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Source: Current Herpetology, 43(1) : 42-54

Published By: The Herpetological Society of Japan

URL: <https://doi.org/10.5358/hsj.43.42>

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# Seasonal Emergence and Habitat Pond Use of Invasive American Bullfrog, *Lithobates catesbeianus*, on Sado Island, Japan

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**Abstract:** The American bullfrog *Lithobates catesbeianus* is a well-known invasive alien species recorded in over 40 countries and islands. It is necessary to elucidate their seasonal emergence and habitat water body characteristics for implementing efficient eradication activities. Sado Island, Japan, is home to several endemic and rare species, including the endangered Sado wrinkled frogs (*Glandirana susurra*) that are concerned to be negatively impacted by invasive American bullfrogs. Here, we surveyed 41 ponds for invasive American bullfrogs over the period preceding emergence to the start of oviposition to determine their seasonal emergence and habitat pond use. As in previous studies, we observed initiations of their post-hibernation activities such as emergence, mating call, and oviposition in that order as air temperature increased, and those activities were thought to begin as late as late-April, mid-May, and mid-July, respectively on Sado Island. For their habitat preferences, we confirmed that they mainly inhabit relatively deep ponds surrounded by rice paddy fields. Our results were broadly consistent with those of previous studies, with respect to the seasonal emergence and habitat pond characteristics of American bullfrogs on Sado Island. Since our results suggest an overlap in habitat and pond usage season between American bullfrogs and Sado wrinkled frogs, we highly recommend implementing bullfrog eradication activities to conserve the Sado wrinkled frog population. This study provided the basic ecological information for selecting appropriate seasons and ponds for implementing bullfrog eradication activities.

Key words: Habitat pond use; Invasive American bullfrog; *Lithobates catesbeianus*; Sado Island; Seasonal emergence

## INTRODUCTION

American bullfrog, *Lithobates catesbeianus*, originally occurring in Northeast America, is a

well-known invasive alien species listed as one of the “100 of the World’s Worst Invasive Alien Species” (Lowe et al., 2000). They have been introduced over large parts of the globe (Kraus, 2009) and recorded in over 40 countries and islands, mainly in Europe and East Asia (Cooper, 2017; GBIF, 2023). Invasive

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American bullfrogs cause negative impacts on native ecosystems through predation, competition, and the transmission of their pathogens (Kupferberg, 1997; Jancowski and Orchard, 2013; Yap et al., 2018). On islands, which are hotspots of species biodiversity, their invasiveness is markedly higher than on the mainland (Yiming et al., 2006).

To prevent the reproduction of invasive frogs, it is necessary to know the timing of their emergence and oviposition because their high reproductive rate is one of the threats (Pitt et al., 2005). Particularly in American bullfrogs, understanding the characteristics of the water bodies they use provides valid ecological information for their eradication because they are known as highly aquatic frog species (e.g., Orchard, 2011; Kamoroff et al., 2020); they require permanent water bodies to accommodate 2–3 years of tadpole periods and spend almost all their adult lives in aquatic habitats (Degraaf and Rudis, 1983; Bury and Whelan, 1984). Furthermore, they have relatively larger clutch size than other frog species (Bury and Whelan, 1984; Matsui, 2021). Therefore, the top priority in bullfrog eradication is to prevent their reproduction.

In Japan, American bullfrogs were introduced in 1918 and are currently distributed in all prefectures and on many islands, such as the Goto Islands, Ogasawara Islands, and Ryukyu Archipelago (Matsui, 2021; Shimada and Takahashi, 2022). Currently, they are listed as “Regulated Living Organisms under the Invasive Alien Species Act” (Ministry of the Environment of Japan, 2023), and the reports of their impact on the native species through predation continue to increase (Hirai, 2004; Dontchev and Matsui, 2016; Sarashina and Yoshida, 2021).

On Sado Island, Niigata Prefecture, Japan, American bullfrogs were introduced as human food from the Japanese mainland to the Kobie district in the southwestern part of the island on May 30, 1926, with 30 tadpoles brought (Iwasawa, 1960). Currently, they are distributed in most of their potentially suitable habitats, mainly in the southwestern and central parts of

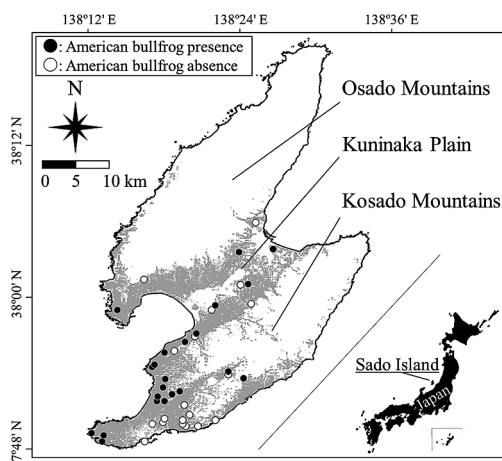


FIG. 1. Study site and surveyed pond locations. Grey areas indicate the probability of invasive American bullfrog occurrence >50% predicted by Sawada et al. (2022).

the island (see Fig. 1), and there are concerns about the increase in their population density within their distribution areas (Kishimoto and Mitsuo, 2020; Sawada et al., 2022). However, there is no clear information on the types of water bodies they use as habitat, and it is necessary to identify the characteristics of the ponds used by invasive American bullfrogs to control their population (Sawada et al., 2022).

Thanks to wildlife-friendly farming, Sado Island maintains a high level of aquatic biodiversity (Usio et al., 2014). As the American bullfrog primarily preys on aquatic organisms (Hirai, 2004; Sarashina and Yoshida, 2021), concerns arise about its impact on native aquatic species. In particular, the Sado wrinkled frog *Glandirana susurra* (Sekiya et al., 2012) is at high risk due to its potential ecological trait overlap (Kobayashi, 2014; Kishimoto and Mitsuo, 2020) and susceptibility to predation by American bullfrogs (Wu et al., 2005; Kobayashi, 2014). Thus, considering the conservation of island biodiversity, prioritizing the Sado wrinkled frogs is crucial which requires understanding the timing and characteristics of water bodies exploited by the invasive American bullfrogs.

Here, we surveyed Sado Island and investi-

gated emergence patterns in American bullfrogs and the characteristics of permanent water bodies inhabited by them to make recommendations for their effective eradication. First, we recorded their seasonal emergence patterns about every two weeks from early spring in March, until oviposition was confirmed in July. Second, we investigated the types of permanent water bodies that invasive American bullfrogs use as their habitat. Finally, we made recommendations for controlling the populations of invasive American bullfrogs on Sado Island, focusing mainly on conserving Sado wrinkled frogs.

## MATERIALS AND METHODS

### *Study site*

This study was conducted on Sado Island, located in Sado City, Niigata Prefecture, Japan (Fig. 1). The island is divided into three main areas: the Osado and Kosado mountain ranges in the northern and southern parts of the island with maximum elevations of 1,172 and 645 m, respectively, and Kuninaka Plain between the two mountain ranges (Sado City, 2012). It is located between 37°48'09" N and 38°20'18" N, and 138°12'11" E and 138°34'28" E and off the western coast of mainland Japan, with an area of 855.25 km<sup>2</sup>, annual precipitation of 1,785 mm, an annual mean temperature of 13.6°C, and a maximum elevation of 1,172 m (Sado City, 2012, 2021). The coldest and hottest months on the island are February and August, respectively (Sado City, 2021). The landscape consists mainly of forests in the mountains and rice paddy fields in the plains (Sado City, 2012, 2021).

### *Survey*

Since the surveyed ponds included privately owned ponds with ornamental carps and other fish, no capture or trapping of invasive American bullfrogs was conducted, and the field survey was restricted to only observational surveys. Field surveys were conducted nine times, almost every two weeks between March and July 2020 for a total of 29 days (see Fig. 2).

Here, we defined a permanent water body with a depth of less than 8 m as a pond, referring to the papers by Oertli et al. (2000) and Biggs et al. (2005). The surveyed ponds were selected to include a variety of environments and be at least 200 m apart from each other because the maximum movement distance of American bullfrogs in a year is slightly greater than 100 m (Sepulveda and Layhee, 2015; Park et al., 2022). The surveyed areas were determined based on the potential distribution probability of the bullfrogs on Sado Island, as predicted by Sawada et al. (2022), with the survey conducted only in areas where this probability was greater than 50% (Fig. 1). A total of 41 ponds were selected for the analysis (Fig. 1).

The first survey of all 41 ponds was conducted between March and May 2020. For the remaining months (i.e., June and July), the second survey was conducted in the ponds where American bullfrogs could not be spotted during the first survey, and the ponds where they were present were monitored randomly multiple times until oviposition was confirmed. During the field survey, we recorded their presence or absence and life stages (i.e., adult, tadpole, or egg mass). For the records of adult bullfrogs, confirmation of only mating calls was treated as presence. The data on local weather conditions were obtained from the Japan Meteorological Agency ([https://www.data.jma.go.jp/obd/stats/etrn/index.php?prec\\_no=45&block\\_no=47682](https://www.data.jma.go.jp/obd/stats/etrn/index.php?prec_no=45&block_no=47682)) to examine the relationship between air temperature and the timing of the occurrence of various developmental stages because their seasonal emergence has been reported to be mainly determined by temperature (Bury and Whelan, 1984; Sepulveda and Layhee, 2015).

To determine the type of permanent ponds that invasive American bullfrogs use as their habitat, the pond perimeter (m), depth (cm), material (artificial or natural), and vegetation cover on the surface of the pond (%) were measured on-site. Measurements of these four items were recorded between November 2 and 5, 2020, after the end of the field survey, to reduce measurement bias due to measurement

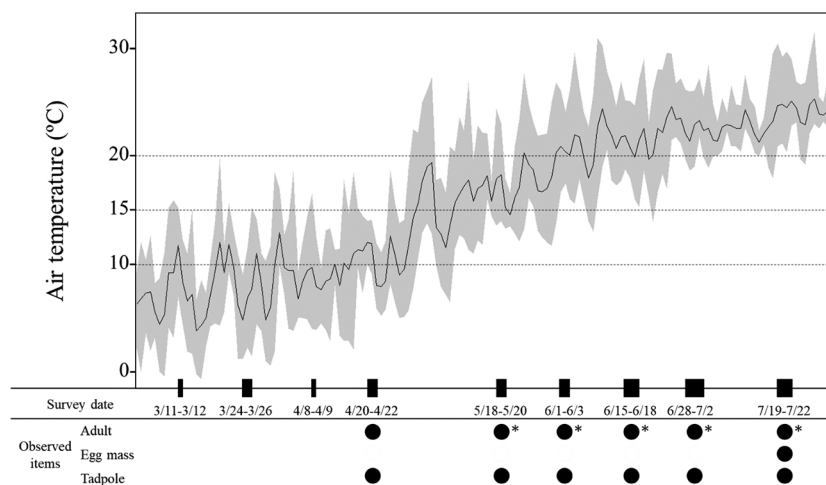


FIG. 2. Air temperatures during the field survey period and seasonal changes in pond use by invasive American bullfrogs. The solid line is the daily mean air temperature, and the shaded area is the interval between the highest and lowest air temperatures. The black rectangles indicate the periods during which the survey was conducted, and the start and end dates of each survey are indicated by month/day under each rectangle. The black circles indicate that the presence of American bullfrogs in corresponding life stages. Asterisks indicate the confirmation of their mating calls.

dates. For classifying the types of ponds based on their bottom material, ponds with rubber or concrete bottoms were considered ponds made of artificial materials, whereas those with gravel or soil bottoms were regarded as ponds made of natural materials. The reason for not classifying the ponds as truly artificial and natural ponds is that most ponds, approximately 1,400, on Sado Island, are artificial (Ouchi, 2012), and it was difficult for us to locate and distinguish natural ponds. In addition to the four measurement items, the forest and rice paddy areas ( $m^2$ ) within the 100 m riparian buffer of ponds were estimated. The size of this buffer was determined based on the general maximum movement distance of the American bullfrog, which is 100 m (Sepulveda and Layhee, 2015; Park et al., 2022). The forest and rice paddy area data were calculated using QGIS version 3.6.0 (<https://qgis.org/en/site/forusers/download.html>) based on the map data (Vegetation Survey 2nd–5th, Niigata Prefecture, Natural Environment Information GIS, Biodiversity Center of Japan: <http://www.biodic.go.jp/trialSystem/EN/vg/vg.html>).

#### Statistical analyses

All statistical analyses were performed using RStudio Desktop version 4.3.1 (RStudio Team, 2023). To clarify the habitat pond characteristic of invasive American bullfrogs on Sado Island, we examined the effect of explanatory variables on response variables using a generalized linear model with the Bernoulli family in a Bayesian framework (hereafter, Bayesian model). The response variable was the presence or absence of invasive American bullfrogs (absence: 0; presence: 1). The explanatory variables were the pond perimeter (m), depth (cm), material (artificial: 0; natural: 1), vegetation cover on the surface of the pond (%), forest area ( $m^2$ ), and rice paddy area ( $m^2$ ). Pearson's correlation coefficient (PCC) and variance inflation factor (VIF) were calculated using the package 'psych' (Revelle, 2022) and 'car' (Fox et al., 2022), respectively, to avoid multicollinearity between explanatory variables. Referring to the studies by Wen et al. (2015) and Alin (2010), we determined high collinearity when PCC was more than 0.75 or less than  $-0.75$  and VIF was greater than 10.

As a result of PCC and VIF evaluations, explanatory variables were included in the Bayesian model (Appendix I). Before analyzing the Bayesian model, the continuous explanatory variables were standardized such that their mean became zero and SD became one.

Estimation of the posterior distribution under a non-informative prior distribution through a flat uniform distribution, which ranges over the values that each parameter takes, of the Bayesian model was conducted using the Markov chain Monte Carlo (MCMC) method that was implemented by RStudio and the package “rstan” version 2.31 (Stan Development Team, 2022). Non-informative priors play a crucial role in objective Bayesian analysis (Shemyakin, 2014). We ran four parallel MCMC chains and retained 10,000 iterations after 2,000 burn-in steps for each chain. We determined that MCMC sampling converged when the R-hat value was  $<1.1$ . A coefficient was defined to be significantly affected if the 95% credible intervals (CI) did not overlap zero: positive (containing only positive values) and negative (containing only negative values).

After Bayesian analysis, box-and-whisker plots were constructed for each significant explanatory variable to understand its specific characteristics.

## RESULTS

### *Timing of emergence*

Invasive American bullfrogs were observed in 22 of the 41 ponds surveyed (Fig. 1) between April 20 and July 22, 2020. Their adults and tadpoles were observed between April 20 and July 22, 2020, with their egg masses confirmed between July 19 and 22, 2020 (Fig. 2). The mating calls of adult bullfrogs were recorded between May 18 and July 22, 2020 (Fig. 2). Mating calls were not observed between November 2 and 5 when we measured the ponds, but their adult individuals were observed. The daily mean air temperatures at the time of the first observations of the

adults and tadpoles, mating calls, and egg masses of American bullfrog were  $10.4^{\circ}\text{C}$ ,  $16.9^{\circ}\text{C}$ , and  $24.5^{\circ}\text{C}$ , respectively (Fig. 2).

### *Habitat pond characteristic*

The values of mean $\pm$ SD of each continuous explanatory variable for the Bayesian model were  $104.4\pm 78.6$  m (perimeter),  $94.5\pm 60.0$  cm (depth),  $12.7\pm 24.9\%$  (vegetation cover),  $13,466.4\pm 10,387.4$  m<sup>2</sup> (forest area), and  $12,991.2\pm 9,892.5$  m<sup>2</sup> (rice paddy area). The numbers of artificial and natural material ponds surveyed were 16 and 25, respectively.

The Bayesian model analysis showed that pond depth and rice paddy area significantly correlated with the presence of invasive American bullfrogs (Table 1). The association between their presence and pond depth was positive (95% CI=0.40–2.98) (Table 1). Similarly, the association between their presence and rice paddy areas was positive (95% CI=0.01–2.66) (Table 1). The values for the mean $\pm$ SD of the depths of ponds with and without their presence were  $114.5\pm 59.2$  and  $71.3\pm 51.8$  cm, respectively, and their presence was not recorded in ponds less than 30 cm deep in any of the field surveys (Fig. 3A). The values for the mean $\pm$ SD of rice field area for ponds with and without them were  $16,053.1\pm 8,971.6$  and  $9,445.9\pm 9,723.7$  m<sup>2</sup>, respectively (Fig. 3B). The former average value of  $16,053.1$  m<sup>2</sup> is approximately 50% of that corresponding to a buffer area with a radius of 100 m. Although there were three ponds where American bullfrogs were observed despite having almost no rice paddy area around the pond (Fig. 3B), these three ponds were relatively deeper than the other ponds with almost no paddy field around the pond (Appendix II).

## DISCUSSION

### *Timing of emergence*

Many studies have reported that the activities of American bullfrogs are restricted by ambient temperature (Bury and Whelan, 1984; Sepulveda and Layhee, 2015), and their activities increase positively with increasing ambient

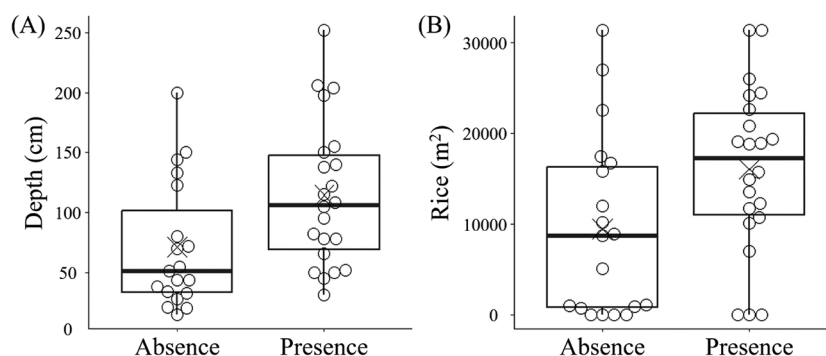


FIG. 3. Box-and-whisker plots with mean values of significant explanatory variable for the absence or presence of invasive American bullfrogs. Box-and-whisker plots with mean values of pond depth (A), and of the area size of rice paddy field within 100 m from surveyed ponds (B). The cross marks indicate the mean values, and the circles indicate each raw data.

TABLE 1. Bayesian estimation of pond characteristics that determine the presence or absence of invasive American bullfrogs, with mean, SD, 95% credible intervals (CI), and  $R$ -hat for each variable. Bold letters indicate significant variables. Details of each explanatory variable are followings: Perimeter: (m); Depth: (cm); Material: (artificial: 0, natural: 1); Vegetation: Vegetation cover on the surface of the pond (%); Forest: Forest area within 100 m from surveyed ponds ( $m^2$ ); Rice: Rice paddy area within 100 m from surveyed ponds ( $m^2$ ).

Variables	Mean	SD	95% CI		$R$ hat
			2.5%	97.5%	
Perimeter	0.19	0.47	-0.70	1.15	1.0
<b>Depth</b>	<b>1.54</b>	<b>0.66</b>	<b>0.40</b>	<b>2.98</b>	<b>1.0</b>
Material	0.58	0.99	-1.29	2.63	1.0
Vegetation	0.84	0.48	-0.01	1.86	1.0
Forest	0.05	0.72	-1.34	1.50	1.0
<b>Rice</b>	<b>1.22</b>	<b>0.67</b>	<b>0.01</b>	<b>2.66</b>	<b>1.0</b>

temperature (Medeiros et al., 2016). Their adults often start to move and emerge when a daily mean air temperature exceeds 10°C (Willis et al., 1956; Govindarajulu et al., 2006; Sepulveda and Layhee, 2015). In this study, adults and tadpoles of the American bullfrog were first observed during the April 20–22 survey when the daily mean air temperature was 10.4°C (Fig. 2), suggesting that their activities after hibernation had started between the April 8–9 survey, when the daily mean air

temperature was 8.6°C, and the April 20–22 survey. For their tadpole activities, although the water temperature is certainly more important than air temperature (Pahor-Filho et al., 2019), the air temperature when adult bullfrogs start their post-hibernation activities may also be the timing for bullfrog tadpoles to be active.

The number of the breeding adults gathering in a pond increase when a daily mean air temperature exceeds 15°C (Willis et al., 1956), and they start to make mating calls when a daily mean air temperature consistently remains over 15°C (Govindarajulu et al., 2006). In our study, their mating calls were first recorded during the May 18–20 survey when the daily mean air temperature was 16.9°C (Fig. 2), suggesting that they had begun to croak between the April 20–22 survey, when the daily mean air temperature was 10.4°C, and the May 18–20 survey. On Sado Island in 2020, a daily mean air temperature often exceeded 15°C from the first half of May (Fig. 2), when we had not conducted surveys, and it is possible that their mating calls had already been made during that period.

As for the timing of egg mass oviposition, there is a report that it occurs when daily mean air temperatures become over 20°C (Jones et al., 2005), and we observed the egg masses during the July 19–22 survey when the daily mean air temperature was 24.5°C (Fig. 2). On the other hand, the daily mean air temperature

on Sado Island in 2020 had been above 20°C on many days since early June (Fig. 2). Since we did not conduct surveys every day and hatching of bullfrogs occurs over about one week (Cook et al., 2013), there is a significant possibility that some egg masses were not observed if some egg masses had oviposited before the July 19–22 survey. Therefore, the date July 19–22 should be considered as the time when their oviposition took place at the latest. Observation of tadpoles occurred well before July 19–22, implying an earlier oviposition in the year. However, the size of these tadpoles was visually 5.0 cm in SVL, which is considerably larger than expected for individuals of the same year, this suggesting that they were likely overwintered tadpoles born in the previous year.

Our results confirmed that the timing of the American bullfrog emergence on Sado Island roughly corresponds with previous studies in terms of the seasonal temperature-dependent development and emergence of this species. We consider that the top priority in bullfrog eradication is to prevent their reproduction. Therefore, on Sado Island, in order to effectively implement their eradication, it is needed to remove adult bullfrogs by July, when they start to oviposit.

#### *Habitat pond characteristic*

We observed that invasive American bullfrogs on Sado Island mainly inhabited relatively deep ponds surrounded by rice paddy fields (Table 1), in agreement with the results reported by previous studies (Bury and Whelan, 1984; Minowa et al., 2008; Wang and Li, 2009). While it is well known that the probability of a pond drying up decreases with increasing water depth, it has been assumed that a depth that does not completely freeze to the bottom of the pond is necessary for the winter survival of hibernating American bullfrogs (Graves and Anderson, 1987). In addition, adult bullfrogs use deep water as a refuge from predators (Graves and Anderson, 1987). Furthermore, the depth of water used by their tadpoles varies with the season (Nie et al.,

1999), and laboratory experiments have shown that they select different water temperatures at different developmental stages (Wollmuth and Crawshaw, 1988). Therefore, water depth plays an important role not only in the rehydration of bullfrogs, which require water throughout their life history, but also in overwintering, predator avoidance, and efficient development, and can be considered a universal and essential factor in American bullfrog habitat selection. On the other hand, regarding specific water depths, the ponds in which bullfrogs were observed in this study had an average depth of 114.5 cm and ranged from 31.0 cm to 252.0 cm (Fig. 3A), but this varied depending on the location and season of the study; Cook and Jennings (2007) showed that the pond depths in which bullfrogs were observed ranged from 37.7 cm to 54.8 cm and the average water depth receded in summer compared to winter. Therefore, it should be assumed that they were most likely to be found in relatively deep ponds in each study area and season.

Rice paddy area within 100 m of the riparian buffer of each pond was also selected as a significant variable in this study (Table 1), but not in the study conducted on Daishan Island, China (Wang and Li, 2009). This difference may be attributed to the regional paddy management practices. On Daishan Island, most rice paddy fields are dry for part or all of the winter or dry season (Wang and Li, 2009). On the other hand, on Sado Island, winter flooding of rice paddy fields and the construction of swales (shallow, unconsolidated diversion ditches in rice fields; called “e” in Japanese) have been applied as a refuge for aquatic organisms including Sado wrinkled frogs in winter and mid-summer (Kobayashi, 2014; Taqumori et al., 2020). American bullfrogs feed on a wide variety of organisms, comprising mainly aquatic organisms (Hirai, 2004; Dontchev and Matsui, 2016; Sarashina and Yoshida, 2021), and they are frequently observed in wet paddy rice fields with deep water in rice paddy areas (Minowa et al., 2008). In addition, there are over 1,400 reservoirs on Sado Island for rice paddies and other



agricultural purposes (Ouchi, 2012; Ministry of Agriculture, Forestry and Fisheries of Japan, 2022). Therefore, although we did not survey rice paddies as a habitat in this study, invasive American bullfrogs may inhabit ponds near rice paddy fields in terms of food availability.

As for the vegetation cover on the surface of the pond, which was not chosen as a significant positive variable in this study by a small margin (95% CI = -0.01–1.86) (Table 1), it is often detected as a significant positive value for their pond selectivity (Bury and Whelan, 1984; Minowa et al., 2008). In previous studies, where vegetation cover was a significant positive variable in the presence of adults or eggs, the mean vegetation coverage in the ponds surveyed was greater than 30% (Minowa et al., 2008; Liu et al., 2016), and Clarkson and Devos (1986) showed that American bullfrog presence was significantly associated with 50% or more bank cover provided by emergent aquatic vegetation (reeds). On the other hand, the mean vegetation coverage was 12.7% in this study and such low vegetation coverage probably did not significantly affect bullfrog habitat selectivity.

#### *Conservation of Sado wrinkled frogs*

In our study, we confirmed that invasive American bullfrogs on Sado Island start their breeding in mid-May and oviposition in mid-July at the latest (Fig. 2), and the ponds they use for habitat are deeper ponds surrounded by rice paddy fields in areas where their potential distribution probability exceeds 50% (Table 1). Sado wrinkled frogs oviposit from June to August and metamorphose from June to August of the following year (Sekiya et al., 2012; Kobayashi, 2014). In addition, they are found in wetlands, such as rice paddy fields, biotopes, and farm ponds and are considered to be adapted to a rice paddy field environment (Kobayashi, 2014). Therefore, the breeding seasons of invasive American bullfrogs and Sado wrinkled frogs overlap, and they use a similar landscape. The overlap of the breeding season and habitat between these two species may be the reason for the negative impact of

American bullfrogs on Sado wrinkled frogs, and we strongly advocate that bullfrog eradication activity should be taken place to conserve Sado wrinkled frogs and the native ecosystem.

#### ACKNOWLEDGMENTS

We are grateful to Dr. Harue Abe, the laboratory members of Silviculture and Nature Conservation in Tsukuba University, the members of Sado Island Center for Ecological Sustainability in Niigata University, and all volunteers who helped us. We would like to thank *Editage* (www.editage.com) for English language editing. No permits were needed to conduct the field surveys in this study. This study was supported by JST SPRING [grant number JPMJSP2124] and JSPS KAKEN [grant number 22J1120].

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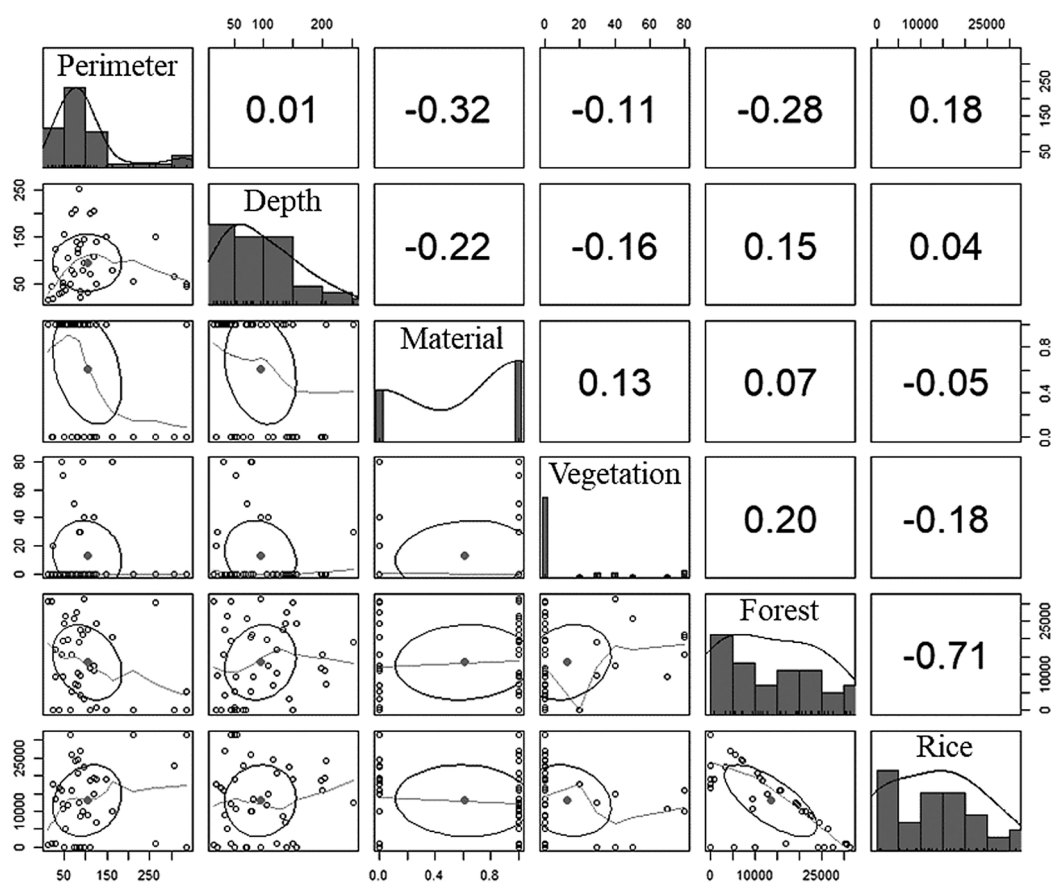
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Accepted: 4 November 2023

## APPENDIX I

Confirmation of multicollinearity among explanatory variables using Pearson's correlation coefficient (PCC). High collinearity was determined when  $PCC > 0.75$  and  $PCC < -0.75$ . The numbers shown in the upper right half of the figure indicate the correlation coefficients between the corresponding explanatory variables. The grey bars indicate the distribution of the corresponding explanatory variables. The graph in the lower left half of the figure shows the scatter plots among the corresponding explanatory variables. Details of each explanatory variables are followings: Perimeter: (m); Depth: (cm); Material: (artificial: 0, natural: 1); Vegetation: Vegetation cover on the surface of the pond (%); Forest: Forest area within 100 m from surveyed ponds ( $m^2$ ); Rice: Rice paddy area within 100 m from surveyed ponds ( $m^2$ ).



## APPENDIX II

Scatterplot showing the relationship between pond depth and rice paddy field area within 100 m from surveyed ponds.

