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Spawning sites of the Japanese Forest Green Tree Frog (*Zhangixalus arboreus*: Rhacophoridae) in Central Japan

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Abstract: We conducted field observations of spawning sites of the Japanese forest green tree frog (*Zhangixalus arboreus*: Rhacophoridae) at four ponds for two years. In 2019, a total of 50 foam nests were made on trees (77%), whereas 15 foam nests were made on the ground (23%). In 2020, 65 nests were arboreal (97%) and there were only two ground nests (3%). About 70% of all ground nests deteriorated or disappeared without their eggs hatching, mostly because of predation. In contrast, only about 4% of all arboreal nests died because of desiccation and 27% died mainly of outside spawning and disappearance before egg hatching for any reason. The relative proportions of arboreal versus ground spawning sites differed significantly between 2019 and 2020. Ground spawning was scarcely observed in 2020; in that year the amount of precipitation during the reproductive period was about 1.5 times that in 2019. Our field experiment using paper-clay models confirmed that arboreal nests were more susceptible to desiccation than ground nests. These results suggest that ground spawning may be of some advantage in resistance to desiccation, whereas arboreal spawning may be less susceptible to predation of foam nests.

Key words: Arboreal spawning; Foam nest; Ground spawning; Spawning site; *Zhangixalus arboreus*

Introduction

Animals can use multiple tactics if there are trade-offs between different mortality factors. When there are multiple spawning environments with different egg mortality factors, choice of the spawning site has consequences on the fitness of both parents and offspring.

The Japanese forest green tree frog (*Zhangixalus arboreus*: Rhacophoridae) is a relatively large species endemic to Honshu and Sado islands, Japan. The frog usually lives in trees, and after mating the female spawns 300 to 800 eggs into a foam nest made most frequently on a tree or shrub near standing water (Matsui and Maeda, 2018).

The fate of foam nests and egg mortality rates are affected by desiccation (Kusano et al., 2005), temperature (Kusano et al., 2006), rainfall (Touchon and Warkentin, 2009; Allingham, 2017), and predation (Fukuyama, 1996; Inoue and Tsuji, 2016). Besides arboreal spawning, the frog sometimes spawns on

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the ground near standing water (Kitano, 2001; Umesako and Nagano, 2005). Between arboreal and ground-surface locations, spawning sites may differ in temperature and humidity as abiotic factors and in predation risk as a major biotic factor. Therefore, reproductive success may also differ depending on whether foam nests are located on tree branches or on the ground. Several studies of *Z. arboreus* have examined spawning behavior and temporal variation in the production of arboreal foam nests (e.g., Kasuya et al., 1996; Takahashi and Sato, 2015), but little is known about the fate of foam nests made on the ground (Kitano, 2001), or about the adaptive significance of such dual patterns of spawning behavior.

We conducted field observations of foam nests of *Z. arboreus* in two reproductive seasons at four ponds. We examined how spawning site (arboreal or ground) affected the fate of foam nests, and we discuss the adaptive significance of spawning-site selection in this species.

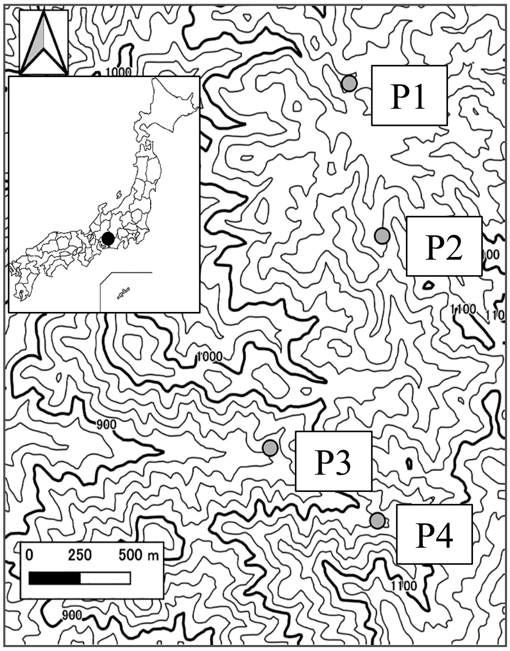

FIG. 1. Locations of the four ponds in the Inabu Experimental Forest in central Japan. The map was produced from data provided by the National Agriculture and Food Research Organization (https://aginfo.cgk.affrc.go.jp/rstprv/rstext.html.ja).

MATERIALS AND METHODS

Study site

This study was conducted at four ponds (P1, P2, P3, and P4) in the Inabu Experimental Forest of Nagoya University, in central Japan (35°11' N, 137°33' E; 980 to 1,000 m a.s.l.) (Fig. 1). P1 has variable surface area, occasionally drying up, but covers about 7.0 m^2 or more in the reproductive season of *Z. arboreus*. P2 has a large surface area of about 20 m^2. P3 is a relatively small pond with surface area less than 2.0 m^2. P4 consists of two separated pools (each area is less than 2.0 m^2).

The mean air temperature was 10.3°C and annual precipitation averaged 2,300 mm (2019 to 2020) (unpublished data from Field Science Center, Bioagric., Nagoya University). All ponds were located more than 500 m apart from each other.

The vegetation is dominated by plantations of about 40- to 60-year-old Japanese cedar (*Cryptomeria japonica*) and Japanese cypress (*Chamaecyparis obtusa*), with small patches of broadleaf trees, mainly *Quercus crispula*, *Fagus crenata*, *Carpinus* spp., and *Acer* spp., that remained along some ridges and streams after conversion to the plantations. The sparse understory vegetation is mainly composed of *Lindera triloba* and *Sasa borealis*. Around the four ponds, there are tall trees, such as *Q. crispula* (P1), *Pourthiaea villosa* (P1), *L. triloba* (P1 and P2), *C. obtusa* (P2), and *Deutzia crenata* (P2 and P4). Around the P3, there was only one tree (*Acer pictum*). Around P2 and P3, there is short vegetation cover, but none around P1 and P4.

Field census of foam nests

We recorded the sites (on tree branches above each pond or on the ground near the pond) of newly spawned foam nests of *Z.*

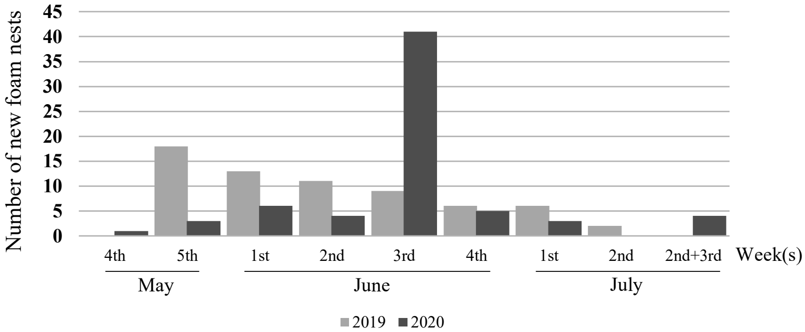

FIG. 2. Numbers of new foam nests observed at the four ponds in a weekly field census during the reproductive periods of *Zhangixalus arboreus* in 2019 and 2020.

arboreus at each of the four ponds every week from 23 May to 19 July 2019 and from 7 May to 29 July 2020. Each foam nest was marked with colored paint or a numbered-flag tag to identify changes in the nest condition with time. If nests were too high to mark, we simply counted them, and at the next census we distinguished them from newly made nests by their surface condition. We also recorded tree species and height above the water surface for each nest. In every field census, we searched for tadpoles and noted their presence. The eggs in foam nests that had deteriorated (dried out, eaten by predators, or spawned outside of a pond) or disappeared before tadpole appearance were treated as dead. Foam nests were judged as dried out from their shriveled appearance, and they were judged as eaten from bite signs or loss of marking tags.

Moisture detection at spawning sites

We conducted a field experiment to detect the moisture at each spawning site (arboreal or ground). We made a total of 40 imitation foam nests (model nests) of paper-clay, weighing about 15 g each. On 31 July 2019, we placed five model nests on tree branches about 1 to 2 m above each pond, and we placed another five on the ground around each pond. About 21 h after placement, the model nests were collected and weighed separately before and after being dried in a convection oven (60°C, 24 h). We used these measurements to determine the percentage loss of moisture in 21 h in each model nest in the arboreal and ground sites. We collected AMeDAS (Automated Meteorological Data Acquisition System) data on monthly precipitation and the number of rainy days (>0.5 mm) for the study area during the field observations in 2019 and 2020 (Japan Meteorological Agency: http://aginfo.cgk.affrc.go.jp/rstprv/rstext.html.ja).

RESULTS

Spawning Period and Number of Foam Nests at Each Spawning Site

In 2019, we observed spawning most frequently in the 5th week of May (29 and 30 May). Spawing then decreased gradually toward early July. In 2020 the peak occurred abruptly in the 3rd week of June (18 June; Fig. 2) and the majority of nests were constructed at P1 (Table 1). In 2019, 50 foam nests were made on trees around the four ponds, whereas 15 were on the ground. In 2020, 65 foam nests were made on trees and only two were on the ground (Table 1). The relative proportions of spawning site selection (arboreal or ground) differed significantly between 2019 and 2020 (Fig. 3: Fisher's exact test, $P=0.0006$).

We observed arboreal foam nests of *Z. arboreus* on 10 tree species. At P1, there was

TABLE 1. Numbers of foam nests in arboreal and ground spawnings of *Zhangixalus arboreus* at each pond each year.

Pond	P1	P2	P3	P4	Total
Year			Arboreal spawning		
2019	17	9	5	19	50
2020	35	9	6	15	65
Total	52	18	11	34	115
Year			Ground spawning		
2019	6	5	4	0	15
2020	0	2	0	0	2
Total	6	7	4	0	17

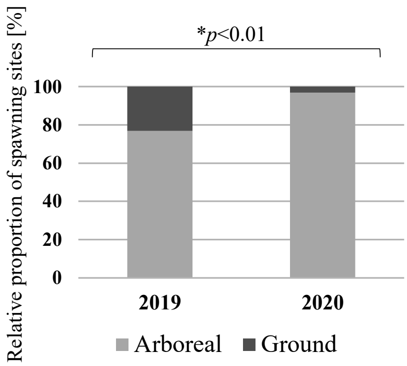

FIG. 3. Spawning-site selection each year by *Zhangixalus arboreus* at the four ponds.

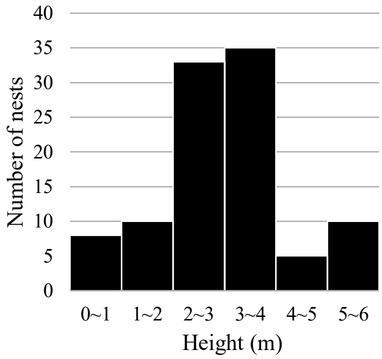

FIG. 4. Height above the ground of foam nests constructed in 2019 and 2020.

a large difference in the species of trees on which foam nests were made between 2019 and 2020. In 2019, all the nests were made on *P. villosa*, whereas in 2020 they were on *Q. crispula*, *L. triloba*, and *Clethra barbinervis*. Spawning on *C. obtusa* at P2 and on a dam site at P4 was observed only in 2019; except for two cases: *Z. arboreus* used the same tree species for arboreal spawning at P2, P3, and P4 in both years. We measured the height above the water surface for 101 arboreal nests. All the nests were made at a height between 0–6 m, with the mean height of about 3 m above the ground (Fig. 4). We could not measure the height for other arboreal nests mainly because they disappeared before measurements could be taken. At P1, the height of arboreal nests was significantly higher in 2020 (t-test, $t=2.68$, $P<0.05$), whereas there were no significant differences between years at the other ponds.

Fate of Foam Nests

Arboreal nests

In most arboreal nests, the spawned positions were so high that we could not confirm the presence of tadpoles in nests. Therefore, among the 115 foam nests made on trees, we could confirm moving tadpoles in only one nest, and therefore eggs in this nest were considered to have hatched successfully. We confirmed 36 dead arboreal nests, and their causes of death were desiccation (five nests),

TABLE 2. Numbers of foam nests those treated as dead in arboreal and ground spawnings of *Zhangixalus arboreus* at each year.

Cause of death	Desiccation	Predation	Outside spawning	Unidentified	Total
Year			Arboreal spawning		
2019	4	1	5	15	25
2020	1	0	9	1	11
Total	5	1	14	16	36
Year			Ground spawning		
2019	0	6	0	4	10
2020	0	2	0	0	2
Total	0	8	0	4	12

predation (one nest, preyed on the ground after dropping from a branch), and outside spawning (14 nests). The remaining 16 nests were judged to have been dead because the nests disappeared before the appearance of tadpoles, although we were unable to determine the cause of the disappearance (Table 2). At the remaining 78 of the 115 arboreal nests, we could not find any evidence suggesting death or hatching of eggs in field census.

Ground nests

Among the 17 foam nests made on the ground at P1 to P3 in 2019 and 2020 (Table 1), two were hidden between the logs, while the other 15 nests were deposited on the grassland or directly on the ground near the ponds. Among the ground nests, we confirmed the presence of moving tadpoles inside four; the eggs in these four were therefore considered to have hatched successfully. We confirmed 12 dead ground nests. The cause of death for eight nests was predation, while the other four nests were judged to have died because the nests disappeared before the appearance of tadpoles, although we were unable to determine the cause of the disappearance (Table 2). At the remaining four of the 17 ground nests, we could not find any evidence suggesting death or hatching of eggs. We found bite signs or loss of marking tags at the nests preyed upon; death therefore likely occurred through predation, probably by mammals or birds.

Moisture Levels at Arboreal and Ground Spawning Sites

Twenty-one hours after placement of the model nests, we collected 19 placed on trees (excluding one that had fallen into P1) and 20 placed on the ground. No precipitation was recorded on 31 July and 1 August when model nests were placed outside, and the mean temperature of the two days was about 23.5°C (unpublished data from Field Science Center, Bioagric., Nagoya University).

The paper-clay model nests weighed 14.8±0.1 g (mean±SD) before placement. After collection, the arboreal nests weighed 12.9±0.3 g, whereas those placed on the ground averaged 14.2±0.3 g. The mean weight of all nests after being dried in the convection oven was 6.1±0.1 g. Some nests were weighed after 12 h of drying, but the small SD of dried nest weight means all of nests were equally, completely dried. The model nests placed in the trees lost 22.0±3.3% of their water in 21 h, whereas the nests placed in the ground lost 6.9±3.3% of their water. The model nests placed in trees therefore lost significantly more water than those placed on the ground (Wilcoxon rank sum test, $W=380$, $P<0.0001$). Also, in a comparison of every combination of ponds,

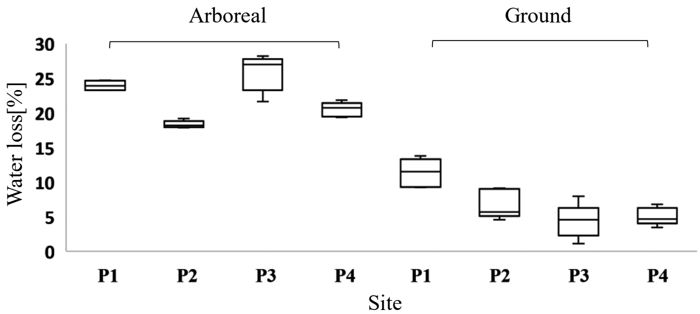

FIG. 5. Mean percentages of water lost compared with initial water content of paper-clay model nests placed on tree branches (Arboreal) or on the ground (Ground) at each pond.

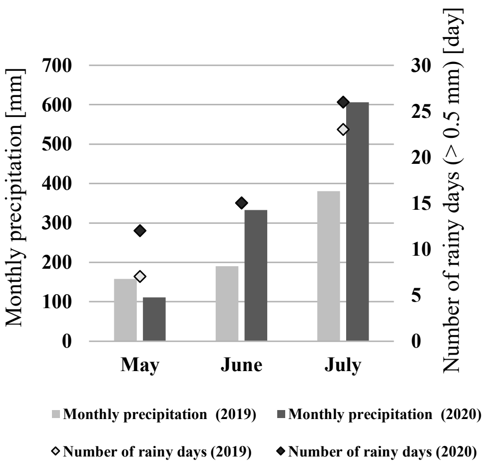

FIG. 6. Monthly precipitation and numbers of rainy days (>0.5 mm) in the study area during the reproductive period of *Zhangixalus arboreus* in 2019 and 2020.

the model nests placed in trees lost significantly more water than those on the ground (Fig. 5; Tukey-Kramer test, $P<0.01$). Analysis of the meteorological data for the study area showed that the monthly precipitation and number of rainy days during May (June) and July were higher in 2020 than in 2019 (Fig. 6).

DISCUSSION

Arboreal spawning in *Z. arboreus* is considered to be an adaptative behavior to avoid predation by terrestrial animals (Kitano, 2001). At our study sites, ground spawning was observed in both 2019 and 2020, although there were many tree branches available for spawning above every pond throughout the spawning periods in both years. As expected, no fewer than 70% of all foam nests made on the ground deteriorated or disappeared without the eggs hatching. Foam nests on the ground are easily accessed by terrestrial animals, and indeed, at our sites, we observed bite signs or loss of marking tags on foam nests as evidence of predation on the ground nests and fallen nests. Predation thus accounted for the loss of about 70% of the deteriorated or dead ground foam nests in 2019 and 2020. Therefore, arboreal spawning is likely more effective than ground spawning for minimizing predation by terrestrial animals.

In contrast, we found that about 4% of all arboreal foam nests in 2019 and 2020 deteriorated through drying out. For *Z. arboreus*, Kitano (2001) listed desiccation risk as a disadvantage of arboreal spawning, and desiccation is considered to be a major cause of mortality of eggs and embryos inside foam nests (Kusano et al., 2005). The results of our field experiment using paper-clay models suggested that arboreal foam nests are more susceptible than ground nests to desiccation, indicating that egg mortality due to desiccation would be higher in arboreal spawning sites near ponds than at ground sites near ponds. Therefore, in the absence of predation, egg mortality would be lower when foam nests were made on the ground than on trees. In our study area, the contents of no fewer than 12% of all arboreal foam nests died because the nests were made on tree branches hanging away from standing water. For almost 14% of arboreal nests we could not determine cause of death. However predation by aquatic animals, such as tadpoles of *Bufo japonicus formosus* that flock to arboreal nests that fall into ponds, may influence the demography of *Z. arboreus*.

At our sites, the relative proportions of spawning sites (arboreal or ground) differed significantly between 2019 and 2020. This difference may have resulted from environmental conditions, such as precipitation. If it rains during the reproductive period desiccation risk of arboreal foam nests will be reduced. Indeed, ground spawning was scarcely observed in 2020—a year in which the precipitation was about 1.5 times that in 2019 during the reproductive period of *Z. arboreus*. Peaks of reproduction in *Z. arboreus* also differed between 2019 and 2020. It is suggested that anuran breeding activity could potentially be influenced by weather conditions, such as temperature (Fukuyama and Kusano, 1992) or rainfall (Fukuyama and Kusano, 1992; Vockenhuber et al., 2008). Thus, the difference in reproductive peaks of *Z. arboreus* may result from a difference of weather conditions during the period.

Our results suggest that ground spawning may be of some advantage in resistance to desiccation, whereas arboreal spawning may be less susceptible to predation of foam nests. However, we have observed spawning of *Z. arboreus* for only two seasons, and more field data are required for evaluation of the relative frequencies of arboreal and ground spawning under different conditions.

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