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Temporal Changes in Number of Breeding Individuals of the Amami Tip-nosed Frog

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Abstract: Amami tip-nosed frog (*Odorrana amamiensis*) is an endangered species endemic to Amami-Oshima Island and Tokunoshima Island, in Japan. This species is threatened by invasive species and habitat destruction. Although action is needed to protect them, the breeding ecology of *O. amamiensis* is poorly understood. A better understanding of its breeding ecology would contribute to conservation efforts. This study reports temporal changes in number of *O. amamiensis* breeding individuals in Kinsakubaru area, Amami-Oshima Island from the 28 Nov. 2015 to the 5 Dec. 2015. A 20 m transect line was set at a stream to count the number of *O. amamiensis* individuals at night and during daytime for eight days. Over 200 individuals were observed and many of them were breeding with lively mating calls around the small stream at night for three days. The high density of individuals decreased rapidly afterwards. This is the first report quantifying the temporal changes in number of breeding individuals of *O. amamiensis*. The species is usually nocturnal, but some individuals were observed at breeding sites during the daytime as well. Field research on *O. amamiensis* is usually conducted at night, but it can be risky because the nocturnal poisonous snake Habu (*Protobothrops flavoviridis*) also inhabits Amami-Oshima Island. The results suggested that field research during daytime was relatively safer and perhaps an easier approach to find breeding sites of *O. amamiensis* compared to searching at night.

Key words: Amami-Oshima Island; Amami tip-nosed frog; breeding ecology; endangered species; endemic species

Introduction

The Amami tip-nosed frog (*Odorrana amamiensis*) is a relatively large (56–101 mm) terrestrial species and endemic to the montane forests on Amami-Oshima Island and Tokunoshima Island, Japan (Matsui, 1994; Matsui et al., 2005; Matsui and Maeda, 2018). It is listed as an endangered species on the IUCN Red List of Threatened Species (Kaneko and Matsui, 2004), the national monument by Kagoshima prefecture, and the endangered species of wild fauna and flora by Amami city. It is negatively impacted by various human-induced factors such as the

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introduction of invasive species and habitat destruction through logging (Utsunomiya and Utsunomiya, 1983; Watari et al., 2008; Matsui and Maeda, 2018). Although action is needed to protect this species, the basic ecology of *O. amamiensis* is poorly understood.

Several studies on *O. amamiensis* have been conducted in recent decades. These studies focused on phylogenetic relationships (Matsui, 1994; Matsui et al., 2005), microsatellite marker development (Igawa et al., 2015), population change caused by invasive mongoose (Watari et al., 2008; Watari et al., 2013), calling site preference (Iwai et al., 2018), and ecological character displacement (Komine et al., 2019). However, little is known on the breeding ecology and breeding sites of *O. amamiensis*. Observing its breeding activity is difficult because they breed around mountain streams for only a few days in autumn to spring (Iwai et al., 2015; Matsui and Maeda, 2018). A better understanding of breeding ecology and breeding sites would contribute to conservation efforts for this species. Although Iwai et al. (2015) reported body size and age of breeding individuals and Sakoda et al. (2007) reported habitat of wild tadpole, there is no study reporting detailed breeding process of *O. amamiensis*. This study describes temporal changes in the number of breeding individuals of *O. amamiensis* on Amami-Oshima Island, Japan.

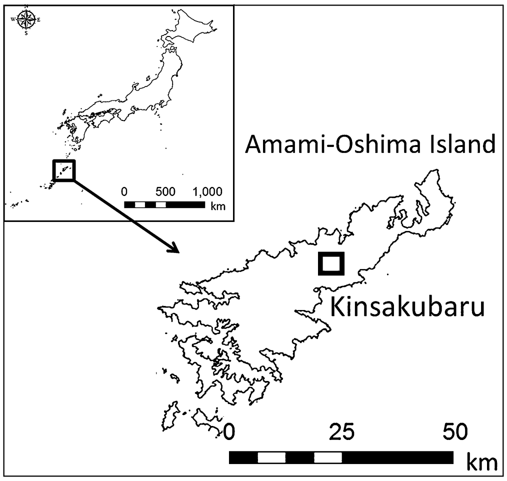

Fig. 1. Map showing the study location in Kinsakubaru area, Amami-Oshima Island in southern Japan.

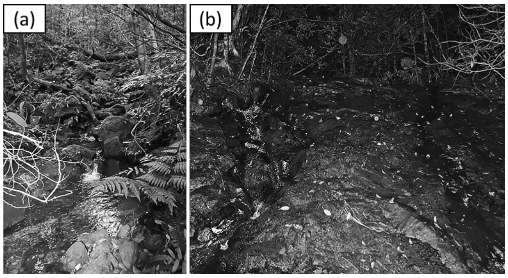

Fig. 2. Photos of the transect line at the breeding stream. The transect line at the stream consists of a flowing section (Fig. 2 [a]) and a cascade (Fig. 2 [b]). The photo (b), which was taken on 30 Nov. 2015, shows several individuals of *Odorrana amamiensis*.

Materials and Methods

Study area

Amami-Oshima Island, approximately 712 km^2 in size, is part of the Nansei Islands in southern Japan (Fig. 1). The average annual temperature is 21.5°C and average annual rainfall is 2,914 mm (Watari et al., 2008). The island is covered with broad-leaved evergreen trees, such as chinquapin (*Castanopsis sieboldii*). The island has a subtropical climate and contains many endemic species. Because of its valuable biodiversity, part of the island is currently nominated to be inscribed on the World Heritage List of the United Nations Educational, Scientific and Cultural Organization.

Field research

The field research was carried out in the Kinsakubaru area, Amami-Oshima Island, Japan from 28 Nov. 2015 to 5 Dec. in 2015. We set a 20 m transect line at a stream with a depth of approximately 20 cm by 80 cm width. The transect line consists of a flowing section (Fig. 2 [a]) and a cascade section (Fig. 2 [b]). The number of *O. amamiensis* individuals was counted on the transect line at night (2000–2300) and during the day (1200–1600) for eight days. We conducted the census once during the day and at night; each census took approximately 30 min. After the census, the mating behavior was observed from one to three hours each day. It was noticed that the number of individuals was likely to be stable during the observation period in each day. Males and females were identified by their morphological features and mating calls. In general, females have a larger body size than males. Males have a nuptial pad used for amplexus (Matsui and Maeda, 2018). Only males produce a mating call (Matsui and Maeda, 2018). Frogs were captured when it was not possible to identify their sex by only visual observation. This study was carried out under permission no. 21 from the Kagoshima government education commission and Amami city. Additionally, we discussed potential relationships between breeding activity and climate factors (e.g. annual change of mean temperature, mean humidity and rain fall). We used open climate data provided by the Japan meteorological agency (https://www.data.jma.go.jp/gmd/risk/obsdl/index.php#, last accessed 1 Oct. 2019).

Results

The number of *O. amamiensis* individuals at night and daytime was recorded for eight days (Table 1). Amplexus of individuals and egg mass were confirmed, hence the frogs apparently gathered at the stream to breed (Fig. 3a, b). Most individuals were males and only several females and amplexus were observed (Table 1). The number of individuals at night exceeded 200 on 29 Nov. 2015, and this extremely high density of breeding individuals at night remained until 2 Dec. 2015. The high density at night strongly decreased on the 3 Dec. and a few individuals were observed on the 4 Dec. The number of individuals recorded during the day exceeded 20 individuals on the 29 Nov, and the high density of breeding individuals continued until the 2 Dec. Only one female and no amplexus were observed during the day through this study period. No individuals were recorded on the 3 Dec. during the day.

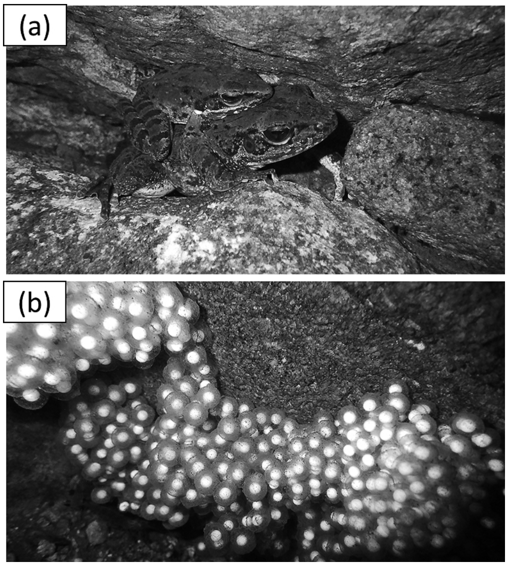

Fig. 3. The photo (a) shows *Odorrana amamiensis* in amplexus and the photo (b) shows frog eggs. Those photos were taken on 3 Dec. 2015.

Discussion

This study recorded over 200 *O. amamiensis* individuals breeding with lively mating calls around a small stream at night for three days (Table 1). Although the number of individuals around the stream before the 28 Nov. was not quantified, we confirmed that there were no lively mating calls on the 23 Sep.–2 Oct., 24 Nov. and 26 Nov, 2015 when we observed the forest road. Since the breeding site is just beside the forest road, we could notice the lively mating calls easily if there was one. Therefore explosive breeding should have started after the 27 Nov. at least. Because *O. amamiensis* did not seem to be usually around the stream, the number of breeding individuals greatly increased between the 27 and 28 Nov. The number of breeding individuals remained high until 1 Dec 2015,

TABLE 1. Temporal changes in number of individuals of *Odorrana amamiensis* (a) at night and (b) at daytime. Number of males and females are only single individuals.

(a)

	Total number of individuals at night	Number of males at night	Number of females at night	Number of amplexus at night
28 Nov.	131	131	0	0
29 Nov.	218	206	0	6
30 Nov.	225	223	0	1
1 Dec.	238	230	0	4
2 Dec.	129	123	0	3
3 Dec.	35	33	0	1
4 Dec.	7	6	1	0
5 Dec.	4	4	0	0

(b)

	Total number of individuals at daytime	Number of males at daytime	Number of females at daytime	Number of amplexus at daytime
28 Nov.	7	7	0	0
29 Nov.	23	23	0	0
30 Nov.	23	23	0	0
1 Dec.	26	26	0	0
2 Dec.	23	22	1	0
3 Dec.	0	0	0	0
4 Dec.	0	0	0	0
5 Dec.	0	0	0	0

after which the density decreased rapidly. Most of the individuals left the stream by 4 Dec. 2015. Although there may be multiple explosive breeding at the same site in a year, most of the individuals left the stream by 4 Dec. 2015 which suggests that the single explosive breeding may have finished by this time. This finding agrees with a previous study describing that *O. amamiensis* mainly breed at mountain streams for a short time period (Iwai et al., 2015; Matsui and Maeda, 2018). The current study is the first to quantify the temporal changes in number of breeding individuals of *O. amamiensis* in a single explosive breeding activity.

Although the number of breeding individuals during daytime was far less than at night, *O. amamiensis* did gather at the breeding site during daytime as well. It was unknown if some breeding individuals would be active during daytime because *O. amamiensis* is usually described as a nocturnal species (Watari et al., 2008). Our observation indicates that the activity pattern of some *O. amamiensis* individuals may change during the breeding period. This finding may be beneficial for the conservation of *O. amamiensis*. We observed no snakes both during the day and at night probably because this study site was seriously damaged by invasive mongoose (Watari et al., 2008; Komine et al., in press). However, it is generally risky to walk around the forests on Amami-Oshima Island at night because of the nocturnal poisonous snake Habu (*Protobothrops flavoviridis*) which inhabits mountain streams as

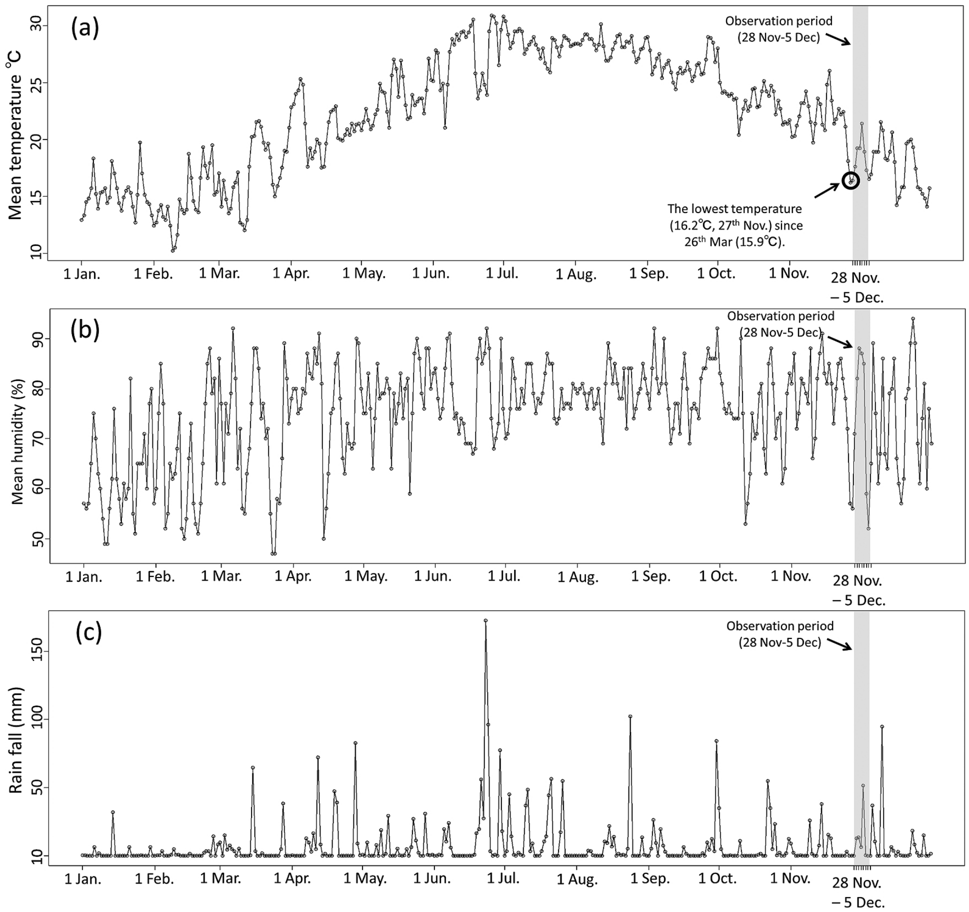

FIG. 4. Annual changes of temperature (a), humidity (b) and rain fall (c) at Naze city close to Kinsakubaru area in Amami-Oshima Island in 2015. Grey bars represent observation period (28 Nov.–5 Dec.).

well (Oumi, 2006). Our observation of *O. amamiensis* breeding activity suggests that searching for breeding sites during daytime may be a relatively safer and useful alternative. Finding breeding sites can lead to better protection of the area and a better understanding of the species' breeding habitat preference. Searching for breeding sites of *O. amamiensis* during daytime may contribute to conservation efforts for this species.

Although it is known that they bred from autumn to spring (Oct to May) (Matsui and Maeda, 2018), it is unknown what factors relate to their breeding activity. The current study does not sufficiently address the potential factors influencing the breeding activity of *O. amamiensis*, while breeding activity might be related to temperature. The observed breeding activity started on 27 or 28 Nov. when there was the lowest mean temperature (27 Nov: 16.2°C, 28 Nov: 16.4°C) since 26 Mar. (26 Mar: 15.9°C) (Fig. 4a). Decreasing temperature after summer might be related to the start of the breeding activity. On the other hand, it seemed to have little relationship between breeding activity and other climate factors (humidity and rain fall) (Fig. 4b, c). However, current study was conducted only a single year hence it is difficult to draw the general conclusion. Year-round monitoring over the years will be necessary to reveal the potential factors influencing breeding activity and the seasonal pattern.

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