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Advertisement calls attract invasive cane toads in Japan

Takashi Haramura[1,*]

Abstract. Cane toads (*Rhinella marina*) are an invasive species in many parts of the world, including the Japanese island of Ishigaki. Extensive research in Australia has identified promising methods for control such as using acoustic signals. Can the control approaches developed in Australia be applied to invasive cane toads in Japan? In this study, I conducted field research to test the applicability of artificial advertisement calls to attract wild adult cane toads. The number of cane toads gathered around a breeding pond increased when an acoustic signal was played, demonstrating that artificial advertisement calls are useful for attracting cane toads. Therefore, artificial advertisement calls could be an effective method to attract invasive cane toads in Japan.

Keywords. advertisement call, anuran, *Rhinella marina*, invasive species

Introduction

Invasive species can spread far outside their native range and can have major ecological impacts on native taxa. Especially on islands that are refuges for endemic fauna, invasive species pose a major threat (Mack et al., 2000; Cox, 2004). Thus, effective control methods to reduce the abundance of invasive species are needed. Eradication is often difficult or impossible, but reducing local densities of invasive species is important to mitigate their environmental impacts (Bank et al., 1998).

The cane toad (*Rhinella marina*) is a large anuran native to South and Central America and was introduced to more than 40 countries worldwide in ill-advised attempts at biocontrol of pests (Lever, 2001). Several approaches to reduce toad numbers have been explored such as genetically-engineered viruses (Pallister et al., 2007) and larval-suppressant pheromones (Clarke et al., 2015, 2016). The optimal approach to reduce cane toad numbers is the capture and removal of adult toads, which also results in a reduction in recruitment (Yeager et al., 2014).

Although cane toads have spread worldwide, the majority of research on cane toad impact and control has been conducted in Australia (e.g., Shine, 2010; Tingley et al., 2017). Cane toads in Australia show strong geographic variation in many traits such as advertisement calls, dispersal behaviour, locomotor performance, thermal acclimation, and developmental strategies (Alford et al., 2009; Llewelyn et al., 2010; McCann et al., 2014; Ducatez et al., 2016; Yasumiba et al., 2016). Several methods to control cane toads have been developed in Australia, however, methods that work well in one population may be less effective in other populations. Therefore, we need to evaluate whether methods developed in Australia can also be applied to other parts of the world where cane toads have been introduced.

Male anuran amphibians typically rely on vocalisation as a mechanism to attract mates during breeding choruses (Bee, 2007; Swanson et al., 2007). Male cane toads produce such advertisement calls, and often form choruses of calling individuals around waterbodies during the breeding season (Schwarzkopf and Alford, 2007; Bowcock et al., 2008). In response, breeding females and other males are attracted to these advertisement calls (Gerhardt, 1994). In anurans, call frequency is correlated with body size, with larger males producing lower frequency calls (Davies and Halliday, 1978; Morris and Yoon, 1989; Gerhardt, 1991). Breeding female anurans often exhibit a strong preference for low-frequency calls, thus resulting in increased mating success for larger males (Márquez and Tejedo, 1990; Richardson et al., 2010). Given these responses, advertisement calls have the potential to attract many toads of both sexes efficiently. Indeed,

[1] Department of Environmental Sciences, Rakuno Gakuen University, Ebetsu, Hokkaido, 069-8501 Japan.
[*] Corresponding author. E-mail: t-haramura@rakuno.ac.jp

an experiment has shown that cane toads in Japan are attracted to artificial advertisement calls, with male toads being attracted to a chorus call while female toads are attracted to low-frequency calls (Haramura et al., 2017). However, it has not been confirmed whether such artificial advertisement calls attract free-ranging toads in the field. In this paper, I report the effect of artificial advertisement calls to attract wild cane toads in Japan.

Materials and Methods

Study species.—Cane toads are among the largest anurans; adults generally average around 100 to 300 g, and females grow larger than males (Lever, 2001; Alford et al., 2009). Cane toads were introduced to the Daito islands in Japan in 1939 (Lever, 2001). In 1978, approximately 10-15 toads were moved from the Daito islands to Ishigaki in Okinawa prefecture (Ota, 1999). Cane toads are now found throughout the island, with the total population likely to exceed 20,000 individuals (H. Ota, pers. comm.). On Ishigaki, cane toads breed in a wide range of waterbodies such as rice paddy fields, ponds, and streams. Sugarcane fields occupy much of Ishigaki, and many artificial pools are installed in these fields as drainage basins. These artificial pools are also frequently used as breeding sites by cane toads.

Playback experiment in the field.—From a previous study on Ishigaki, it was determined that adult cane toads of both sexes move towards the acoustic stimuli of male toad calls, but the optimal stimulus differed between sexes (males were attracted to a chorus call, while females were attracted to a low-frequency call; Haramura et al., 2017). Therefore, in the present research, I used the same two sound types (chorus and low-frequency call) in a playback experiment to confirm whether advertisement calls attract cane toads to a specific location (breeding area) in the field. The range of frequency in the chorus was 591 to 715 Hz, and the low-frequency call was 564 Hz. The chorus call was obtained by recorded a natural chorus (five to eight male toads) in the field. The low-frequency call was generated by artificially manipulating the recorded call of a single male toad. Further details about the chorus and artificial low-frequency call are provided in Haramura et al. (2017).

The playback experiment was conducted between February 2016 and October 2019 in an area of sugarcane fields on Ishigaki (24˚26´N, 124˚06´E). The whole sugarcane area is approximately 0.35 km^2 and contains 10 separated sugarcane fields. In this area, five artificial pools have been installed for each two sugarcane divisions. The size of each artificial pool is approximately 100m^2. In the present study, I selected one artificial pool where many cane toads have been observed (personal observation), and all experimental trials were conducted at this artificial pool. Because this artificial pool was located in the centre of the sugarcane area, cane toads in the surrounding cane fields would be able to hear the advertisement calls.

For each trial, I initially conducted a route census by slowly walking around the artificial pool to count the number of adult cane toads at the water's edge (within 5 m from water body) and in the water. Next, I switched on the IC recorder (Olympus, LS-14) to play two call types (chorus and low-frequency calls) through a speaker. These two call types were contiguous and looping, and automatically replayed until manually switched off. The Speaker (Elecom, LBT-SPP20BK) was placed at the water's edge (<1 m), and the mixed call was played for several hours (11 trials, average: 208 min, range: 152-269 min experiment month: May, September, October, November). After the playback calls, I conducted a second route census to count toads, as described above. I did not conduct trials using a neutral noise, such as pink noise, because cane toads do not respond to such noise (Schwarzkopf and Alford, 2007). However, as a control, I conducted the same route census as above without playing the artificial calls through the speaker (13 trials, average: 178 min, range: 92-264 min, experiment month [January, February, October, November, December]). The start time of experimental trials was less than one hour after sunset (except for two control trials that started three hours later after sunset). The date of playback and control trials was randomly selected.

Data analysis.—After the sound call (or control) was played, the number of cane toads observed at the waterbody could be increased or decreased relative to the initial census. The Quasi-Poisson GLM regression was used to analyse the relationship between the change in the number of cane toads and multiple explanatory variables. The explanatory variables were several physical factors (see below) as well as sound treatment (Playback or No: Control) and duration of playing time (the time calls were played using the IC recorder). Initially, I selected seven physical factors to assess effects on the number of toads observed (average air temperature, maximum air temperature, minimum air temperature, daily precipitation, average wind speed, average air humidity, average atmospheric pressure)

Table 1. Comparison between playback and control treatments. The increased number of cane toads was calculated by subtracting the number of toads observed before versus after playback calls. Data are mean ± SE. Parentheses show ranges.

	Increased number of cane toads	Duration of playing time (min)	Average air temperature (˚C)	Daily precipitation (mm)	Average wind speed (m/s)	Average air humidity (%)	Average atmospheric pressure (hPa)
Playback (N=11)	3.55 ± 0.84 (-1 – 8)	208.09 ± 42.13 (152 – 269)	27.07 ± 0.55 (22.8 – 29.1)	15.4 ± 8.18 (0 – 79.5)	4.68 ± 0.61 (2.1 – 7.7)	82.36 ± 2.18 (72 – 91)	1009.44 ± 0.94 (1004.8 – 1014.9)
No (Control) (N=13)	0 ± 0.53 (-3 – 0)	178.92 ± 13.31 (92 – 264)	20.83 ± 1.09 (16.6 – 28.5)	1.5 ± 1.12 (0 – 13.5)	5.49 ± 0.44 (2.9 – 8.1)	70.85 ± 3.18 (55 – 87)	1165.34 ± 99.62 (1012.2 – 2023.2)

because activity patterns of anurans are influenced by several environmental factors (Bertoluci, 1998; Maciel et al., 2003). However, because average, maximum and minimum air temperatures were highly correlated (average vs maximum air temperature: $R^2 = 0.991$, $P < 0.0001$, average vs minimum air temperature: $R^2 = 0.982$, $P < 0.0001$), both maximum and minimum air temperature were removed as explanatory variables. The remaining four physical factors were not highly correlated (e.g. average air temperature vs daily precipitation: $R^2 = 0.005$, $P = 0.749$, average air temperature vs average wind speed: $R^2 = 0.061$, $P = 0.246$). Therefore, five physical factors were used in the analysis (average air temperature, daily precipitation, average wind speed, average air humidity, average atmospheric pressure). Average data were based on recordings over 24-hours. The data for physical factors on Ishigaki were obtained from the Japan Meteorological Agency (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). The distance from this weather station to the study site is approximately 13 km.

Quasi-Poisson GLM regression analysis requires non-negative integer data. In the present study, the number of cane toads observed during the second route census (after playback or control trials) was usually greater than the number observed in the initial census (before playback or control trials; i.e., the response was a positive integer). However, for some trials, the number of toads observed during the second census decreased (i.e., the response value was a negative integer; Table 1). The maximum reduction in the number of toads observed during a second census was three. To account for this, all data for the change in the number of cane toads observed were adjusted by adding a value of 3 prior to conducting Quasi-Poisson GLM regression analyses. This resulted in the lowest response number for change in toad numbers being zero. I conducted statistical analyses using JMP 9.0. The level of statistical significance was set at $P = 0.05$.

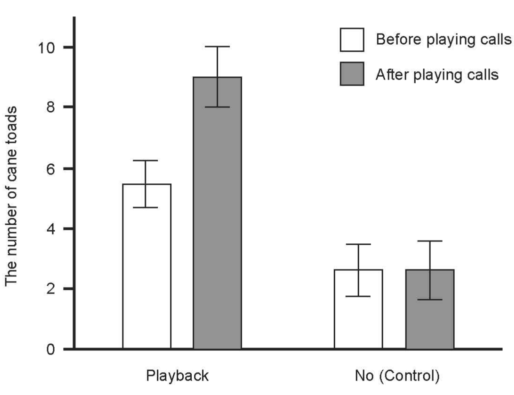

Figure 1. Mean values (±SE) of the number of cane toads observed between playback and control treatments. The playback treatment was a continuous loop of male toad calls broadcast through a speaker. The control treatment did not broadcast male calls (see Methods for full details).

Results

Data for the change in the number of cane toads observed between the initial and final censuses, and explanatory variables, during playback versus control trials are shown in Table 1. During playback trials, the average number of toads increased after advertisement calls were played. In contrast, there was almost no change in the number of toads at the end of control trials (Fig. 1).

Quasi-Poisson GLM regression revealed that the increased number of cane toads was correlated with playback treatment (artificial calls versus control), with the number of cane toads at the artificial pond increasing when advertisement calls were played ($P = 0.003$; Table 2). Toad abundance patterns were not significantly influenced by any of the other explanatory variables (Table 2).

Table 2. Result of Quasi-Poisson GLM regression on the increased number of cane toads.

	Likelihood ratio chi-square	SE	*P*
Playback treatment	8.757	0.279	0.003
Duration of playing time	2.996	0.003	0.084
Average air temperature	1.473	0.068	0.225
Daily precipitation	1.538	0.009	0.215
Average wind speed	0.120	0.071	0.729
Average air humidity	0.480	0.018	0.489
Average atmospheric pressure	0.285	0.001	0.594

Discussion

In the present study, the increase in the number of cane toads at the waterbody was correlated with sound treatment, demonstrating that the acoustic signal played through a speaker was effective for attracting adult cane toads. Research in Australia has reported that both male and female cane toads are attracted to traps by playing quiet recordings of a small toad chorus, suggesting that this response could be useful for controlling populations of invasive cane toads (Schwarzkopf and Alford, 2007). In the present study, I assessed changes in cane toad abundance using visual censuses rather than traps. Nonetheless, the results demonstrate that artificial advertisement calls using a playback system could also be effective for capturing wild invasive cane toads in Japan.

Several physical factors influence the activity patterns of anuran species (Bertoluci, 1998; Maciel et al., 2003). In particular, the calling activity of anurans is affected by variation in air temperature, rainfall, and photoperiod (Vaira, 2005; Both et al., 2008; Canavero et al., 2008; Canavero and Arim, 2009; Van Sluys et al., 2012; Caldart et al., 2013). In general, temperature is the most common predictor of anuran reproductive activity (Oseen and Wassersug, 2002). The present study showed that acoustic signals are also an important factor influencing the behaviour of cane toads, with artificial male reproductive calls attracting other cane toads to the waterbody. Acoustic communication plays a key role in the life-history of anurans, being directly linked to breeding activity, and ultimately to reproductive success (Gerhardt, 1991; Gerhardt and Huber, 2002; Tárano and Herrera, 2003). Therefore, broadcasting artificial advertisement calls using a playback system acts to replicate the acoustic signal of the toad's natural breeding activity, and, in Japan, is an effective method for attracting cane toads, as it is in Australia (Yeager et al., 2014). Additionally, the physical environment could influence cane toads movement activity and therefore attraction responses. In the present research, the playback treatment was tested under relatively warm and humid conditions (compared the control treatment). The Quasi-Poisson GLM regression analysis that incorporated the effect of these factors showed that playback of artificial male calls is important to attract cane toads. Further study is required to identify the optimal weather conditions to maximise the attraction of cane toads to acoustic playback lures.

Habitat structure greatly influences the propagation of sound and its attenuation (Marten and Marler, 1977). Male cane toad calls, which attract conspecifics to breeding aggregations, carry over a large distance (Buxton et al., 2015; Muller et al., 2016). The sugarcane fields (and rice paddies) where many cane toads breed on Ishigaki are comparatively flat and have few obstacles to restrict sound propagation. In such environments, advertisement calls carry far and may be heard by many cane toads located some distance from the playback source. Such habitat factors enhance the use of artificial advertisement calls as a lure to attract (and subsequently capture/remove) cane toads in Japan.

Acoustic signals play an important role in intraspecific communication for most anurans. Male anurans use the calls of others to locate waterbodies for hydration or mating opportunities (Oldman, 1967; Schwarzkopf and Alford, 1996; Pfenning et al., 2000; Semlitsch, 2008; Bernal et al., 2009). Increased chorus activity is associated with increasing mating probabilities for males (Ryan et al., 1981; Forester and Thompson, 1998; Halliday and Tejedo, 1995), and females exhibit a strong preference for low-frequency calls which are made by larger males (Márquez and Tejedo, 1990; Richardson et al., 2010). In Australia, female cane toads prefer low-frequency calls and low pulse rate (Yasumiba et al., 2015). Adult cane toads are an ideal life-history stage to target for capture/removal in invasive populations because doing so removes their potential future offspring from the population. In particular, female cane toads can carry clutches of 5,000 to more than 35,000 eggs (Hagman and Shine, 2008); thus, targeting adult females will be the most effective management strategy. The new attraction method of synthetic calls based on low frequency or low pulse rate to selectively remove female toads can be applied to achieve this.

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