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Research article

Do captive golden mantella frogs recognise wild conspecifics calls? Responses to the playback of captive and wild calls

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Abstract

With so many species being threatened with extinction, captive breeding programmes are becoming an important aspect of ex-situ conservation. Captive populations are important for species conservation and for reintroduction back into the wild. Some of the most important wild behaviours to maintain in captive animals are those associated with sexual reproduction, such as courtship and mating. Amphibian reproductive behaviour is associated with call patterns, with studies demonstrating that male advertisement calls elicit positive behavioural responses from females. This study evaluated the response of captive golden mantella frogs *Mantella aurantiaca* to playback calls from different wild and captive populations (one generation in captivity and more than five generations in captivity). During the experiment, three different calls were used as treatments: one from wild populations, and two from captive populations. Generalised linear mixed models were used to evaluate the effects of the playback treatments on the behaviour of captive frogs: replicates and enclosures were used as random factors. The model showed that vocalisations from wild individuals led to an increase in movement and social behaviours while calls from captive frogs did not. This was especially true of frogs bred for more than five generations in captivity. This could have negative consequences on the reproduction of captive frogs if released to the wild.

Introduction

In the wild, many species of amphibians are threatened with extinction, thus captive breeding programmes are an important aspect of ex-situ conservation (Bloxam and Tonge 1995; Griffiths and Pavajeau 2008). Maintaining captive populations is not only important in terms of species conservation, but also for potential reintroduction into the wild (Harding et al. 2015). One of the main goals of captive animal management is the promotion of natural behaviours and the prevention of abnormal behaviours (Farmer et al. 2011) in order to facilitate successful reintroduction programmes (Jule et al. 2008).

In management terms, some of the most important behaviours to maintain are those associated with sexual reproduction, such as courtship and mating (Farmer et al. 2011). Amphibian reproductive behaviour is associated with each species' vocalisations (Bee 2007; Caldart et al. 2016). For instance, advertisement calls of male frogs are essential to elicit positive behavioural responses from mature females, leading to them moving towards preferred signals (i.e. phonotaxis) (Mayer et al. 2014). Acoustic signals convey important information about the sender's fitness (Dullman and Trueb 1986; Ryan 1988; McClelland et al. 1996) and individual reproductive success is directly proportional to calling effort (McClelland et al. 1996; Witte et al. 2001; Prohl 2003).

Playback experiments under field conditions have demonstrated that vocalisations also play an important role in sexual selection during male-male competition in many species (Marshal et al. 2003; Narins et al. 2003). For example, among male frogs, vocalisations allow the identification of

the resource holding potential of an opponent (Bee et al. 1999), facilitate inter-male spacing (Marshal et al. 2003) and allow recognition of territorial neighbours (Bee 2007).

Males gathering at ponds during the rainy season and, competitively, calling to attract females characterise the golden mantella frog's *Mantella aurantiaca* breeding behaviour. In this scenario, it is usual to observe males showing aggressive behaviour toward other males as a sign of competition for females (Edmonds et al. 2015). This aggressive behaviour has been described in the wild and observed in captive populations (Edmonds et al. 2015).

Animals bred in captivity for conservation purposes, such as reintroduction programmes, should have a natural behavioural repertoire and be able to recognise wild conspecific calls (Kraaijeveld-Smit et al. 2006). *Mantella aurantiaca* is a critically endangered frog with captive breeding for reintroduction identified as a key part of its conservation plan. Thus, understanding the effects of captivity on its behaviour and the ability to recognise wild frogs is essential for the conservation of the species. This study evaluated the group response of captive *M. aurantiaca* to playback calls from different wild and captive populations to verify if a captive colony would recognise calls and show the same breeding behaviour as described for wild frogs.

Methodology

Study subject

Mantella aurantiaca is a critically endangered species (Vence and Raxworthy 2009), found only in Madagascar, with a distribution restricted to a fragment of forest that is under severe threat from mining, agriculture, timber extraction and over-collecting for the pet trade (Randrianavelona et al. 2010). According to the Amphibian Ark, ex-situ assistance is vital for the long-term survival of *M. aurantiaca* (Johnson 2008).

Recording calls

Calls were recorded using a digital audio recorder (H4n Handheld Digital Recorder, Zoom USA) with an omnidirectional microphone (NTG1 microphone, Rode Microphones, Australia). Calls were recorded during the breeding season without disturbing animals. Each recording represents a breeding population with multiple males calling simultaneously. Wild populations were recorded with the microphone positioned 50 cm above the calling individuals. Calls were recorded as a wave file, using a sampling frequency of 44 KHz and a resolution of 16 bit. Captive colonies were recorded by putting the microphone on the mesh the covers on the top of the tanks. Before recording calls, a pilot study was undertaken at the University of Manchester with their captive colony of *M. aurantiaca* to ensure the microphone and recorder had the appropriate sensitivity (i.e. could record all the frequencies emitted by the subjects).

Mantella aurantiaca calls (Figure 1) were recorded from three different populations during the breeding season: wild calls from Mangabe, Madagascar and captive calls from Mitsinjo captive breeding centre (Madagascar) and Chester Zoo (Table 1). A previous analysis has shown statistical differences on call's parameters between these captive and wild populations (Passos et al. 2017). This playback experiment aimed to observe if such differences would provoke dissimilar responses on golden mantella frogs kept at Chester Zoo. It is known that environmental conditions, such as temperature and humidity, can affect calling behaviour in frogs, so calls were recorded under the same conditions. Information on temperature and humidity were also collected during recordings and no difference was observed.

Mangabe area (Madagascar): This is a conservation priority site for *M. aurantiaca* where most breeding ponds are found. Mangabe, also known as the 'blue forest', is a site of international biodiversity importance, divided into two administrative districts, Moramanga in the north and Anosibe An'ala to the south. Data sampling for this study was done in the Moramanga region, during the month of November, the breeding season for *M. aurantiaca*.

Mitsinjo Association Captive Breeding Centre (Madagascar): This community-run conservation organisation operates around the village of Andasibe in east-central Madagascar and it holds the first Malagasy biosecure facility to protect endangered amphibians. Fifteen local species, including a genetically viable population of the golden mantella frog collected from the wild (i.e., genetic founders) in the Ambatovy area and their F1 offspring, are currently being kept at Mitsinjo. Only calls from the F1 frogs were used. Animals were being kept in breeding conditions when calls were recorded.

Chester Zoo (UK): The zoo currently maintains two visually and acoustically isolated ex-situ groups of *M. aurantiaca*, one is on public display at the zoo's Tropical Realm exhibit, from which calls were recorded, and a second group is kept off show in a biosecurity container specifically designed for conservation-related research, where the playback experiment was conducted. Animals have been in captivity for more than five generations. Animals were being kept in breeding conditions when calls were recorded.

Playback experiments

Three different tanks at the biosecurity facilities at Chester Zoo with similar number and sex ratio of frogs were used during the experiment (Tank 1: 11 males, 6 females; Tank 2: 10 males, 5 females; Tank 3: 10 males, 5 females). This study was conducted during the breeding season. Calls from three different populations were used as treatments: wild populations of *M. aurantiaca* from Mangabe, and two from captive populations; Chester Zoo and Mitisnjo for each treatment (Wild, Mitsinjo and Chester). Calls from different animals were used during the experiment, to be a representation of a population and not only one individual.

Table 1. Call characteristics results for different wild and captive populations of golden mantella frogs. sd=standard deviation

Population	Origin	Duration (s) ±sd	Period (s) ±sd	Pulse rate ±sd	Interpulse (s) ±sd	Dominant frequency (Hz) ±sd
Mangabe	Wild	0.043 ±0.004	0.090 ±0.05	2.92 ±0.27	0.008 ±0.002	4875.00 ±0.00
Chester Zoo	Captive	0.033 ±0.011	0.750 ±0.620	3.90 ±0.72	0.010 ±0.006	5198.01 ±172.84
Mitsinjo	Captive	0.062 ±0.008	0.120 ±0.063	4.04 ±0.19	0.005 ±0.001	4941.96 ±146.25

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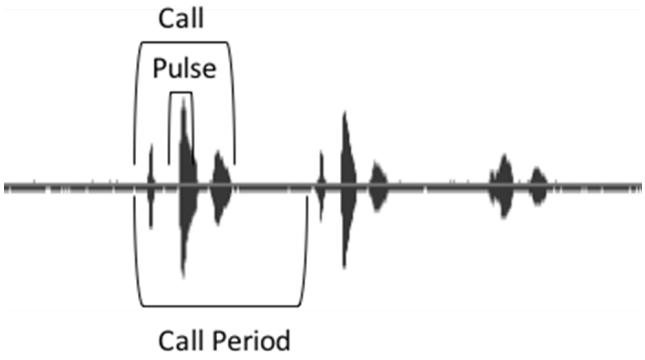

Figure 1. Wild *M. aurantiaca* call waveform showing some call characteristics.

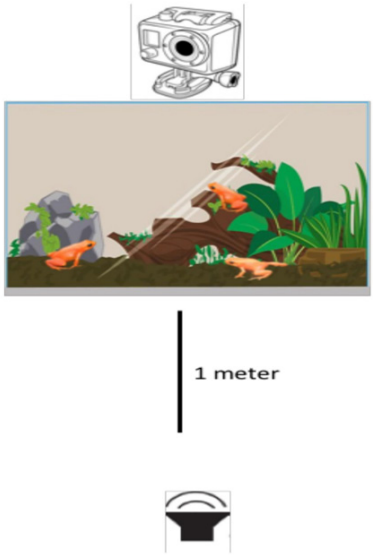

Figure 2. Schematic representation of the playback experiment set up with *M. aurantiaca* kept in Chester Zoo's (UK) biosecurity container.

Playbacks were replicated five times on non-consecutive days to avoid overstimulation and calls were presented using a randomised block design. During the experiment, Bluetooth speakers (model HX-P240PK, Jam Plus, USA – response frequency: 100-18,000 Hz) were placed at a distance of one metre to each tank, calls were played for 10 min as a playback stimulus (Figure 2). The animals' responses were videotaped for 10 min before the experiment, during the experiment and for 10 min after the playback for behavioural analyses. Playback experiments were always performed during the morning, between 0900 and 1100, to match the time *M. aurantiaca* are active in the wild (Andreone and Luiselli 2003; Piludu et al. 2015). Behavioural data were collected using instantaneous scan sampling with 20-sec intervals. This experiment was designed to mimic wild conditions: during the breeding season, male *M. aurantiaca* aggregate to call and attract females. Videos were analysed using the BORIS software (Friard and Gamba 2016).

Prior to any experimentation, measurements of sound pressure (noise) levels that animals are already exposed to during routine husbandry at Chester Zoo were taken using a sound pressure meter (SIP95 Sound Level Logging Meter FFT Audio Analyser, Balkon Technology) to avoid exposing animals to any extreme situations. Sources of sound pollution included visitors, keepers, heathers, fan and others. Recordings were played in a similar amplitude (i.e. volume) to that which the animals were already exposed in captivity. The sound-level meter recorded the noise

Table 2. *Mantella aurantiaca* ethogram used for behavioural analysis during playback experiments.

Behaviour	Category	Description
Jumping	Movement	Forward whole body movement in which all four limbs briefly leave contact with the surface substrate
Crawling	Movement	Forward whole body movement in which at least two limbs retain contact with surface substrate
Calling	Social	Vocalisation, single or series of audible calls
Eating	Other	Ingestion of food
Fighting	Social	Offensive or defensive social interaction/s may include displacement from position, lunging/leaping at another individual or wrestling
Chasing	Social	The act of following another individual in close proximity
Active	Other	Stationary, no obvious activity beyond perching/sitting
Breeding	Social	Male rubbing femoral glands on the dorsum of the female
Others	Other	Other behaviour not listed
Non-visible	Other	Animal cannot be seen by the observer

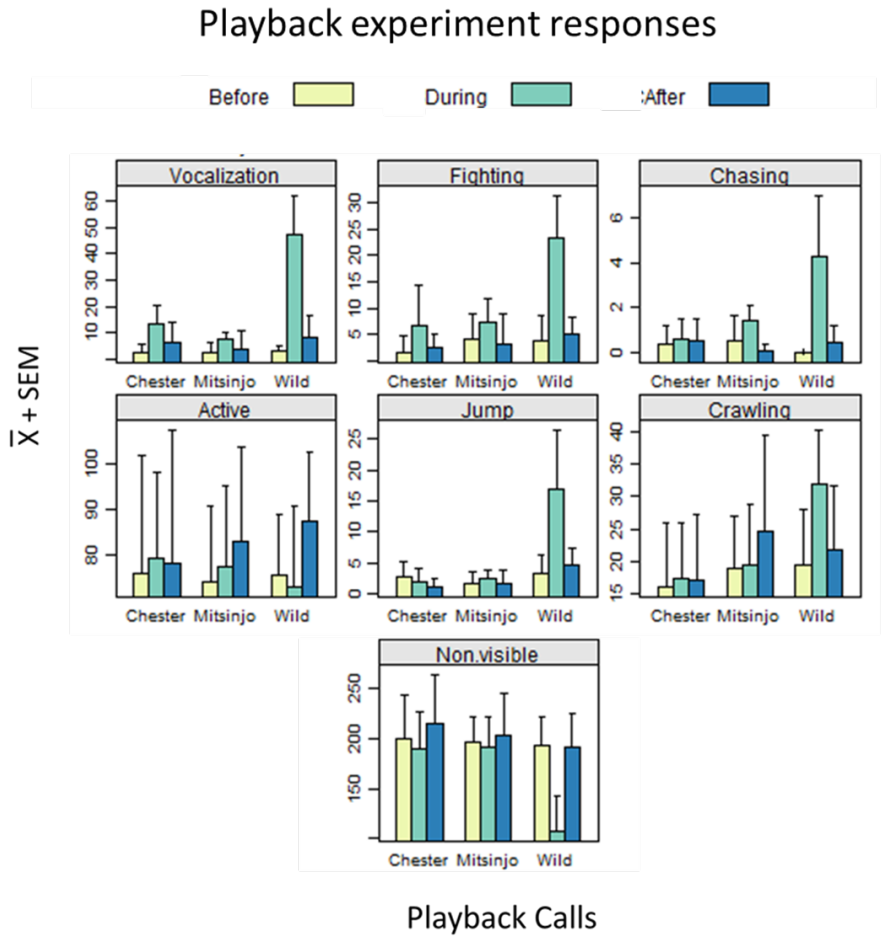

Figure 3. *Mantella aurantiaca* kept in Chester Zoo's (UK) biosecurity container behavioural patterns before, during and after playback experiment using calls recorded from Chester Zoo, Mitsinjo Breeding Centre and wild colonies.

values inside the tanks in decibels every 5 sec for a 15-min measurement period. A sound level of Leq (Linear weighting) 85.4 dBLin was observed.

Mantella aurantiaca were videotaped 30 min a day for a week prior to playback experiments; this footage was used to construct an ethogram (Table 2).

All social interactions were monitored by the researchers to ensure that no frogs became injured or ill as a consequence of the playback experiments. The frogs were monitored for several weeks after the experiments and none became ill or showed any signs of distress. All experimentation was done in compliance of the relevant animal welfare laws of the country where conducted and followed the Association for the Study of Animal Behaviour's Guidelines for the care of animals (ASAB 2014).

Statistical analysis

Data were tested for normality using a Shapiro-Wilk test; data did not show a normal distribution even after transformations. Generalised linear mixed models (GLMM) were used to compare behavioural patterns before, during and after the playbacks with call origin as a random factor. All statistical analyses were done using R Studio (R Studio Team 2015).

Results

Chester Zoo's *M. aurantiaca* spent the majority of their time as active (25%) or non-visible (65%) in the 10 min prior to the playback calls. During the playback, an increase of behaviours being displayed was observed, especially when the wild calls were played. After the 10-min playback, *M. aurantiaca* returned to the same behaviour pattern as before the playback (Figure 3). The GLMM showed no differences between before and after behaviour patterns. However, a statistical difference was found between before and during playback and also between during and after playback for all behaviours ($P<0.005$). Captive frogs were kept at temperatures to mimic their natural habitat, no differences were observed between wild and captive temperatures.

The GLMM model (see Table 3) showed that vocalisations from wild individuals lead to a significant increase ($P<0.005$) in fighting, vocalisations, chasing, jumping and crawling behaviours, and a significant decrease ($P<0.005$) of non-visible individuals. Playback experiments using calls from Mitsinjo Breeding Centre, led to a significant increase of fighting, chasing, jumping and calling behaviours. Calls from Chester Zoo only resulted in an increase in calling behaviour ($P<0.005$).

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Table 3. Parameter estimates for the Generalised Linear Mixed Models describing the relationship between call origin and behaviour as the predictor variable for *M. aurantiaca.*

Behaviour	Call	Mean(n)	St.Deviation	P-value
Calling	Mangabe	19.61	±22.56	<0.001
Calling	Mitsinjo	11.05	±13.13	0.004
Calling	Chester	8.08	±7.62	0.008
Fighting	Mangabe	10.91	±10.84	<0.001
Fighting	Mitsinjo	4.91	±5.43	0.004
Fighting	Chester	3.73	±5.44	ns
Chasing	Mangabe	1.64	±2.55	0.001
Chasing	Mitsinjo	0.70	±0.97	0.018
Chasing	Chester	0.50	±0.92	ns
Jumping	Mangabe	8.61	±8.63	<0.001
Jumping	Mitsinjo	2.02	±1.69	0.050
Jumping	Chester	2.08	±2.15	ns
Crawling	Mangabe	24.79	±10.27	<0.001
Crawling	Mitsinjo	21.52	±11.07	ns
Crawling	Chester	17.17	±9.40	ns
Non-visible	Mangabe	164.02	±52.81	<0.001
Non-visible	Mitsinjo	195.14	±32.67	ns
Non-visible	Chester	198.79	±39.00	ns

Discussion

The playback experiment showed that captive *M. aurantiaca* do recognise and respond to calls from wild *M. aurantiaca*. Wild vocalisations created a significant increase in movement and social behaviours from captive frogs, whereas calls from captive populations did not lead to such an increase in these behaviour patterns. It was also observed that calls from animals that were in captivity for more generations (more than five generations; e.g. Chester colony) provoked fewer responses from *M. aurantiaca* than calls from frogs that were in captivity for only one generation (e.g. Mitsinjo population).

The behavioural response observed during the playback experiment using wild frog calls was similar to the behavioural patterns described for wild individuals during the breeding season (Edmonds et al. 2015). However, the same reaction was not observed when captive frogs were subject to playback using calls from captive frogs. A previous study has shown that captive *M. aurantiaca* can have their calls altered by captive conditions. Animals kept in captivity for many generations had their calls significantly affected by their environment, while frogs that had been in captivity for only one generation possessed calls similar to wild ones (Passos et al. 2017). Captive animal calls were shorter, at a higher frequency and had a longer period.

The difference between wild and captive calls would explain some of the results found during the playback experiment, with calls from Mitsinjo frogs leading to a greater increase in social behaviours, while calls from Chester Zoo animals did not lead to such responses. In anurans, significant information about the individual's fitness is transmitted by acoustic signals (Duellman and Trueb 1986; Ryan 1988), which plays an important role in sexual selection (Marshall et al. 2003). The calls of captive frogs are not as attractive as calls from wild individuals and, as a result, do not elicit full reproductive behaviour, which in a reintroduction programme could lead to negative consequences (Sun and Narins 2005). A low frequency of breeding between captive-bred and wild animals would also mean no improvement of the wild population's genetic diversity (Slade et al. 2014; Edmonds et al. 2015).

Maintaining wild-type behaviours, such as communication, courtship and male-male combat, is relevant for successful reproduction in captivity and for reintroduction programmes (Farmer et al. 2011; Schulte-Hostedde and Mastromonaco 2015). Chester Zoo's *M. aurantiaca* captive colony, besides being in captivity for over five generations, has retained its natural breeding behaviour (i.e. responding appropriately to wild conspecific calls). It is now necessary to understand how the changes to vocalisations could influence male reproductive opportunities if animals were released back into the wild. A playback experiment with wild animals is necessary to fully understand the consequences of the observed changes.

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