The Preference of Domestic Alien Toads, Bufo japonicus formosus, for Spawning Ponds on Sado Island

Authors: Sawada, Kiyoto, and Kamijo, Takashi

Source: Current Herpetology, 42(2) : 99-115

Published By: The Herpetological Society of Japan

URL: https://doi.org/10.5358/hsj.42.99

Current Herpetology 42(2): 99–115, August 2023 doi 10.5358/hsj.42.99

The Preference of Domestic Alien Toads, *Bufo japonicus formosus*, for Spawning Ponds on Sado Island

Kiyoto SAWADA[1,*] and Takashi KAMIJO[2]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Ten-nodai 1–1–1, Tsukuba, Ibaraki 305–8572, JAPAN*
[2]*Faculty of Life and Environmental Sciences, University of Tsukuba, Ten-nodai 1–1–1, Tsukuba, Ibaraki 305–8572, JAPAN*

Abstract: Although the global population of many native frog species has declined in recent years, alien frog species have spread rapidly worldwide. In order to eradicate alien frogs, characterizing their spawning sites is highly important. In recent years, eastern-Japanese common toads, *Bufo japonicus formosus*, have caused negative impacts on native ecosystems though predation and poisoning deaths as a domestic alien species. However, their spawning sites preference is little known. Here, to clarify when and what kind of ponds *B. j. formosus* prefers for spawning, we conducted a field survey on Sado Island which is one of the invaded regions of this species. We recorded their breeding season and analyzed what kind of ponds they use mainly focusing on the pond structure and landscape to obtain basic information for their eradication. Our results clarified that alien *B. j. formosus* on Sado Island have the same reproductive features as the native population according to the environmental conditions (lunar phase, wind speed, and rainless period), and the ponds they prefer for spawning are in areas with more surrounding forest and less rice paddy area regardless of pond structures. In addition, we identified that *B. j. formosus* on Sado Island currently have not yet formed large populations. These findings provide useful information for their eradication not only Sado Island but also in areas where *B. j. formosus* is distributed as an alien species. It is possibile that *B. j. formosus* on Sado Island can be eradicated completely if extermination activity is started immediately.

Key words: Breeding season; *Bufo japonicus formosus*; Domestic alien species; Sado Island; Spawning pond preference

Introduction

Although the global population of many native frog species has declined in recent years (Wake and Vredenburg, 2008; Collins, 2010; Pimm et al., 2014), alien frog species have spread rapidly worldwide (Capinha et al., 2017). They cause negative impacts on native ecosystems through predation, diseases, and poisoning deaths (e.g., Daszak et al., 2004; Shine, 2010; Sarashina and Yoshida, 2015). A study focusing on the life history traits contri-

* Corresponding author.
E-mail address: kiyoto.3816@gmail.com

buting to the invasion success of alien amphibians revealed that small body size and large clutch size promote their population establishment, and early maturity and higher introduction effort promote their population spread (Allen et al., 2017). Meanwhile, Measey et al. (2016) showed that the alien amphibian species with large body size and clutch size have significant environmental impacts. These comprehensive studies focused on invasion success and environmental impacts, respectively, and showed that large clutch size is common in invasive alien amphibians.

In general, frogs spend their larval stages in water and live on land in adult stages, but adult frogs need to migrate back to water area for breeding (Semlitsch, 2002). In addition, most frogs spend their adult stages near water bodies that serve as spawning sites (Rittenhouse and Semlitsch, 2007). Thus, characterizing their spawning sites is important in order to predict the potential habitats of alien frogs for eradicating them. In fact, many studies successfully eradicated invasive frog populations by intensively removing them from their habitat ponds (e.g., Kraus, 2009; Gray, 2009; Orchard, 2011).

Many alien species of the toad family Bufonidae Gray, 1825, such as cane toads and guttural toads, are invasive alien species that pose significant threats to native ecosystems worldwide (e.g., Mandrillon and Saglio, 2007; Shine, 2010; Ravalo et al., 2019). The global assessment of alien amphibian impacts conducted by Measey et al. (2016) showed that the toad family has relatively higher ecological and socio-economic impacts on native ecosystems than other amphibian taxa. In addition to the high predation pressure, which is common to many invasive alien frogs, alien toads cause poisonous death in upper predators due to steroid toxins stored in the parotoid glands (Letnic and Ward, 2005; Lodhi, 2018). Furthermore, tadpoles and egg masses of some toad species also possess deadly toxins (e.g., Crossland, 1999; Hayes et al., 2009; Shine, 2010). These findings imply that alien toads pose significant threats throughout their life stages, ranging from water to land.

Many species of toads known as "explosive breeders" concentrate their reproductive activity over short periods (Wells, 1977), and have a homing instinct to the pond where they were born (Sinsch, 1992). Also, a large clutch size is one of the promoters of range expansion among toads (Van Bocxlaer et al., 2010). Therefore, it would be effective to conduct extermination activities at the spawning ponds during the breeding season. For instance, on Nonsuch Island in Bermuda, extermination activities focused on spawning ponds have successfully eradicated the population of alien cane toads, *Rhinella marina* (Wingate, 2011). The preferences of toads for spawning ponds vary by species and are often characterized by the pond's structure and surrounding landscape. For example, the common toad, *Bufo bufo*, prefers permanent ponds, while the natterjack toad, *Epidalea calamita*, and European green toad, *Bufotes viridis*, prefers ephemeral ponds in Europe (Buskirk, 2003). In comparison, alien cane toads prefer ponds located in open areas with gently sloping banks and shallow water in the different climate regions in Australia (Semeniuk et al., 2007). Moreover, several factors have been found to be associated with the onset of breeding in some toad species, explosive breeders, such as precipitation, temperature and lunar phase (e.g., Vaira, 2005; Arnfield et al., 2012). Therefore, it is necessary to understand the breeding season and pattern, and spawning ponds preferences of each alien toad species for effective eradication.

In July 1963, three domestic alien toads (unknown sex), *Bufo japonicus formosus*, were introduced into pond at a temple called Kouninji, in the southwestern part of the Sado Island, Japan from the former Yunotani village (now Uonuma city), Niigata Prefecture, Japan (Nakamura, 1966). The following year, in March 1964, an additional 14 male and four female toads were introduced into the same pond from Sendai city, Miyagi Prefecture, Japan (Nakamura, 1966). Although *B. j. formosus* had already been introduced before 1963 in 1922 and 1961, it has been reported that these introductions did not lead to the establishment of populations (Okada, 1930; Iwasawa, 1960; Nakamura, 1966). Currently, they are distributed within 10 km from the point of introduction (Sawada et al., 2022). According to the species distribution model, there are concerns about their further expansion (Sawada et al., 2022). However, there have been no reports of the *B. j. formosus* eradication activities until now. On Sado Island, there is an abundance of ground beetles (Baba, 1963), which are the main prey taxa of *B. j. formosus* (Sarashina and Yoshida, 2015). Furthermore, an endangered species, the Japanese crested ibis, *Nipponia nippon*, which mainly eats frogs (Li et al., 2002), and the rare endemic species the Sado wrinkled frog, *Glandirana susurra*, which could be a potential competitor of *B. j. formosus* also live on Sado Island (Sado City, 2012). The negative impacts of *B. j. formosus* on the native ecosystems on Sado Island have not yet been reported. However, they will likely cause significant negative impacts on their potential preys, predators, and competitors. In other areas where *B. j. formosus* has invaded as a domestic alien species, there has been a serious impact on native ecosystems. For example, in Hokkaido, high predation pressure, mainly among insects (Sarashina and Yoshida, 2015), and poisonous death of endemic predators have been reported (Kazila and Kishida, 2019; Oyake et al., 2020). Recently, Okamiya et al. (2021) found that the egg of *B. j. formosus* also possesses toxins. On Izu Islands, they have been introduced on Oh-shima Island, Miyake-jima Island, Nii-jima Island, and Hachijo-jima Island (Goto and Iwasaki, 2012; National Institute for Environmental Studies, 2022). Particularly, on Hachijo-jima Island, the local media has reported large number of *B. j. formosus* (Goto and Iwasaki, 2012). Although many studies focus on the reproductive traits of *B. j. formosus* and its subspecies *Bufo japonicus japonicus*, such as breeding season, movement, and population fluctuation (e.g., Okuno, 1973; Kusano et al., 2015; Okamiya and Kusano, 2018), only a few studies report on their spawning pond preference (e.g., Ishikuro and Iwai, 2018; Zheng et al., 2021), which is important information for their eradication.

Bufo japonicus tadpoles metamorphose into very small froglets, less than 1 cm (Okuno, 1984), and adults spend most of their life on land except during breeding season (Matsui, 2021). Therefore, we hypothesized that their spawning ponds tend to be in the forests and shallow wetlands to prevent juvenile desiccation and adult drowning. In fact, fatal cases of juvenile desiccation and adult drownings of toads have been reported in their native regions (e.g., Miyamae and Matsui, 1979; Shinohara, 1992).

To clarify when and what kind of ponds *B. j. formosus* prefers for spawning in invaded areas, we conducted a field survey on Sado Island, where negative impacts on native ecosystems by alien *B. j. formosus* are feared in the future. We recorded their breeding season and analyzed what kind of ponds they use mainly focusing on the pond structure and landscape to obtain basic information for their eradication.

Materials and Methods

Study site

This study was conducted on Sado Island (Sado City, Niigata Prefecture, Japan) (see Fig. 1), which is divided into three main areas: the Osado Mountains in the northern part of the island (maximum elevation 1,172 m), the Kosado Mountains in the southern part of the island (maximum elevation 645 m), and the Kuninaka Plain between the two mountain ranges (Sado City, 2012). Sado Island is located off the western coast of Niigata Prefecture, with an area of 855.25 km^2, annual precipitation of 1,785 mm, annual mean temperature of 13.6°C (Sado City, 2012, 2021).

Surveys

Field surveys were conducted nine times for a total of 29 days between March and July 2020 (see Table 1) because previous studies conducted in Niigata and Nagaoka city in Nii-

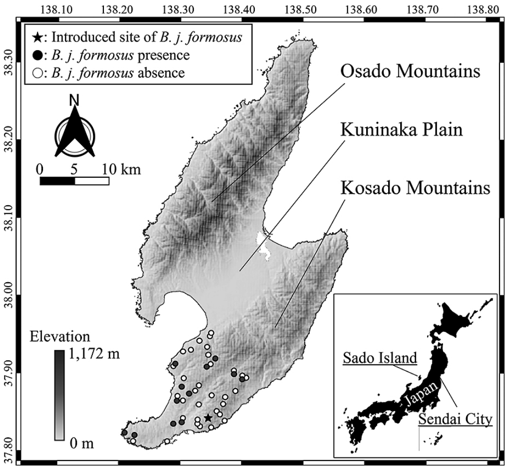


FIG. 1. The elevation map of Sado Island and field survey sites. The star and circles indicate the locations where the pond surveys were conducted. The star indicates the Kouninji where *Bufo japonicus formosus* were introduced. White circles indicate sites where no *B. j. formosus* were found, and black circles and a star indicate sites where *B. j. formosus* were found. Sendai City is the origin site of the alien *B. j. formosus* on Sado Island (Nakamura, 1966). Elevation data were obtained from the digital national land information (Ministry of Land, Infrastructure, Transport and Tourism, 2009) and compiled on Geographic Information System.

gata Prefecture reported early April to early May as the start of the *B. j. formosus* breeding season (Iwasawa and Miyazaki, 1984; Nakano, 2001). Surveyed ponds were selected to include a variety of environments and to be at least 200 m apart from each other because *B. j. formosus* is known to have an average home range of 20 m to 100 m (Kusano et al., 1995; Okuno, 1995; Okamiya and Kusano, 2018). Here, we defined a permanent water body with less than 8 m depth as a pond, referring to the studies by Oertli et al. (2000) and Biggs et al. (2005). We focused on ponds in the southwestern part of the island based on the previous study which confirmed that *B. j. formosus* was only distributed in that area (Sawada et al., 2022), and selected 45 ponds for the analysis (Fig. 1). All 45 pond field surveys were completed in March and April, and were monitored in May and June to ensure the presence or absence of *B. j. formosus*. Moreover, the ponds where *B. j. formosus* were found were visited during every survey after their initial observation. Alien *B. j. formosus* found during the survey were captured whenever possible and euthanized by freezing approximately –10°C (Lillywhite et al., 2017), and all egg masses we found were collected from the pond and disposed of so they would not hatch.

During the field surveys, we recorded the presence or absence of *B. j. formosus* and their life stages, and period of pond use of *B. j. formosus*. Toad presence data were recorded with three stages information (adults, tadpoles, or egg masses). To collect pond structure data, the pond's perimeter (m), depth (cm), material (artificial or natural), and vegetation cover on the surface of the pond (%) were measured on site. As for pond material, ponds with rubber or concrete bottoms were considered artificial material ponds, and ponds with gravel or soil bottoms were considered natural material ponds. Most ponds on Sado Island are artificial (approximately 1,400) (Ouchi, 2012), and therefore it was difficult for us to find and distinguish natural ponds. After the field survey, to examine the relationship between weather conditions and the timing of the *B. j. formosus* breeding initiation, weather data was obtained from the Japan Meteorological Agency (2020) and compared with previous studies. Also, the forest and rice paddy area (m^2) within a 100 m riparian buffer of each pond was calculated to collect landscape data. The size of this buffer was determined with reference to the average home range of *B. j. formosus* being 100 m (Kusano et al., 1995; Okamiya and Kusano, 2018). The forest and rice paddy area data were calculated using a QGIS version 3.6.0 (QGIS Development Team, 2019) based on the vegetation map data (Ministry of the Environment, 1999).

Statistical analysis

All statistical analyses were performed using RStudio Desktop 1.3.1093 (RStudio Team,

TABLE 1. Seasonal changes in pond use by *Bufo japonicus formosus*. Numbers in parentheses indicate the number of adult females (F) and males (M) captured. The alien *B. j. formosus* found during the survey were captured whenever possible and euthanized by freezing approximately –10°C, and all egg masses we found were collected from the pond and disposed of so they would not hatch.

Survey span		Observed number		
Start date	End date	Adult	Egg mass	Tadpoles*
11 Mar.	12 Mar.	12 (M: 10/F: 1/Uncaptured: 1)		
24 Mar.	26 Mar.	9 (M: 3/F: 4/Uncaptured: 2)	6	
8 Apr.	9 Apr.		6	1
20 Apr.	22 Apr.			1
18 May	20 May			
1 Jun.	3 Jun.			
15 Jun.	18 Jun.			
28 Jun.	2 Jul.			
19 Jul.	22 Jul.			

*: 1 tadpole means 1 clutch size individual visually.

2021). To clarify the pond preference of alien *B. j. formosus* on Sado Island, we examined the effect of explanatory variables on response variable using the generalized linear model (GLM). The response variable was the presence or absence of *B. j. formosus* (absence: 0; presence: 1). The explanatory variables were the pond's 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). To avoid multicollinearity between explanatory variables, Pearson's correlation coefficient (PCC) and variance inflation factor (VIF) were calculated (Appendixes I and II) using the package 'psych' (Revelle, 2022) and 'car' (Fox et al., 2022), respectively. Referring to the studies by Wen et al. (2015) and Alin (2010), we determined high collinearity when PCC was greater than 0.75 or less than –0.75 and when VIF was greater than 10. GLM was performed with binomial distribution and logit link function using RStudio's default function 'glm' (RStudio Team, 2021). The correlation strength between the response variable and each explanatory variable was evaluated using Wald statistics (z-value). We determined the explanatory variable to be significant for the response variable if the P-value calculated from the z-value was less than 0.05. Akaike's information criterion (AIC; Akaike, 1973) was used to select the best model. The set of models were generated with all possible combinations of the explanatory variables using the package 'MuMIn' (Bartoń, 2022). The model with the smallest AIC was selected as the best model and Model 1. The models with differences of 2 or less from the AIC of Model 1 (ΔAIC$\leq$2) were also selected as the best models and Model numbers were assigned in descending order of ΔAIC values. In addition, to evaluate the most important explanatory variables in them, model averaging was performed on the best models using the package 'MuMIn' (Bartoń, 2022).

Finally, after the GLM analysis, box-and-whisker plots were created for each significant explanatory variable, with and without *B. j. formosus*, to understand its specific characteristics.

RESULTS

Field survey

Bufo japonicus formosus were observed in 13 of the 45 ponds surveyed (Fig. 1; Appendix

TABLE 2. Detailed records of ponds where *Bufo japonicus formosus* were observed. The Kouninji is the introduced site of *B. j. formosus* (Nakamura, 1966). Numbers in parentheses indicate the number of adult females (F) and males (M) captured.

Distance from the Kouninji (km)	Observed number		
	Adult	Egg mass	Tadpoles*
0	15 (M:10/F:5)	3	0
3.3	1 (M:1/F:0)	0	0
3.6	2 (Uncaptured)	0	0
4.3	0	1	0
4.6	0	0	1
5	1 (M:1/F:0)	0	0
7.2	0	1	0
7.4	0	1	0
7.6	0	1	0
8.3	1 (Uncaptured)	0	0
8.6	0	3	0
10.5	0	2	0
11.8	1 (M:1/F:0)	0	0
Total	21 (M: 13/F: 5/Uncaptured: 3)	12	1

*: 1 tadpole means 1 clutch size individual visually.

III). They were observed in the ponds between 11 March and 22 April 2020, with adults observed between 11 March and 26 March, egg masses between 24 March and 9 April, and tadpoles from 8 April to 22 April (Table 1). The highest numbers of *B. j. formosus*, i.e., 15 adults (10 males and five females) and three egg masses (Table 2), were observed in the pond at Kuoninji where they were introduced; the number of adults observed from 11 March to 12 March was nine males and one female and from 24 March to 26 March, one male and four females. For the other 12 ponds, no correlation between distance from the Kouninji and the number of observed *B. j. formosus* was found (Adult: PCC=–0.61, Egg mass: PCC= –0.06, Tadpoles: PCC=–0.16) (Table 2).

Spawning pond preference

All explanatory variables were included for the GLM as a result of PCC and VIF evaluation, and seven GLMs were selected as the best models using AIC best model selection (Table 3). In the best models, there were no significant relationships between the *B. j. formosus* presence and pond structure variables (Table 3). On the other hand, the most frequently used explanatory variable was forest area, which was used in six of the seven best models (Table 3).

Forest area was significantly positively correlated with the presence of *B. j. formosus* in all models including forest area except model 5 (Table 3). The values for the mean±standard deviation (SD) of forest area for ponds with and without *B. j. formosus* were 22,010± 7,960 m^2 and 15,220±8,730 m^2, respectively, and *B. j. formosus* were never observed at the ponds with less than 5,346 m^2 (≈15%; for a buffer area of 31,400 m^2 with a radius of 100 m) of forest area (Fig. 2A).

In Model 2, the rice paddy area was significantly negatively correlated with the presence of *B. j. formosus* (Table 3). The values for the mean±SD of the rice paddy area for the ponds with and without *B. j. formosus* were

TABLE 3. Results of GLMs estimation of pond characteristics that determine the presence or absence of *Bufo japonicus formosus*. Coefficients for models where the ΔAIC was within two are shown. Asterisks (*) denote coefficients for which the P-value from the Wald test was less than 0.05. Details of each explanatory variables are followings: Perimeter: (m); Depth: (cm); Material: (artificial: 0, natural: 1); Plant: Vegetation cover on the surface of the pond (%); Forest: Forest area within 100 m from survey ponds (m^2); Rice: Rice paddy area within 100 m from survey ponds (m^2).

Model	(Intrc)	Perimeter	Depth	Material	Plant	Forest	Rice	ΔAIC
1	–2.657					**0.094***		0.0
2	–0.073						**–0.087***	1.2
3	–2.845				0.010	**0.095***		1.4
4	–3.051			0.559		**0.091***		1.6
5	–1.842					0.068	–0.035	1.8
6	–2.714		4.0×10^{-4}			**0.095***		2.0
7	–2.672	1.0×10^{-4}				**0.094***		2.0

6,740±7,320 m^2 and 12,370±8,280 m^2, respectively, and excluding the one outlier, *B. j. formosus* were never observed in the ponds with more than 12,781 m^2 (≈40%; for a buffer area of 31,400 m^2 with a radius of 100 m) of rice paddy area (Fig. 2B).

The results of averaging the seven best models showed that the explanatory variables could be ranked in the following order according to their importance: forest area, rice paddy area, vegetation cover, material, depth, and perimeter (Table 4). In particular, forest area was remarkably important compared to the other explanatory variables (Table 4).

DISCUSSION

Invasion state of B. j. formosus

The nine-year monitoring study of Okuno (1973), focused on the native *B. j. japonicus* until its decline, reported that the largest breeding population included 209 adults in one year in a pond, and the smallest breeding population included 18 adults in a pond. In addition, on Hachijo-jima Island and Hokkaido, where *B. j. formosus* are invasive, 1,471 and 3,079 adults were captured at one site during a year (Goto and Iwasaki, 2012; Tokuda, 2021). Therefore, it is highly likely that alien *B. j. formosus* on Sado Island have not yet formed large populations, since the maximum number of adult *B. j. formosus* observed in one pond was 15 in this study (Table 2).

However, there has been a steady expansion of *B. j. formosus* populations on Sado Island; as of 2020, 56 years after the invasion, it was estimated that the expansion has reached areas approximately 10 km from the point of introduction (Sawada et al., 2022). In addition, Sado Island is included in both the latitude and longitude of the Japanese toads' natural distribution area (Matsui, 2021), and Sawada et al. (2022) predicted that the *B. j. formosus* populations will expand into the south-eastern part of the Island along the Kosado Mountains in the near future. Hence, although the *B. j. formosus* population on Sado Island may be suppressed by non-climatic factors, such as abiotic and topographic conditions, it is undeniable that it will not increase in the future.

Breeding season

In the pond at the Kouninji, where the highest number of *B. j. formosus* was observed, we found that females appeared more frequently in the later stages of the breeding season (Table 1). Based on Okuno (1973), which showed that the peak breeding period is between the first and second days when many female toads appear in the pond, in our study, end of March may have been the peak breeding season.

Kusano et al. (2015) showed that lunar

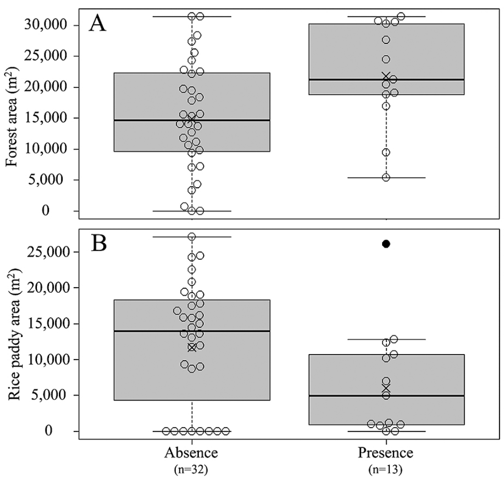


FIG. 2. Relationship between the presence or absence of *Bufo japonicus formosus* and the area of forest and rice field within 100 m from the ponds. Box-and-whisker plots and mean value of the forest area (A) and rice paddy area (B) within 100 m of the ponds. The values for the mean±standard deviation (SD) of forest area for ponds with and without *B. j. formosus* were 22,010±7,960 m^2 and 15,220±8,730 m^2, respectively, and those of the rice paddy area were 6,740±7,320 m^2 and 12,370±8,280 m^2, respectively. The horizontal lines represent (from the top) the maximum, the third quartile, the median, the first quartile and the minimum. The cross mark indicates the average value. The shaded box represents the middle 50% of the distribution (between the first and third quartiles). The circles indicate each raw data, and a black circle indicates an outlier.

phase significantly influences the reproductive activity of *B. j. formosus*, which increases as the new moon approaches, even after taking into account the effects of weather conditions. During study, 24 March 2020 was the new moon on Sado Island (Japan Meteorological Agency, 2020). Assuming the peak breeding period was end of March, the main activating factor of reproductive behavior for alien *B. j. formosus* on Sado Island would be the new moon, similar to native populations.

In addition, Kusano et al. (2015) showed that the breeding activity of *B. j. formosus* increases when daily mean wind speed becomes stronger and the rainless period lasts longer, although they are not as influential as the lunar phases. On Sado Island, the daily mean wind speed in March 2020 was 2.3 m/s, whereas that on 11 March was 3.1 m/s, making it the second strongest day between 1 and 11 March (Japan Meteorological Agency, 2020). Incidentally, maximum instantaneous wind speed of 7.0 m/s on 11 March was the strongest between 1 and 11 March (Japan Meteorological Agency, 2020). Furthermore, the longest rainless period in March 2020 lasted three days from 7 to 9 March, while the last time there were three or more days without rain before this date was three days from 25 to 27 January (Japan Meteorological Agency, 2020). These results confirmed that the breeding season activity of alien *B. j. formosus* on Sado Island corresponds to that of populations living in their native habitat.

Spawning pond preference

In terms of landscape characteristics, the forest area within 100 m of the pond was significantly positively correlated with the presence of *B. j. formosus* (Table 3). Zheng et al. (2021), who conducted a study in an urban-to-rural gradient area, showed that *B. j. formosus* prefer to spawn in forested areas within 100 m of the study site at the landscape level, as they did in this study. Moreover, at the pond structure level, shallow water depth, greater cover of aquatic vegetation, and increased water area were deemed to be important variables to influence on the number of *B. j. formosus* egg masses (Zheng et al., 2021). Furthermore, as our study focused only on permanent ponds, our results may differ from those of Zheng et al. (2021) as they only focused on paddy fields (including abandoned ones). As for forest area, it has been demonstrated that adult *B. j. formosus* prefer closed canopies as their habitats during the non-breeding season because they use them to retain body water (Okamiya and Kusano, 2018). In addition, forests are essential ecosystems for tadpoles and frog juveniles as they help avoid desiccation (Osawa and Katsuno, 2001a; Rothermel and Semlitsch,

TABLE 4. Model averaging parameter estimates of standardized partial regression coefficients. The predictors are sorted by the relative importance. Model averaging was performed from the seven best models shown in Table 3. The relative importance for a predictor is the sum of Akaike weights of the models in which the predictor was present, referring to Kusano et al. (2015). Details of each explanatory variables are followings: Perimeter: (m); Depth: (cm); Material: (artificial: 0, natural: 1); Plant: Vegetation cover on the surface of the pond (%); Forest: Forest area within 100 m from survey ponds (m^2); Rice: Rice paddy area within 100 m from survey ponds (m^2).

Valuables	Estimate	Stdard Error	Relative importance
Forest	0.090	0.048	1.00
Rice	–0.065	0.061	0.45
Plant	0.010	0.012	0.27
Material	0.559	0.916	0.18
Depth	4.0×10^{-4}	0.006	0.00
Perimeter	2.0×10^{-4}	0.011	0.00

2002); with this in mind, and considering that *B. japonicus* tadpoles metamorphose into especially small froglets measuring less than 1 cm (Okuno, 1984), one could easily assume that a pond with landscape structures that help block sunlight, such as a forest, is indispensable for preventing desiccation, which was one of our initial hypotheses.

Rice paddy fields are important spawning sites for frogs in Japan, such as the Japanese tree frog, *Dryophytes japonica*, and Japanese brown frog, *Rana japonica* (e.g., Osawa, 2008; Watabe et al., 2021). While toads have often been seen in wetlands near rice paddy fields (e.g., Miyamae and Matsui, 1979; Kusano et al., 1995; Fukuyama et al., 2007), there is little evidence showing that they use rice paddy area for spawning (e.g., Zheng et al., 2021); besides, our results showed a negative correlation between rice paddy area and presence of *B. j. formosus* (Table 3). It is generally believed that organisms in the same taxonomic group avoid interspecific competition with the temporal and spatial division of reproductive resources (Crump, 1971; Maiorana, 1976), which has often been observed in frogs as well (e.g., Donnelly and Guyer, 1994; Gillespie et al., 2004). On Sado Island, the montane brown frog, *Rana ornativentris*, has their breeding season in early to late March (Kato et al., 2010; Uruma et al., 2012), sharing the breeding season with *B. j. formosus* (Table 1), and they are generally found in forests and grasslands and mainly use stagnant water such as rice paddy fields as its spawning site (Osawa and Katsuno, 2001b). Therefore, alien *B. j. formosus* may avoid rice paddy fields used by *R. ornativentris* for spawning.

Measures for eradication

We confirmed that alien *B. j. formosus* on Sado Island have the same reproductive requirements as the native population based on environmental conditions (lunar phase, wind speed, and rainless period: Table 1), and the ponds they prefer for spawning are in areas with more surrounding forest and less rice paddy area regardless of the pond structure (Fig. 2; Table 3). Moreover, we identified that the population on Sado Island is currently very small (Table 2). Early countermeasures are the most effective way to control the impact of alien species and reduce the eradication cost, time, and efforts (e.g., Simberloff et al., 2013; Russell et al., 2017). For these reasons, extermination activities on Sado Island should be conducted immediately before a large population forms. Numerous studies have demonstrated the effectiveness of intensive eradication efforts focused on spawning ponds during the breeding season (e.g., Wingate, 2011). Such measures have also successfully captured many alien toads in the short term on Hokkaido and Hachijo-jima Island, where *B. j. formosus* has invaded as a domestic alien species (Goto and Iwasaki, 2012; Naito and Shiga, 2016). *Bufo j. japopnicus* spawns in ponds as well as small water bodies such as puddles, but the ponds become large breeding sites (Kuwabara et al., 2019). To implement the eradication activities more efficiently, it is important to select priority management ponds and seasons based on the results of this study and the study of potential distribution areas by Sawada et al. (2022). We consider it a top priority to eradicate *B. j. formosus* in the 13 ponds where they were found and ponds in forests within their potential distribution areas need attention. In the ponds where *B. j. formosus* are particularly abundant, it should be necessary to consider installing traps for mass capture of toads, such as the drift fence with large pit-falls trap (Suzuki et al., 2018) that prevent toads from getting out once they enter the pond (Tokuda, 2021) minimizing the impact of traps on native species.

The Australian invasive frog, the cane toad, has a similar preference for spawning ponds even in different climatic regions (Semeniuk et al., 2007). Therefore, it is highly likely that *B. j. formosus* will show similar spawning pond preferences in Hokkaido and the Izu Islands as they did on Sado Island, and the results of this study will provide useful information for eradication activities not only on Sado Island but also in areas where *B. j. formosus* is distributed as an alien species.

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. This work was supported by JST SPRING [grant number JPMJSP2124] and JSPS KAKEN [grant number 22J1120].

Literature Cited

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. p. 267–281. *In*: B. N. Petrov and F. Csaki (eds.), *Second International Symposium on Information Theory*. Akadémiai Kiadó, Budapest.

Alin, A. 2010. Multicollinearity. *Wiley Interdisciplinary Reviews: Computational Statistics* 2: 370–374.

Allen, W. L., Street S. E., and Capellini, I. 2017. Fast life history traits promote invasion success in amphibians and reptiles. *Ecology Letters* 20: 222–230.

Arnfield, H., Grant, R., Monk, C., and Uller, T. 2012. Factors influencing the timing of spring migration in common toads (*Bufo bufo*). *Journal of Zoology* 288: 112–118.

Baba, K. 1963. A review on the insects of Sado Island. *Bulletin of the Sado Museum* 5: 1–11.

Bartoń, K. 2022. *MuMIn: Multi-Model Inference. Version 1.46.0*. Available via https://CRAN.R-project.org/package=MuMIn

Biggs, J., Williams, P., Whitfield, M., Nicolet, P., and Weatherby, A. 2005. 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15: 693–714.

Buskirk, J. V. 2003. Habitat partitioning in European and North American pond-breeding frogs and toads. *Diversity and Distributions* 9: 399–410.

Capinha, C., Seebens, H., Cassey, P., García–Díaz, P., Lenzner, B., Mang, T., Moser, D., Pyšek, P., Rödder, D., Scalera, R., Winter, M., Dullinger, S., and Essl, F. 2017. Diversity, biogeography and the global flows of alien amphibians and reptiles. *Diversity and Distributions* 23: 1313–1322.

Collins, J. P. 2010. Amphibian decline and extinction: what we know and what we need to learn. *Diseases of Aquatic Organisms* 92: 93–99.

Crossland, M. R. and Azevedo-Ramos, C. 1999. Effects of *Bufo* (Anura: Bufonidae) toxins on tadpoles from native and exotic *Bufo* habitats. *Herpetologica* 55: 192–199.

Crump, R. G. 1971. Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. *Journal of Experimental Marine Biology and Ecology* 7: 137–162.

Daszak, P., Strieby, A., Cunningham, A. A., Longcore, J. E., Brown, C. C., and Porter, D. 2004. Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *Herpetological Journal* 14: 201–208.

Donnelly, M. A. and Guyer, C. 1994. Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* 98: 291–302.

Fox, J., Weisberg, S., Price, B., Adler, D., Bates, D., Baud-Bovy, G., Bolker, B., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., Graves, S., Heiberger, R., Krivitsky, P., Laboissiere, R., Maechler, M., Monette, G., Murdoch, D., Nilsson, H., Ogle, D., Ripley, B., Venables, W., Walker, S., Winsemius, D., Zeileis, A., and R-Core. 2022. *Car: Companion to Applied Regression. Version 2.25*. Available via https://CRAN.R-project.org/package=car

Fukuyama, K., Abe, M., Matsuda, H., and Sasaki, F. 2007. Monitoring of long term trends in *Rana ornativentris* and *Bufo japonicus formosus* in the Segami Valley, Yokohama, Japan. *Bulletin of the Herpetological Society of Japan* 2: 146–153.

Gillespie, G. R., Lockie, D., Scroggie, M. P., and Iskandar, D. T. 2004. Habitat use by stream-breeding frogs in south-east Sulawesi, with some preliminary observations on community organization. *Journal of Tropical Ecology* 20: 439–448.

Goto, Y. and Iwasaki, Y. 2012. The eradication of *Bufo japonicus formosus* on Hachijo Island in 2012. *Bulletin of the Herpetological Society of Japan* 2: 112–114.

Gray, I. 2009. Breeding pond dispersal of interacting California red-legged frogs (*Rana draytonii*) and American bullfrogs (*Lithobates catesbeianus*) of California: A mathematical model with management strategies. *Unpublished master's thesis*. Humboldt State University, California.

Hayes, R. A., Crossland, M. R., Hagman, M., Capon, R. J., and Shine, R. 2009. Ontogenetic variation in the chemical defenses of cane toads (*Bufo marinus*): toxin profiles and effects on predators. *Journal of Chemical Ecology* 35: 391–399.

Ishikuro, H. and Iwai, N. 2018. Effects of noise and light on breeding and oviposition site selection by frogs in urban ponds. *Japanese Journal of Conservation Ecology* 23: 177–186.

Iwasawa, H. 1960. Memorandums on amphibia of Sado and a list of vertebrata of Sado Island. *Bulletin of the Sado Museum* 4: 21–25.

Iwasawa, H. and Miyazaki, H. 1984. Breeding emergence of the toad *Bufo japonicus formosus* in Donchi-ike, Niigata city, in the delayed spring of 1984. *Japanese Journal of Herpetology* 10: 104–106.

Japan Meteorological Agency. 2020. *Historical Weather Data Search*. https://www.data.jma.go.jp/obd/stats/etrn/index.php?prec_no=45&block_no=47682 (accessed 12 April 2022)

Kato, N., Yoshio, M., Kobayashi, R., and Miyashita, T. 2010. Differential responses of two anuran species breeding in rice fields to landscape composition and spatial scale. *Wetlands* 30: 1171–1179.

Kazila, E. and Kishida, O. 2019. Foraging traits of native predators determine their vulnerability to a toxic alien prey. *Freshwater Biology* 64: 56–70.

Kraus, F. 2009. Management Responses. p. 95–110. *In*: F. Kraus (ed.), *Alien Reptiles and Amphibians: A Scientific Compendium and Analysis*. Springer, Dordrecht.

Kusano, T., Maruyama, K., and Kaneko, S. 1995. Post-breeding dispersal of the Japanese toad, *Bufo japonicus formosus*. *Journal of Herpetology* 29: 633–638.

Kusano, T., Miura, T., Terui, S., and Maruyama, K. 2015. Factors affecting the breeding activity of the Japanese common toad, *Bufo japonicus formosus* (Amphibia: Bufonidae) with special reference to the lunar cycle. *Current Herpetology* 34: 101–111.

Kuwabara, K., Itou, A., Kano, T., Okuda, M., Kakuya, E., Oka, S., Matsukawa, J., Hirakawa, S., Morioka, H., and Doi, T. 2019. Disappearance of egg-laying sites of Western-Japanese common toad, *Bufo japonicus japonicus* in Ohnan-cho, Shimane prefecture. p. 23–30. *In*: A. Iwasawa, M. Kumakura, A. Sasaki, N. Sato, M. Sano, B. Takayama, H. Takahashi, T. Nomura, Y. Hasegawa, and H. Fujita (eds.), *Enhanted by* *the Amphibians*. Nippon Batrachological Society, Niigata.

Letnic, M. and Ward, S. 2005. Observation of freshwater crocodiles (*Crocodylus johnstoni*) preying upon cane toads (*Bufo marinus*) in the Northern Territory. *Herpetofauna* 35: 98–100.

Li, X., Li, D., Li, Y., Ma, Z., and Zhai, T. 2002. Habitat evaluation for crested ibis: a GIS-based approach. *Ecological Research* 17: 565–573.

Lillywhite, H. B., Shine, R., Jacobson, E., DeNardo, D. F., Gordon, M. S., Navas, C. A., Wang, T., Seymour, R. S., Storey, K. B., Heatwole, H., Heard, D., Brattstrom, B., and Burghardt, G. M. 2017. Anesthesia and euthanasia of amphibians and reptiles used in scientific research: should hypothermia and freezing be prohibited? *Bioscience* 67: 53–61.

Lodhi, F. L. 2018. A fatal case of toad (*Bufo melanostictus*) intoxication in a german shepherd dog and review of literature. *Advances in Animal and Veterinary Sciences* 6: 156–160.

Maiorana, V. C. 1976. Reproductive value, prudent predators, and group selection. *The American Naturalist* 110: 486–489.

Mandrillon, A. L. and Saglio, P. 2007. Herbicide exposure affects the chemical recognition of a non native predator in common toad tadpoles (*Bufo bufo*). *Chemoecology* 17: 31–36.

Matsui, M. 2021. Bufonidae. p. 37–42. *In*: Herpetological Society of Japan (ed.), *Amphibians and Reptiles of Japan*. Sunrise Publishing, Hikone.

Measey, G. J., Vimercati, G., De Villiers, F. A., Mokhatla, M., Davies, S. J., Thorp, C. J., Rebelo, A. D., and Kumschick, S. 2016. A global assessment of alien amphibian impacts in a formal framework. *Diversity and Distributions* 22: 970–981.

Ministry of Land, Infrastructure, Transport and Tourism. 2009. *Digital national land information, Elevation and Slope mesh data.* Available via https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-G04-d.html

Ministry of the Environment. 1999. *Vegetation survey 2nd–5th, Niigata Prefecture.* Available via http://www.biodic.go.jp/trialSystem/EN/vg/vg.html

Miyamae, M. and Matsui, M. 1979. Larval growth and development of the Japanese toad, *Bufo bufo formosus*, at Iwakura, Kyoto. *Contributions from the Biological Laboratory, Kyoto University* 25: 273–294.

Naito, H. and Shiga, K. 2016. Immigrant of *Bufo japonicus* into the left bank region of Ishikari estuary, Hokkaido, Japan. *Bulletin of Ishikari Sakyu no kaze Museum* 6: 65–70.

Nakamura, Y. 1966. Invasion of *Rana nigromaculata* and *Bufo japonicus formosus* on Sado Island. *Bulletin of the Sado Museum* 2: 10–11.

Nakano, M. 2001. Spawning behavior of Azuma toads in the Nagaoka Higashiyama area and the influence of valley stops. *Journal of the Niigata Society of Engineering Geology* 56: 49–56.

National institute for Environmental Studies. 2022. *Invasive Species of Japan*. https://www.nies.go.jp/biodiversity/invasive/DB/etoc4_amphibians.html (accessed 12 April 2022)

Oertli, B., Joye, D., Castella, E., Juge, R., and Lachavanne, J. B. 2000. Diversité biologique et typologie écologique des étangs et petits lacs de Suisse. OFEFP, University of Geneva, Geneva. Available via http://archive-ouverte.unige.ch/unige:26123

Okada, Y. 1930. Topics: Sado Island. p. 209–211. *In*: Y. Okada (ed.), *Japanese Frog Review*. Iwanami Shoten. Tokyo.

Okamiya, H. and Kusano, T. 2018. Evaluating movement patterns and microhabitat selection of the Japanese common toad (*Bufo japonicus formosus*) using fluorescent powder tracking. *Zoological Science* 35: 153–160.

Okamiya, H., Takai, K., and Kishida, O. 2021. Japanese common toad, *Bufo japonicus formosus*, contains venom in the egg stage. *Current Herpetology* 40: 103–106.

Okuno, R. 1973. The records of the Japanese toad, *Bufo japonicus japonicus*, in Kanazawa castle. *Annual report of Botanic Garden, Faculty of Science, Kanazawa University* 11: 5–17.

Okuno, R. 1984. Studies on the natural history of the Japanese toad, *Bufo japonicus japonicus*: IV. Post-metamorphic growth and age of maturity. *Japanese Journal of Ecology* 34: 445–455.

Okuno, R. 1995. *The Records of the Japanese Toad*, Bufo japonicus japonicus, *in Kanazawa Castle*. Doubutsusya, Tokyo.

Orchard, S. A. 2011. Removal of the American bullfrog, *Rana* (*Lithobates*) *catesbeiana*, from a pond and a lake on Vancouver Island, British Columbia, Canada. p. 217–221. *In*: C. R. Veitch, M. N. Clout, and D. R. Towns (eds.), *Island Invasives: Eradication and Management*. IUCN, Gland.

Osawa, S. 2008. The spawning of *Bufo japonicus formosus* and *Rana ornativentris* from 1997 to 2001 in the Segami-Yato valley, southern Tama-Hills. *Bulletin of the Herpetological Society of Japan* 1: 10–12.

Osawa, S. and Katsuno, T. 2001a. Dispersal of brown frogs *Rana japonica* and *R. ornativentris* in the forests of the Tama Hills. *Current Herpetology* 20: 1–10.

Osawa, S. and Katsuno, T. 2001b. The relationship between habitats of brown frogs and floor conditions in hill's woodland. *Landscape Research Japan* 64: 611–616.

Ouchi, H. 2012. A report on the "6th Tameike Symposium in Sado Island". *Nature of Irrigation Pond* 52: 18–22.

Oyake, N., Sasaki, N., Yamaguchi, A., Fujita, H., Tagami, M., Ikeya, K., Tagami, M., Kobayashi, M., Abe, H., and Kishida, O. 2020. Comparison of susceptibility to a toxic alien toad (*Bufo japonicus formosus*) between predators in its native and invaded ranges. *Freshwater Biology* 65: 240–252.

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., and Sexton, J. O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344: 1246752.

QGIS Development Team. 2019. *QGIS geographic information system. Open Source Geospatial Foundation.* Available via https://qgis.org/en/site/forusers/download.html

Ravalo, D. D., Gersava, J. R., Alojado, J., Achondo, M. J. M., and Gamalo, L. E. 2019. Predation of Samar Cobra *Naja samarensis* Peters, 1861 on the invasive Cane Toad *Rhinella marina* (Linnaeus, 1758) in Davao City, Philippines. *Herpetology Notes* 12: 1023–1025.

Revelle, W. 2022. *psych: Procedures for Psychological, Psychometric, and Personality Research. Version 2.2.5.* Available via https://CRAN.R-project.org/package=psych

Rittenhouse, T. A. and Semlitsch, R. D. 2007. Distribution of amphibians in terrestrial habitat surrounding wetlands. *Wetlands* 27: 153–161.

Rothermel, B. B. and Semlitsch, R. D. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology* 16: 1324–1332.

RStudio Team. 2021. *RStudio: Integrated Development for R*. RStudio, PBC, Boston. Available via http://www.rstudio.com/

Russell, J. C., Meyer, J. Y., Holmes, N. D., and Pagad, S. 2017. Invasive alien species on Islands: impacts, distribution, interactions and management. *Environmental Conservation* 44: 359–370.

Sado city. 2012. *Environmental compendium of Sado Island*. Sado city, Niigata.

Sado city. 2021. *Statistical data: Topography*. Sado city. https://www.city.sado.niigata.jp/soshiki/2015/2522.html (accessed 4 April 2022)

Sarashina, M. and Yoshida, T. 2015. Predation impacts of four invasive frogs in Hokkaido, Japan, assessed by an index of relative importance. *Japanese Journal of Conservation Ecology* 20: 15–26.

Sawada, K., Aihara, T., and Kamijo, T. 2022. Current and future predicted distributions of invasive toads (Anura: Bufonidae) and bullfrogs (Anura: Ranidae) on Sado Island. *Journal of Asia Pacific Biodiversity* 15: 345–353.

Semeniuk, M., Lemckert, F., and Shine, R. 2007. Breeding-site selection by cane toads (*Bufo marinus*) and native frogs in northern New South Wales, Australia. *Wildlife Research* 34: 59–66.

Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. *Conservation Biology* 16: 619–629.

Shine, R. 2010. The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology* 85: 253–291.

Shinohara, N. 1992. *Bufo japonicus* in Megi and Ogi Island, Takamatsu. *Kagawa Seibutsu* 19: 41–44.

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., and Vilà, M. 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28: 58–66.

Sinsch, U. 1992. Amphibians. p. 213–233. *In*: F. Papi (ed.), *Animal Homing*. Springer, Dordrecht.

Suzuki, A., Sarashina, M., and Yoshida, T. 2018. Examining the effects of invasive Japanese common toad (*Bufo japonicus formosus*) trapping on drift fense with large pit-falls in Ishikari Coast. *Journal of Rakuno Gakuen University* 43: 29–32.

Tokuda, R. 2021. Establishment and effectiveness of the enclosure trap for the domestic invasive species Japanese common toad *Bufo japonicus formosus*: a case study in Hokkaido Prefecture. *Bulletin of the Herpetological Society of Japan* 1: 44–47.

Uruma, H., Kobayashi, R., Nishijima, S., and Miyashita, T. 2012. Effectiveness of conservation-oriented agricultural practices on amphibians inhabiting Sado Island, Japan, with a consideration of spatial structure. *Japanese Journal of Conservation Ecology* 17: 155–164.

Vaira, M. 2005. Annual variation of breeding patterns of the toad, *Melanophryniscus rubriventris* (Vellard, 1947). *Amphibia-Reptilia* 26: 193–199.

Van Bocxlaer, I., Loader, S. P., Roelants, K., Biju, S. D., Menegon, M., and Bossuyt, F. 2010. Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. *Science* 327: 679–682.

Wake, D. B. and Vredenburg, V. T. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* 105 (Supplement 1): 11466–11473.

Watabe, K., Nakashima, N., and Koizumi, N. 2021. Conservation of frog habitats for land consolidation projects in paddy field areas. *Ecology and Civil Engineering Society* 24: 95–110.

Wells, K. D. 1977. The social behavior of anuran amphibians. *Animal Behaviour* 25: 666–693.

Wen, L., Saintilan, N., Yang, X., Hunter, S., and Mawer, D. 2015. MODIS NDVI based metrics improve habitat suitability modelling in fragmented patchy floodplains. *Remote Sensing Applications: Society and Environment* 1: 85–97.

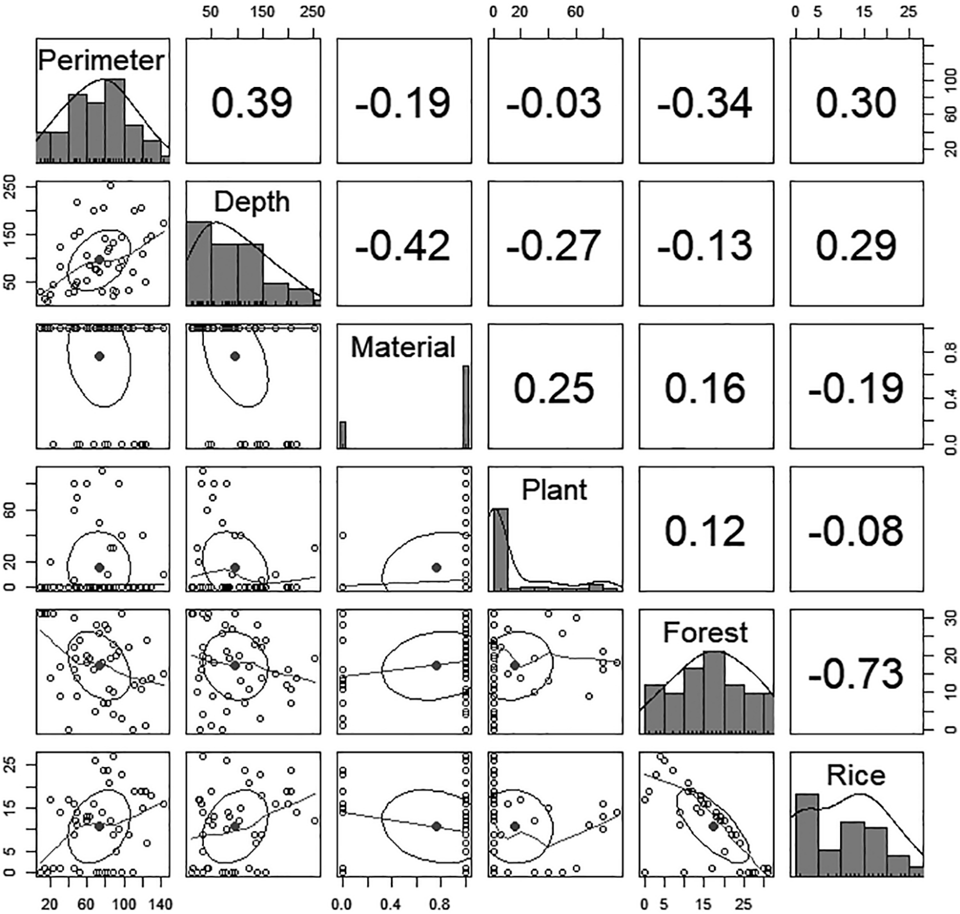
Wingate, D. B. 2011. The successful elimination of cane toads, *Bufo marinus*, from an island with breeding habitat off Bermuda. *Biological Invasions* 13: 1487–1492.

Zheng, X., Natuhara, Y., Li, J., Li, G., Du, Y., Jia, H., Dai, Z., Du, D., Zhong, S., and Qin, D. 2021. Effects of multiple stressors on amphibian oviposition: Landscape and local determinants in central Japan. *Ecological Indicators* 128: 107824.

Accepted: 2 January 2023

Appendix I

Confirmation of multicollinearity among explanatory variables using 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.



Appendix II

Confirmation of multicollinearity among explanatory variables using VIF. If the VIF between explanatory variables was less than 10, multicollinearity was not considered.

	Perimeter	Depth	Material	Plant	Forest
Depth	1.184504				
Material	1.036381	1.216488			
Plant	1.000822	1.079946	1.065144		
Forest	1.126967	1.018147	1.027299	1.013687	
Rice	1.099355	1.089163	1.038893	1.005735	2.160617

Appendix III

Data of the ponds where field surveys were conducted. Each valuable was measured as follows. *B. j*: Occurrence of *Bufo japonicus formosus* (absence: 0, presence: 1); Perimeter: (m); Depth: (cm); Material: (artificial: 0, natural: 1); Plant: Vegetation cover on the surface of the pond (%); Forest: Forest area within 100 m from survey ponds (m^2); Rice: Rice paddy area within 100 m from survey ponds (m^2).

B. j	Perimeter	Depth	Material	Plant	Forest	Rice
0	88.5	21.0	1	30	9,808	0
0	52.0	155.0	0	0	24,251	0
0	97.6	144.0	0	0	3,278	22,526
0	40.0	28.0	1	0	0	16,719
0	73.2	72.0	1	50	25,544	0
0	105.0	33.0	1	0	22,455	8,945
0	47.2	44.0	1	0	19,430	11,970
0	82.4	115.0	0	0	6,960	24,440
0	111.2	198.0	0	0	11,742	18,797
0	45.2	148.0	1	5	22,089	9,311
0	46.2	31.0	1	80	15,661	15,739
0	70.2	77.0	1	0	28,385	0
0	83.6	122.0	1	0	10,607	20,793
0	30.8	82.0	1	0	9,326	13,569
0	48.0	216.0	0	0	14,066	14,388
0	118.8	204.0	0	0	11,167	19,389
0	120.0	108.0	0	40	12,696	14,934
0	77.5	206.0	1	0	7,209	24,191
0	87.7	133.0	1	0	22,724	8,676
0	60.2	105.0	1	0	19,698	11,702
0	87.7	34.0	1	0	4,328	27,072
0	124.0	50.0	0	0	691	18,968

Appendix III

(continued)

B. j	Perimeter	Depth	Material	Plant	Forest	Rice
0	109.6	70.0	1	0	0	0
0	66.6	200.0	0	0	15,574	15,826
0	129.6	148.0	1	0	13,638	17,762
0	82.8	90.0	1	10	27,361	0
0	19.0	24.0	1	20	13,982	17,418
0	143.6	174.0	1	10	15,267	16,133
0	60.0	55.0	1	80	18,394	13,006
0	76.8	32.0	1	90	17,837	13,563
0	8.8	32.0	1	0	31,400	0
0	16.4	11.0	1	0	31,400	0
1	97.0	95.0	1	40	31,400	0
1	78.8	140.0	0	0	27,586	0
1	13.6	15.0	1	0	30,651	749
1	30.0	123.0	1	0	16,941	967
1	126.4	138.0	1	0	24,435	6,965
1	48.2	52.0	1	70	9,430	10,725
1	68.8	78.0	1	0	5,346	26,054
1	91.2	32.0	1	0	20,465	4,965
1	94.2	80.0	1	80	21,244	10,156
1	84.8	252.0	1	30	19,112	12,288
1	23.0	44.0	0	0	30,518	882
1	44.6	42.0	1	60	30,249	1,151
1	62.4	86.0	1	0	18,759	12,781