explained 61.4% of the variation and PC2 explained 28.5% of the variation. The loadings of the different principal components are indicated with the arrows (N=10 calls per individual).

4.3.2 High quality-calls of few individuals

A permuted discriminant function analysis (pDFA) was performed on a data set of high-quality whistle calls of six individuals (N= 9-10 calls per individual). The cross-validated pDFA correctly classified 96.4% of the whistle calls to the six individuals (Fig. 11). This result was statistically significant (P= 0.0001) compared to the expected correct classification of 16.5 % by randomly assigning a call to an individual. The percentage of correct classification per individual ranged from 81% (Tarka, Fig. 11) – 100% (Ivo, Fig. 11) with males having a higher average correct classification percentage than females (males vs. females: 99.2% vs. 85.6%).

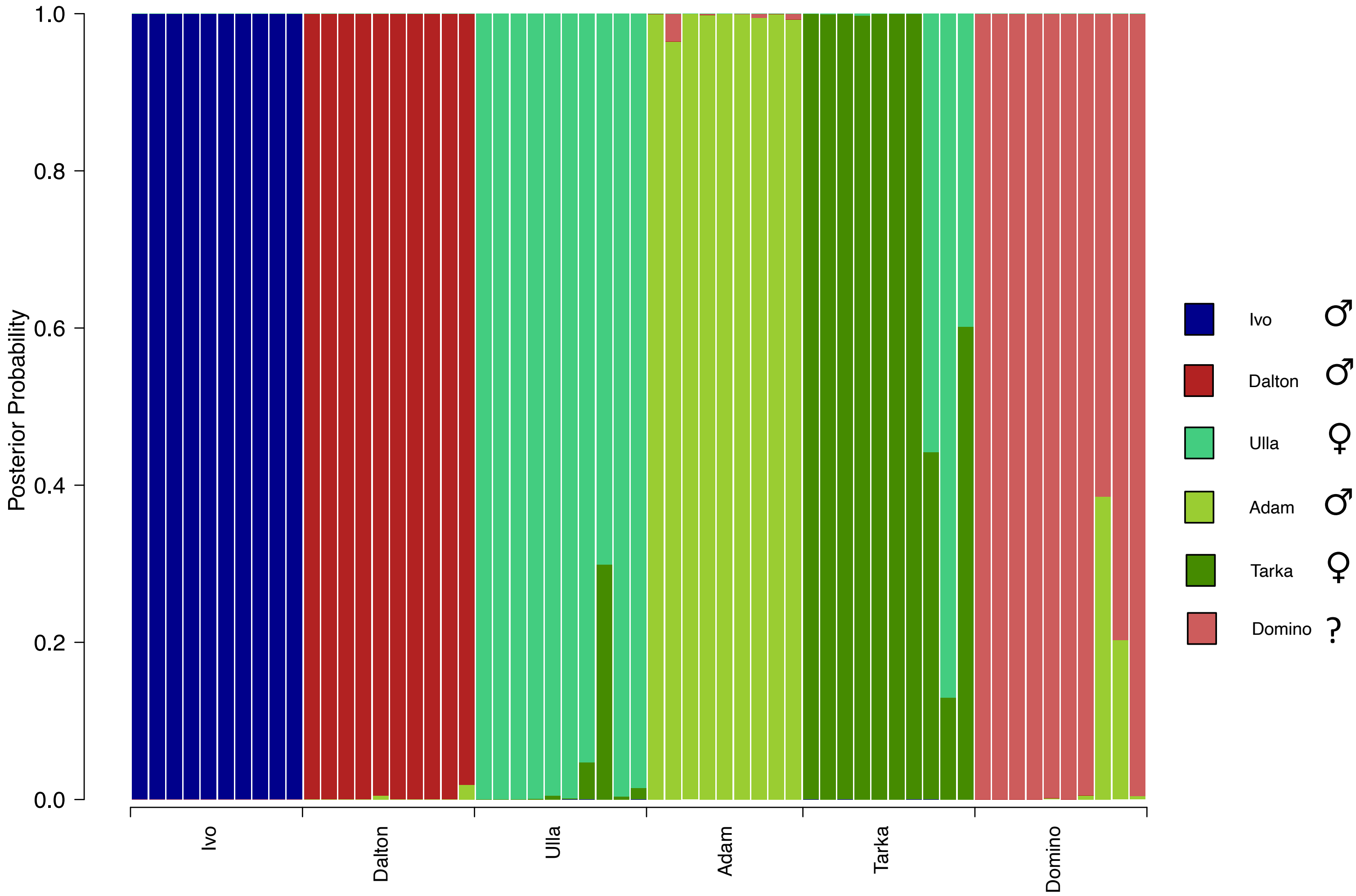

Figure 11 Correct classification of each whistle call to the six individuals with high quality recordings with a permuted discriminant function analysis. Nine to ten high quality calls per individual were analysed. Each bar corresponds to a single call. The colour code of a bar indicates the probability with which the call was assigned to an individual.

4.3.3 Low-quality calls of more individuals

A pDFA was performed on a data set of less high-quality whistle calls of eleven individuals (N= 4-6 calls per individual). Overall, 79.3% of all the recorded whistle calls were correctly attributed to the correct individual (Fig. 12). Compared to the expected correct classification of 9.1% with randomly assigned calls, the results were statistically significant (P= 0.0001). One individual (Lulu, female) showed only 2% of correct classification. Otherwise, the correct classification of each individual varied from 61% - 99%. The results differed between the sexes with 87.3% for the males, 88% for the wild individuals where the sex was unknown and 69.6% for the females. However, when excluding Lulu (4 calls with very low quality), the females achieved an average correct classification of 86.5%. In this case, the correct classification of all three categories (male, female, unknown sex) is very similar, leading to an overall average correct classification of 87.3%.

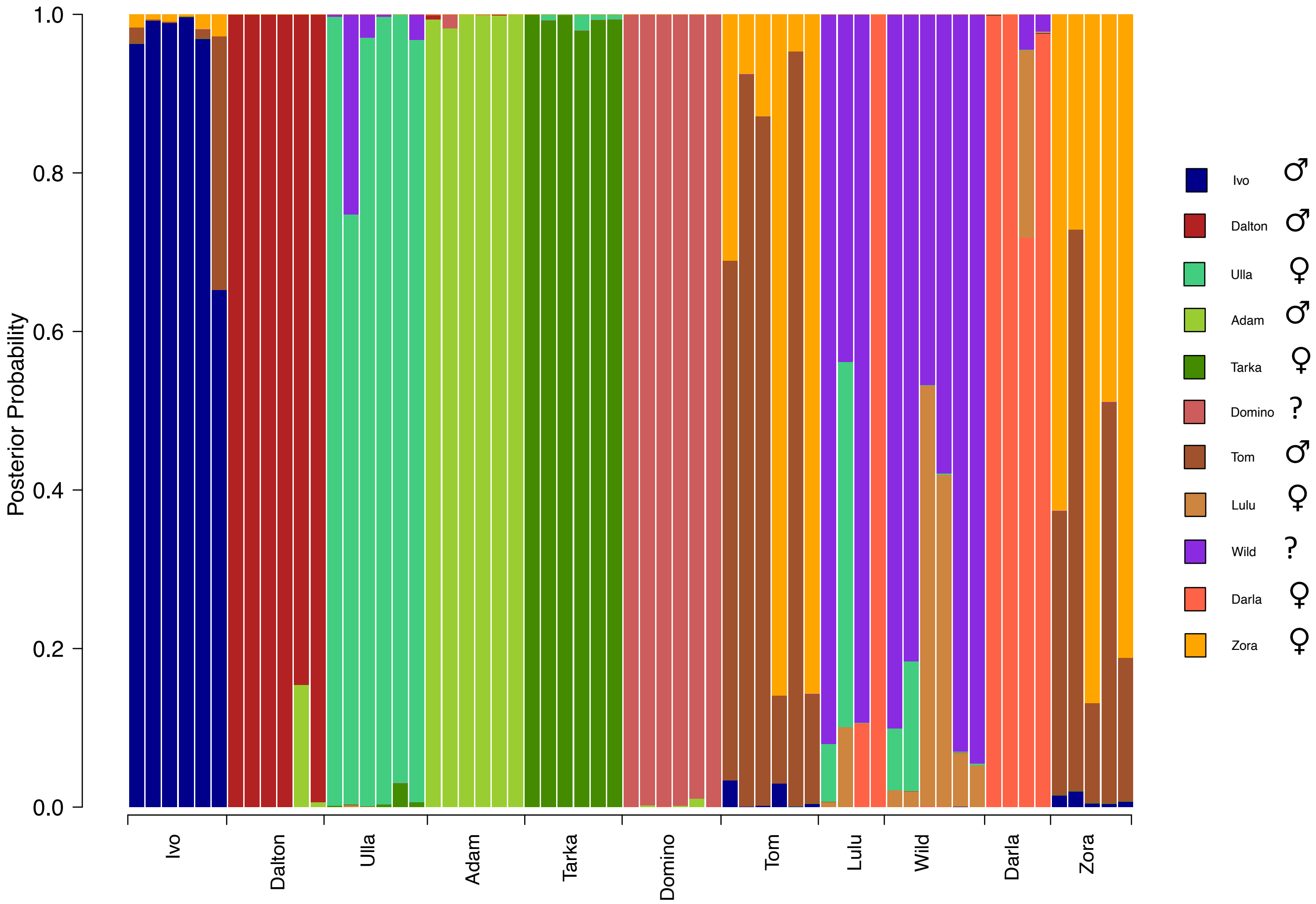

Figure 12 Correct classification of each call for the extended data set with a permuted discriminant function analysis. 4 to 6 recordings per individual of different quality were analysed. Each call is represented by a bar. The different colours of the bars show how often an individual has been allocated to a call.

The variable that explained most of the correct classification of the DFA was the mean fundamental frequency (Fig. 13). The fundamental frequency of the individuals ranged from 1700 Hz to 4900 Hz (mean across all individuals = 3377 ± 930 Hz). The lowest mean fundamental frequency belonged to Ivo (1801 ± 35 Hz) a male from Männedorf while the highest mean fundamental frequency belonged to the female Darla from Tierpark Dählhölzli (4609 ± 139 Hz). Four of the five females had a higher fundamental frequency than 3500 Hz while all of the males had a lower fundamental frequency than 3500 Hz. An exception was the female Zora from the Wildnispark Zürich. She had a mean fundamental frequency of 2201 ± 43 Hz, this clearly differed from the fundamental frequencies of the other females.

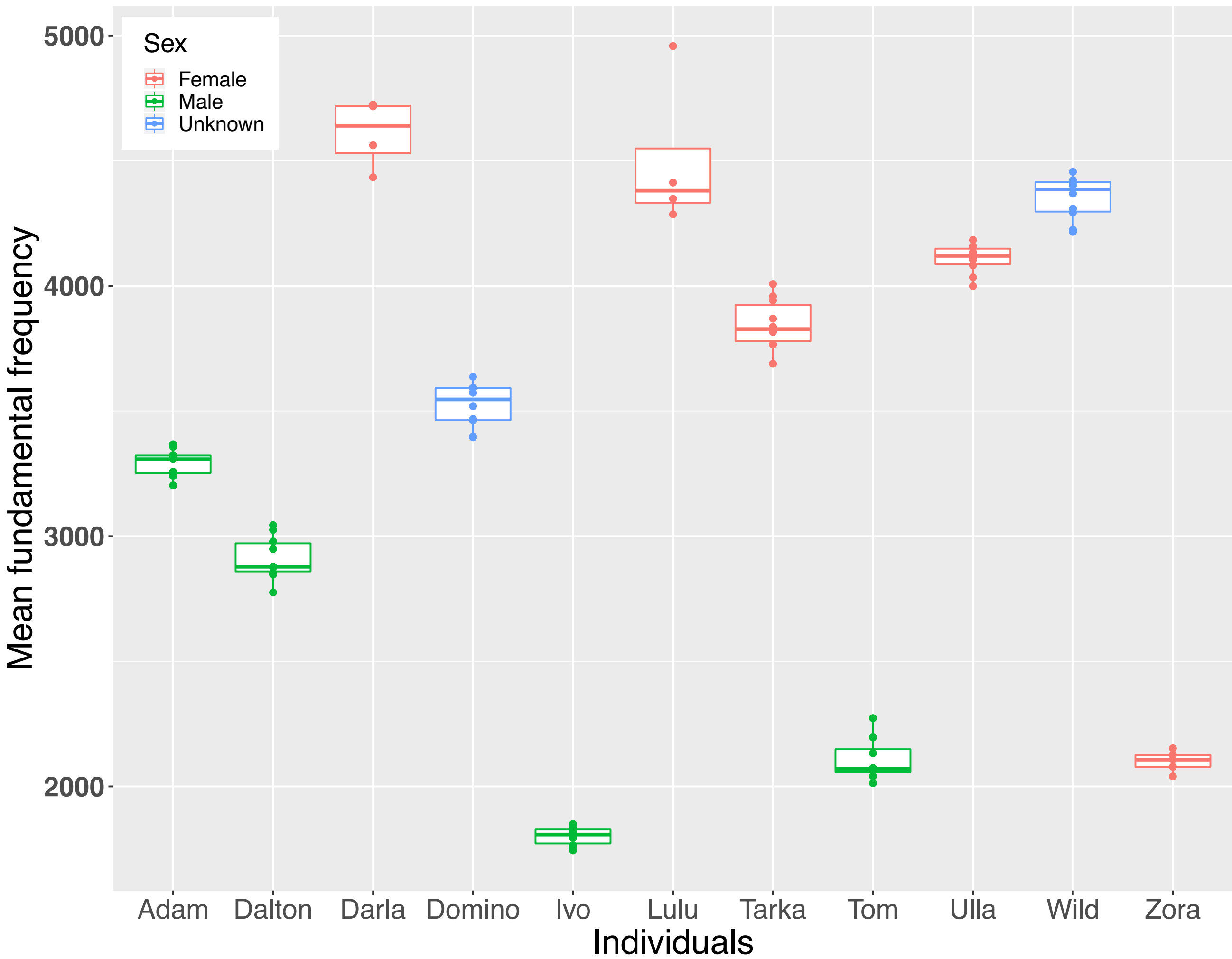

Figure 13 The mean and variation (SD) of the fundamental frequency of the eleven individuals. The variation of the fundamental frequency within an individual was usually 300 to 350 Hz in all individuals.

4.4 Playback experiment

Of the total of 23 playbacks conducted in the five of the six zoos the response to the playback was different in each zoo, but none of the tested subjects produced a tonal vocalization as a result of the playback. However, in the Tierpark Dählhölzli, both the male and the female emitted blows in response to the playback, as they swam slowly towards the speaker. There were similar behavioural reactions at the Zoo Zurich and the Wildnispark Zurich, but no blows were recorded there: The animals turned their heads towards the loudspeaker and approached slowly. Due to the circumstances (high background noise, remote location of individual and water noise) I was not able to record blows from these animals if they had produced any. The strongest reaction was shown by the female in the Zoo Zürich. She ran directly towards the loudspeaker during a playback sequence (whistle calls from the male from Männedorf). When playing back sequences of other individuals: The female turned her head towards the speaker and approached slowly, without emitting a call. In both zoos Tierpark Goldau and La Garenne, the focal animals did not show any obvious reaction to the playback.

5 Discussion

In this study I investigated whether the calls of Eurasian otters collected with stationary recording devices and directional microphones are individually discernible, and which acoustic parameters are most important in discriminating individuals. I found that whistle calls contained characteristics that enabled discrimination between individuals with a rate that was significantly higher than expected by chance. In addition, I used playback experiments to trigger animals to voice whistle calls. Although visible behavioural reactions were observed, no additional whistle calls were recorded.

5.1 Individual consistency

When comparing the variation within the call structure of two individuals across six consecutive days, both individuals showed consistency in the 20 examined acoustic parameters. Variation was present within all the investigated parameters, but overall the variation between days was not greater than the variation within days. Even though the sample size of two individuals was very small, the number of parameters was high, and furthermore all parameters were consistent over time for both individuals. Whether the calls of adult individuals are consistent over a longer time period, such as weeks, months or years, or in relation to specific life history stages, is still unclear. However, the acoustic parameters of Eurasian otter pups are most likely not consistent, as a study showed that the change of the fundamental frequency of pups is non-linear (Barbara Rogoshik 1987, unpublished PhD thesis). Although, the fundamental frequency of pups is on average higher than that of adults, the fundamental frequency increases for a few weeks after birth before it then decreases. However, further studies are needed to examine whether the fundamental frequency changes after individuals reached adulthood or stays the same.

If body size and fundamental frequency correlate, as it would be predicted according to the source-filter theory (Chiba and Kajiyama 1941), and Eurasian otters are able to extract this information from the whistle call of conspecifics, this could affect the behaviour of the receiving individual. Smaller males or females could avoid larger conspecifics of the same sex, and not to risk physical confrontation. Alternatively, the fundamental frequency could also decrease with age. This might explain the fundamental frequency of two older individuals Ivo (Männedorf) and Zora (Wildnispark Zürich) having the lowest fundamental frequency of all individuals. This hypothesis, however, could not be investigated as the age was not known for all individuals. However, a study done on Asian small clawed otter supports the theory that older, heavier and larger individuals emit lower-fundamental frequency calls (Lemasson et al. 2014). Studies on other species have shown that the fundamental frequency of individuals can also be influenced by hormones (great partridge [*Perdix perdix*], Beani et al. 1995), dominance status (meerkat [*Surictta suricatta*], Mausbach et al. 2017) or social status (paired vs. single individuals, pygmy marmoset [*Cebuella pygmaea*], Snowdon and Elowson 1999). Even though social status might not be as relevant for a solitary species, information on dominance might be conveyed through calls. For example, Koelewijn et al. (2010) showed that dominant Eurasian otter males reproduce more often. Also, the hormonal balance changes throughout the different life stages and could therefore have short-term or long-term influences on the fundamental frequency.

5.2 Individual distinctiveness

My results show that whistle calls of individual Eurasian otters can statistically be discriminated based on only two acoustic parameters: mean fundamental frequency and call duration. This was shown using the data set with only high-quality recordings (96.4% correct classification) of six individuals as well as for an extended data set including lower quality calls of eleven individuals (79.3% correct classification). The most discriminating parameter was mean the fundamental frequency, however, all investigated fundamental frequency measurements can be used for the discrimination of Eurasian otters as indicated by the results of the PCA. However, individuals with similar frequencies could not be distinguished as reliable.

The lower correct call classification in the extended data set may be the result of several factors, such as sample size of individuals, calls per individual, or call quality (Budka et al. 2014). Discrimination of individuals may be improved by comparing call sequences of whistles. The use of several whistle calls in a row might help to reduce the within individual variation while at the same time increasing between individual variation. Furthermore, by analyzing call sequences instead of single calls, additional parameters such as inter-call interval or calls per second can be considered.

The correct classification percentage of the discriminant function analysis in my study is higher compared to studies in social living otter species. McShane et al. (1995) found a 80% correct classification in screams of adult sea otters (*Enhydra lutris*) and a 75% correct classification in screams of sea otter pups. In giant otters (*Pteronura brasiliensis*), the correct classification of cohesion calls was 56% (Mumm et al. 2014), while in Asian small clawed otters the correct classification was 51% (Lemasson et al. 2014). The differences between the correct classification percentage found in these studies can have several reasons. First, the studies investigated different call types. Second, the sample sizes differed a lot between the studies. Third, different acoustic parameters were used in each of the studies. Although the four studies used call duration and fundamental frequency measurements for the analysis, the studies on the social otters included further parameters in their calculations: Asian small clawed otters: maximum frequency, dominant frequency, (Lemasson et al. 2014); sea otters: maximum frequency, intecall interval (McShane et al. 1995); giant otter: time to peak frequency, time to peak amplitude, peak amplitude, minimum and maximum frequency (Mumm et al. 2014). Nonetheless, individual discrimination was achieved in all four otter species with correct classifications higher than expected by chance.

Similar high correct classification rates have been found in studies of many animal species, such as birds (up to 94.5% large-tailed nightjars [*Caprimulgus macrurus*], Chang et al. 2018; 88% in great spotted woodpeckers [*Dendrocopos major*], Budka et al. 2018), canids (up to 90% in Asiatic wild dogs [*Cuon alpinus*], Durbin 1998b; 99% in swift foxes [*Vulpes velox*], Darden et al. 2003), ungulates (up to 99.4% in saiga antelopes [*Saiga tatarica tatarica*], Sibiryakova et al. 2017; 77% in red deer, Sibiryakova et al. 2015) and primates (up to 84% in Western gorillas [*Gorilla gorilla*], Salmi et al. 2014; 83.5% in lar gibbons [Hylobates lar], Terleph et al. 2015). However, not all investigated vocalizations achieve a high correct classification percentage using the discriminant function analysis, for example the grunts of piglets (*Sus scrofa domesticus*) achieved a low correct classification with 13% – 31% (Syrová et al. 2017) or the zip calls of rifleman (*Acanthisitta chloris*) with a correct classification of 26% (Khwaja et al. 2019). Nevertheless, even those studies achieved a higher correct classification percentage than expected by chance. However, some vocalizations cannot be attributed to individuals, as in the case of the hum call of the giant otter, where the correct classification percentage was not greater than expected by chance (Mumm et al. 2014).

5.3 Eliciting calls with playback experiments

The playback experiments were conducted to elicit additional whistle calls from otters that did not call over the several recording days. The reaction of the focal animals to the playbacks was different in every zoo and except for non-tonal blows, no vocalization was recorded. All seven animals but two individuals showed a visible behavioral reaction. The two exceptions were the male from the Tierpark Goldau and the female from La Garenne. While the female from La Garenne could have shown no reaction because she was stressed due to being under a medical treatment, no explanation can be found about the male. The two animals from the Tierpark Dählhözli swam towards the microphone and emitted blows. Similar behaviors were observed in the Wildnispark Zürich and the Zoo Zürich, but no blows were detected in the recordings. However, potentially the reason why no blows seemed to be recorded was more likely because the background noise masked the blows rather than the otters not uttering any.

A strong behavioral reaction was shown by the female (Lulu) from the Zoo Zürich. While the playback was still running, Lulu ran towards the speaker and tried to find the source of the calls. This behavior was only shown when the playback sequence from Ivo (male from Männedorf) was played back. Although the two animals did not live in the same zoo over the recording period, they had in previous years successfully reproduced several times. They had met last in spring 2014. The reaction of Lulu indicates that Eurasian otters may be able to identify another individual by its whistle call. Moreover, the reaction suggests that Eurasian otters may be potentially able to remember conspecifics they have met before for at least five years. Though more controlled experiments need to be done to exclude any other reasons for the strong response. This hypothesis is supported by Kruuk's (2006) observations in which marked individuals visited each other several times before they mated. This suggests that Eurasian otters interact with conspecifics of the opposite sex not only for mating and are therefore more social than assumed. A study conducted by Quaglietta et al. (2014) supports the hypothesis that the social system of the Eurasian otter is better described as facultative social as they radiotracked individuals during several years. They documented many friendly non-mating related interactions. Studies have shown that social discrimination is important for social living species (Tomasello and Call 1994). In closely related Asian small clawed otters it was shown that individuals recognize each other acoustically and olfactorily, but not visually (Lemasson et al. 2013). This might also be true for Eurasian otters. The reaction of Lulu indicates that Eurasian otters may be able to acoustically discriminate at least between known and unknown individuals. Further playback experiments could test whether Eurasian otters are able to discriminate between known, unknown and even related conspecifics.

5.4 Vocal repertoire and timing

The captive Eurasian otters in this study emitted all call types that have been described for this species (whistle, cry, murmur, mewing, twitter and blow, see Gnoli and Prigioni 1995) including graded calls, as well as a new vocalization that was emitted in the context of feeding. Except for the call type given in the context of food, more calls were recorded at night-time than during daylight. The fact that the feeding context call was only recorded from one individual could be an indication that this is an individual related call type or a context specific call type. Most likely, not all call types of the Eurasian otter have been described yet. The vocal repertoire of the solitary Eurasian otter is smaller than that of the socially living sea otter (10 different call types, McShane et al. 1995) or the socially living giant otter (15 different call types including graded calls, Leuchtenberger et al. 2014). However, the described number of basic call types for the socially living Asian small clawed otter (Lemasson et al. 2014) is equal in number to the described call types so far for the Eurasian otter, and the spectro-temporal properties of the call types are also very similar. The Asian small clawed otters emit vocalizations resembling the blow, mewing, whistle and cry, furthermore they also merge these different call types (Lemasson et al. 2014) similarly to what I found in my recordings of the captive Eurasian otter.

The 'social complexity hypothesis' states that animals living in more complex social structures require a more complex vocal systems to interact with group members (reviewed in Freeberg et al. 2012). A comparison between closely related mongoose species showed that the vocal repertoire of discrete call types differed between solitarily and socially living species, with social species having a larger vocal repertoire (Manser et al. 2014). Since Asian small clawed otters and giant otters live in a complex social system and have a complex communication system, it can be concluded that the Eurasian otter which have a similar vocal repertoire to Asian small clawed otters should have a similar complex social system. Either the social system is much more complex than assumed (Quaglietta et al. 2014) or the vocal repertoire is rudimentary. If the vocal system is a rudiment, this would indicate a group-living social ancestor. Phylogenetically, the Eurasian otter is more closely related to the Asian small clawed otter than to the sea otter or to the giant otter (Koepfli and Wayne 1998) and closely related species should have a more similar vocal repertoire. These findings suggest that the common ancestor of the Eurasian otter and the Asian small clawed otter had a similar vocal repertoire. To support this hypothesis, the vocal repertoire of the related otter species would have to be investigated: The vocal repertoire of the Eurasian otter should be compared to the closely related hairy-nosed otter (*Lutra sumatrana*); the vocalization of the Asian small clawed otter should be compared to his closest relative, the smooth-coated otter (*Lutrogale perspicillata*).

The most common call type recorded in the zoos and wildlife parks was the whistle. This result coincides with Kruuk's (2006) observation of wild-living Eurasian otters, where he reported these calls to be the most frequently heard. As a mainly solitarily living species, Eurasian otters most likely rely more on long-distance calls than on close-distance. Although the function of the whistle call is not yet fully known, I propose that the whistle call is used as a contemporary signal that covers a large area to inform conspecifics about the location of the sender. In contrast to acoustic cues, the chemical information in spraints is restricted in space but present for a longer time period and informs conspecifics about a presence of an individual in the area (Kruuk 1992). I assume that whistle calls have a territorial function. Eurasian otters may thus provide information about themselves (age, size, health status) to avoid physical confrontations. According to observations of an animal keeper (personal communication, Jörg Wick, Zoo Zürich), captive females also signalled readiness to mate through whistle calls. In captivity, the initiative for mating rarely came from the male. Thus, the reproductive status of a female may not only be encoded in spraints (Kean et al. 2011), but also within the finer structure of the whistle call. In order to fully understand the exact function and context in which Eurasian otters emit whistles, more detailed investigations are required on this call type. The Eurasian otter called significantly more often at night (18:00 – 06:00 hrs) than during the day (06:00 – 18:00 hrs, 1891 vs. 72 whistles). Even though Eurasian otters are described as mostly nocturnal animals, they are flexible in their behaviour. For example, in Shetland they hunt their preferred prey at day-time in a marine environment (Kruuk et al. 1991). Eurasian otters may be mainly active when their prey is asleep, and where the success rate for hunting is high. I thus expected to record more calls during day-time in zoos and wildlife parks due to the feeding during day-time. My results, however, contradict my initial assumptions to find more calls at day-time suggesting that captive Eurasian otters maintain their nocturnal activity pattern.

5.5 Potential for use of PAM to monitor elusive species

In this study I showed that it is possible to statistically discriminate Eurasian otter individuals based on their whistle calls with only two parameters (mean fundamental frequency and sound duration). The correct classification was high with both a data set of high-quality calls and a data set with lower-quality calls. The quality of the latter data set is more likely to reflect calls typically recorded in nature with a PAM. The effect of more individuals reducing the correct classification rate of a DFA might have a small influence on the application of monitoring wild Eurasian otter. The expected number of recorded individuals of one stationary recording device will most likely be low due to the low density of otters (1 adult otter per 7.09-14.36 km river length, Quaglietta et al. 2015). Even though, the density probably depend on the aquatic habitat, since the density is lower in flowing rivers (Durbin Durbin 1996) than in pond landscapes (Dulfer et al. 1996).

High-quality calls are thus of crucial importance. Background noise can mask, disrupt or lower the quality. Hence, the quality of the calls was influenced by either rain, water noise, wind, vocalizing birds or visitors more often in summer than in winter. Therefore, I suggest that an acoustic monitoring of wild Eurasian otters should take place in winter assuming that they are as vocal in winter as in summer. The files recorded in winter months were processed faster than recordings from spring and summer. This was mainly due to the larger sound scape recorded in warmer months. Even in winter, the recorders should be placed as far away as possible from disruptive noises such as waterfalls or roads. In addition, Eurasian otters are more vocal at night, therefore the monitoring can be limited to the night. While the investigated parameters are highly consistent for at least six consecutive days, future studies have yet to show if the investigated parameters will remain constant over several years and over different life history stages. In order to obtain as much information as possible from acoustic monitoring, the function of the whistle call has to be examined in more detail. In addition, transmission playback experiments should be conducted in natural Eurasian otter habitats to obtain information on transmission properties of the whistle call.

Passive acoustic monitoring has a high potential for monitoring wild Eurasian otters and should be added as a potential complementary tool to the existing monitoring methods. In order to implement PAM successfully, more information on calling behaviour, call transmission properties and call function is needed. However, PAM might not replace the other monitoring methods, but it can provide additional data and an exceptional insight into the ecology and behaviour of the monitored species. Conservation management on elusive species and species living in difficult accessible habitats like rain forests or aquatic environments could profit from passive acoustic monitoring by gaining knowledge on key factors such as abundance, range, density, population size and population structure. Though, the characteristics of the calls and their transmission properties in relation to background noise and the spatial distribution must be appropriate from the point of detecting calls reliable and within an economically justified array of microphones.

7. References

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8. Appendix

Proportion of the close contact calls murmur (Fig 14), mewing (Fig 15) and cry (Fig. 16) found within half hour periods compared to half hour periods any call was recorded. Murmurs and mewings were recorded more often at night-time. The cry was more evenly distributed throughout the day. Besides, the cry was the most frequent recorded close contact call.

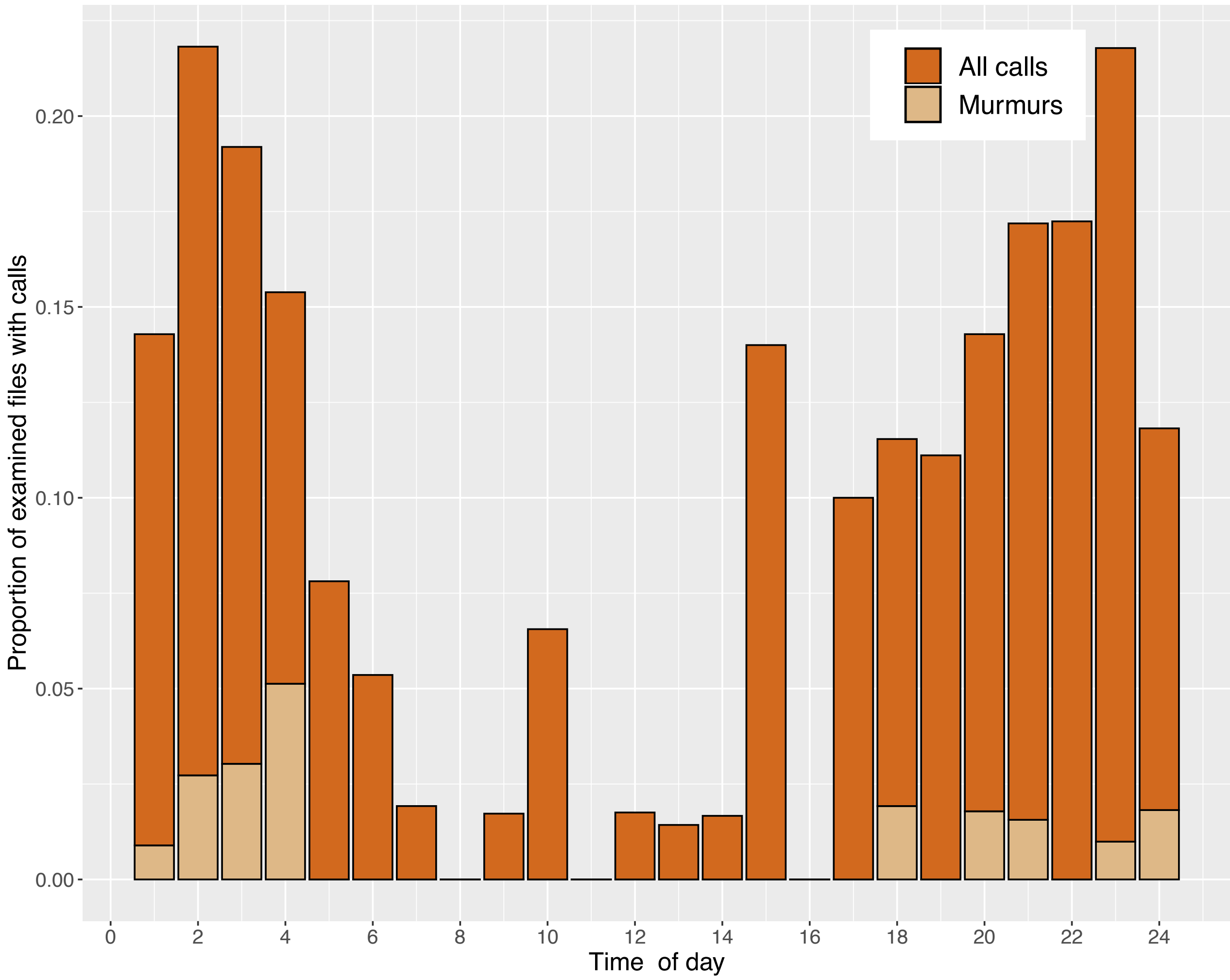

Figure 14 Overview at which time of day audio files with at least one call/murmurs were recorded. The x-axis indicates the time of day, while the y-axis shows the proportion of audio files containing a call. The orange bars indicate the percent of the examined 30 min audio files that contained at least one call of any type. The beige bars show what percent of the examined files contained at least one murmur call. (N = 80.5 record days of effort in 6 zoos with 11 individuals)

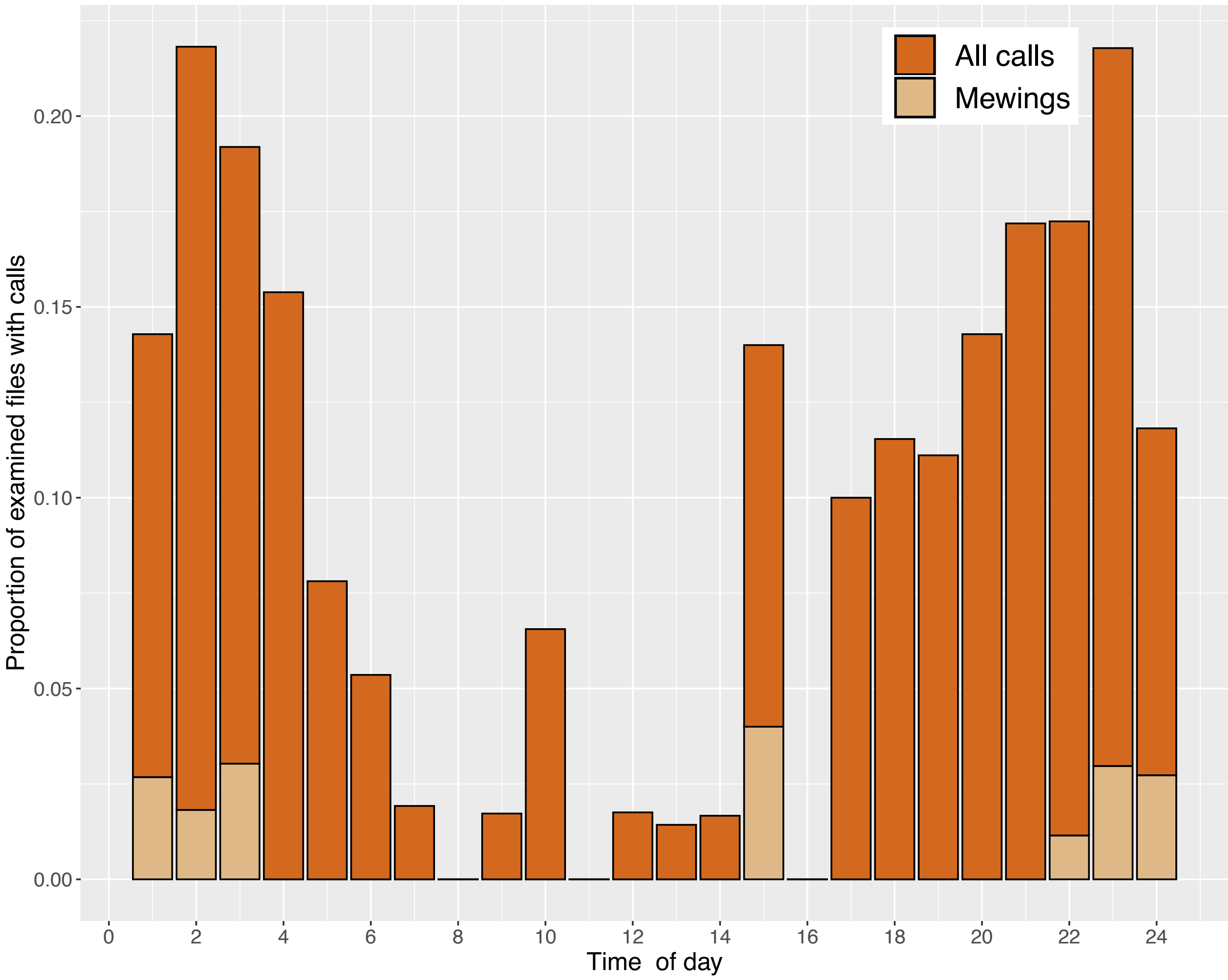

Figure 15 Overview at which time of day audio files with at least one call/mewing were recorded. The x-axis indicates the time of day, while the y-axis shows the proportion of audio files containing a call. The orange bars indicate the percent of the examined 30 min audio files that contained at least one call of any type. The beige bars show what percent of the examined files contained at least one mewing call. (N = 80.5 record days of effort in 6 zoos with 11 individuals)

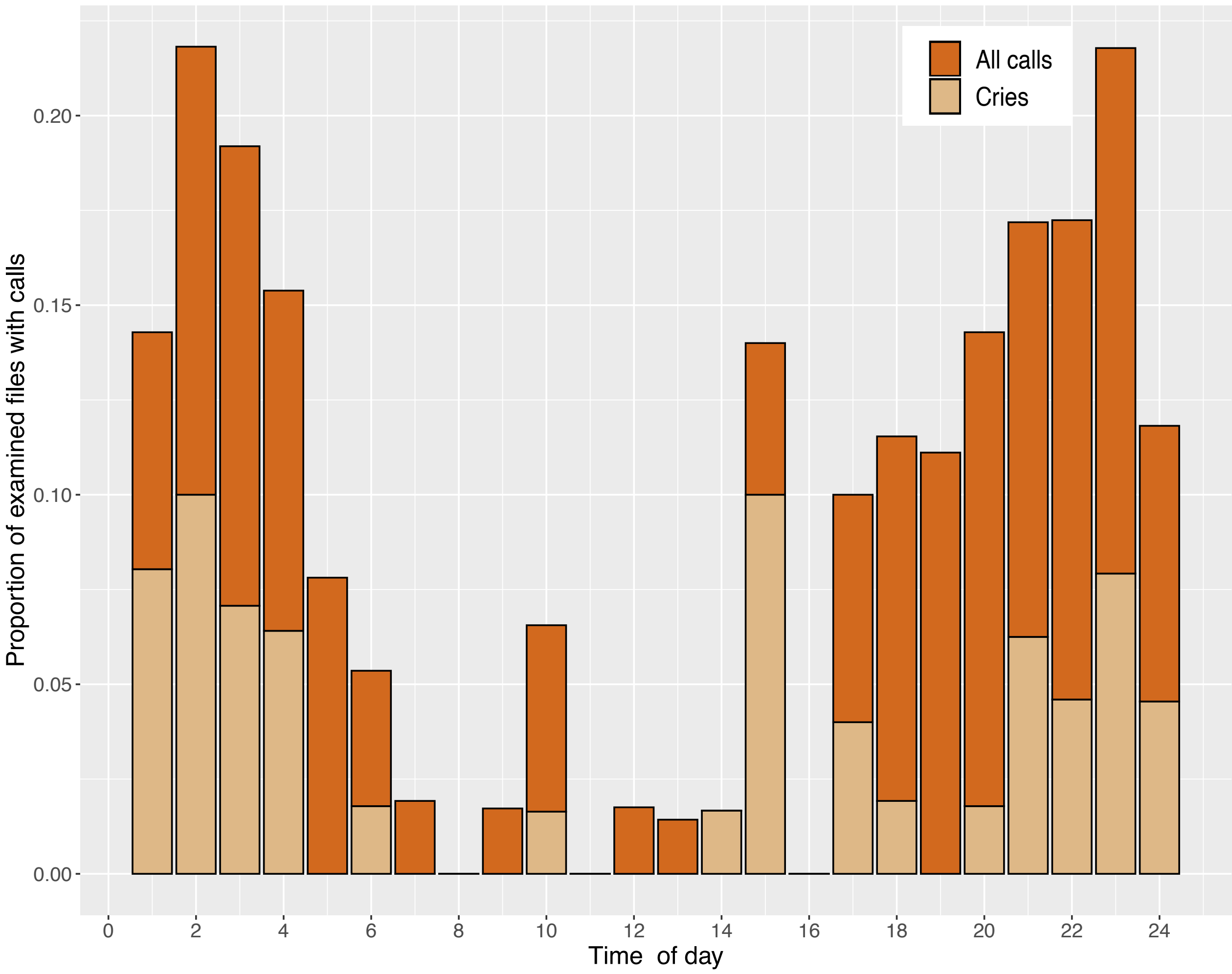

Figure 16 Overview at which time of day audio files with at least one call/cry were recorded. The x-axis indicates the time of day, while the y-axis shows the proportion of audio files containing a call. The orange bars indicate the percent of the examined 30 min audio files that contained at least one call of any type. The beige bars show what percent of the examined files contained at least one cry call. (N = 80.5 record days of effort in 6 zoos with 11 individuals)

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I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

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