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# Factors Affecting the Surface Activity of Japanese Common Toad, *Bufo japonicus formosus* (Amphibia: Bufonidae) during the Non-breeding Season

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**Abstract: Lunar phase and weather conditions are known to affect breeding activity of the Japanese common toad, *Bufo japonicus formosus*. However, information on toad activity during non-breeding season is very scarce. In this study, we investigated the influence of weather conditions and lunar phase on toad activity during the non-breeding season (April-November). Surface activity during the non-breeding season was monitored in Minami-ohsawa, Hachioji Tokyo from 2010 to 2014. The effects of weather conditions and the lunar phase on the number of toads captured were analyzed using Random Forests models (an ensemble machine learning method). Toads began to forage on the soil surface from late April and activity lasted until mid-November. Some meteorological variables such as temperature, wind, and atmospheric pressure affected toad activity, but the lunar phase, which is the most important variable in breeding activity, did not have an effect on surface activity of the toads during non-breeding season. However, differences were observed among adult males and females with respect to their response to meteorological variables: males responded positively to rainfall, but females did not. A distinct difference in the effect of temperature was also observed between adults and juveniles: juveniles became more active when daily minimum temperature was >15°C, although adults did not respond to an increase in temperature. Our results on sexual differences in weather-mediated activity and lack of the lunar phase effects during non-breeding season provide useful information for our understanding of life history of these toads.**

Key words: *Bufo japonicus formosus*; Lunar cycle; Non-breeding activity; Sexual differences; Weather conditions

## Introduction

The influence of many exogenous factors on the activity of amphibians is well documented (Wells, 2007). Because amphibians are ectotherms, nearly every aspect of their physiology and behavior is affected by changing weather conditions. The number of adults participating in breeding activities vary every day, and this fluctuation is influenced by temperature (Kusano and Fukuyama, 1989; Fukuyama and Kusano, 1992), rainfall (Kluge, 1981; Todd

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and Winne, 2006), wind (Robertson, 1986), or a combination of these (Okuno, 1985b; Semlitsch, 1985). Another variable that has gained much attention recently is the lunar cycle, which influences the breeding and behavior of several amphibian species (Deeming, 2008; Grant et al., 2009; Yetman and Ferguson, 2011; Vignoli and Luiselli, 2013). In some species, the full moon increases amphibian activity, but in other species, it decreases their activity: there is no significant difference between the numbers of species which increase, and those which decrease activity during a full moon (Grant et al., 2013). Responses to the lunar cycle cannot be generalized across taxonomic group, but instead are highly species specific and relate directly to the species' ecology. The primary reasons for changes in amphibian behavior in response to the lunar cycle appear to be temporal synchronization of breeding and predator avoidance (Grant et al., 2013). To the best of our knowledge, we cannot find any reports which compare the effects of lunar cycle between breeding and non-breeding seasons in the same populations. This comparison may assist our understanding of adaptive significances of amphibian responses to the lunar cycle.

The Japanese common toad, *Bufo japonicus formosus*, is a large and robust species that reaches a snout-vent length (SVL) in excess of 100 mm (Matsui and Maeda, 2018). This species is very common in eastern Japan; its distribution ranges from southern Hokkaido to the Kinki and San'in districts of Honshu. The toad inhabits a variety of habitats, from the seas to the high mountains, and breeds explosively during early spring in still waters such as roadside ditches, lakes, marshes, and small ponds (Matsui and Maeda, 2018). The breeding biology of *B. j. formosus* is well documented; many aspects of its reproductive ecology such as breeding site fidelity (Kusano et al., 1999), migration to and from the breeding sites (Hisai et al., 1987; Kusano et al., 1995), and age structure of the breeding populations (Kusano et al., 2010) etc., have been clearly established. The breeding activity of this species is triggered by an increase in soil temperature (Aoyagi et al., 1977; Hisai and Sugawara, 1978). However, the daily fluctuations of the number of breeding adults subsequent to the onset of breeding have are also strongly affected by the lunar phase (Kusano et al., 2015; Kusano, 2021).

Owing to the difficulty in observing toads outside their breeding season, most studies have been limited to the breeding activity. As with many Japanese amphibians, little is known about the biology of this species during its non-breeding season. Although a few studies have focused on the growth, movement patterns, microhabitat use, and food habits (Hisai, 1975; Okuno, 1985a; Hirai and Matsui, 2002; Okamiya and Kusano, 2018), the seasonal patterns of activity during non-breeding season in this species are yet to be established.

We conducted a long-term survey of a population of *B. j. formosus* over five non-breeding seasons from 2010 to 2014. In the current paper, the term "non-breeding activity" means the activity on the ground surface during the non-breeding season. The context of this surface activity was not known, but we assume that it was primarily foraging and/or movement behaviors. We analyzed the relationships between the surface activity of the toads and the associated environmental factors, including climatic variables and the lunar phase. The results obtained were compared to those on the breeding activity of the species; seasonal and sexual differences observed in response to environmental factors were discussed in order to determine their ecological significance.

## Materials and Methods

### *Study sites*

We conducted this study over five years from July 2010 to July 2014 on the campus grounds (area approximately 40 ha) of the Tokyo Metropolitan University in Hachioji Tokyo (35°37'10" N, 139°22'56" E) at an altitude of 110–140 m. The study area includes various types of habitats, such as secondary deciduous forests, planted coniferous forests, grass fields, paved areas, and abandoned paddy fields. During the breeding season (late February–March), adults of *B. j. formosus* aggregate in an artificial pond located near the center of the campus, and subsequently disperse during the non-breeding season to various nearby habitats (Kusano et al., 2015; Miura, 2015). Detailed descriptions of the study area have been provided by Kusano et al. (2006, 2015).

*Monitoring of non-breeding activities and environmental variables*

At the study site, toads overwintered in December–January, and bred in February–March (Kusano et al., 2015; T. Kusano, personal observation). During the non-breeding season from April to November, we surveyed the study area for one or two hours to look for toads appearing on the ground surface, at least once a week shortly after sunset (at approximately 1900 h). Toads were captured by hand, measured for SVL to the nearest 0.1 mm using a slide caliper and weighed to the nearest 0.1 g using an electronic balance. Their sexes were determined by the secondary sexual characteristics such as nuptial pad development and morphology of snout, as well as monitoring release calls (Hisai, 1984; Matsui and Maeda, 2018). The toads were classified as females if they were equal to or larger than 82 mm in SVL, which is the minimum size of breeding females in the population studied (Miura, 2015; T. Kusano, personal observation), and did not show signs of male sexual maturation. When their SVLs were smaller than 82 mm, they were classified as juveniles. All captured toads were released at the points of capture immediately after recording these observations. At the study site, a vast number of just-metamorphosed toadlets emerged from the breeding pond in May every year (T. Kusano, personal observation). We excluded small individuals (≤20 mm in SVL) captured during this period from the census data.

We collected lunar phase data from the National Astronomical Observatory of Japan (http://www.nao.ac.jp/) and daily meteorological variable data from the nearest weather station of the Automated Meteorological Data Acquisition System, Hachioji station, which is located eight km northwest of the study site. Data on humidity and atmospheric pressure were collected from Tokyo regional headquarters, which is located 34 km northeast of the study site, because these data were not provided from the Hachioji station (Japan Meteorological Agency, 2022).

*Statistical analyses*

We analyzed several environmental factors potentially affecting the magnitudes of non-breeding activity using Random Forests models (RF; an ensemble machine learning method; Breiman, 2001). In the present analysis, complicated relationships among the candidate predictor variables such as multicollinearity and the non-linear responses of response variables were suspected; therefore, the application of commonly used Generalized Linear Models was not considered appropriate. In such case, it is recommended that RF models be applied instead (Breiman, 2001; Strobl et al., 2008).

The number of toads captured each day was treated as a response variable. In addition to the lunar phase, six meteorological variables were selected as candidate predictor variables affecting non-breeding activity: total precipitation since the previous day, daily minimum temperature, daily mean wind speed, hours of sunshine, daily mean relative humidity, and daily mean atmospheric pressure (Table 1). Because collinearity severely distorts model estimation and subsequent prediction, pairwise correlations among candidate variables were interrogated. We ascertained that all of the correlations were relatively minor; the absolute values of all correlation coefficients were less than the threshold of 0.7 (Dorman et al., 2013). Because the lunar phase is not a linear variable, but a circular one, the effects of lunar cycle should be analyzed using circular statistics (Pewsey et al., 2013). The lunar phase of each census day was converted to the number of days from the nearest full moon as moon

TABLE 1. Environmental variables affecting the non-breeding activities of *Bufo j. formosus*. Variables were weekly mean values at the census days except for Year and Week.

| Variable | Unit | Abbreviation | Mean (n=103) | SD | Range |
|---|---|---|---|---|---|
| Total amount of precipitation (48 h) since the previous day | (mm) | Rain | 10.91 | 18.31 | 0.00–112.75 |
| Daily minimum air temperature | (°C) | Tmin | 17.38 | 5.18 | 2.10–25.11 |
| Daily mean wind speed | (m/sec) | Wind | 2.84 | 0.84 | 1.25–6.05 |
| Hours of sunshine | (h) | Sunshine | 4.77 | 3.33 | 0.00–12.70 |
| Daily mean relative humidity | (%) | Humidity | 69.88 | 9.35 | 40–89 |
| Daily mean atomospheric pressure | (hPa) | Atomosphere | 1007.4 | 5.2 | 995.5–1020.8 |
| Moon age (the number of days to the nearest full moon) | (day) | Moon | 7.51 | 4.33 | 0.25–14.75 |
| Year (categorical variable) | — | Year | — | — | 2010–2014 |
| Weeks of the year | — | Week | 32.1 | 8.4 | 17.0–48.0 |

age (Moon in Table 1), thereby converting it to a linear variable (Kusano et al., 2015).

Since census data collected in the present study had a time series aspect to it, temporal autocorrelation was suspected. Firstly, the entire daily census data were converted in weekly data by averaging the daily numbers of toads captured; the weekly data were then used for all subsequent analyses. We checked the data for the magnitude of temporal autocorrelation by estimating autocorrelation functions (ACF; Venables and Ripley, 2002). Temporal variables such as year (as a categorical variable) and week of the year were also incorporated into RF models to take the temporal autocorrelation into consideration. In order to examine the difference in responses among the sex categories, the RF models were also separately applied to males, females, and juveniles. As the measure of accuracy of predictions, the root mean squared errors (RMSEs) were also estimated by using 10-fold cross validation technique (Kuhn and Johnson, 2016).

The values of Variable Importance were calculated to evaluate the contribution of each variable to the variation in activity levels. However, to avoid any ill-effects of complicated correlations among predictor variables, a Conditional Inference Tree was used as a classifier in the RF analysis, and Conditional Variable Importance (CVI) was adopted and evaluated (Strobl et al., 2008). The 95% confidence intervals of the CVI were determined by bootstrapping (100 repetitions). In order to assess the relationship between each predictor and the response variable, a partial dependency plot was constructed, which visualized the predicted response of toad activity while accounting for the average effect of other predictors in the model; this is particularly effective with black box models like Random Forests (Friedman, 2001; Greenwell, 2017). We used the software R 4.1.3 (R Core Team, 2022) for statistical analyses. RF analysis was performed using the function "cforest" in the "party" package (Hothorn et al., 2006). The significance level for all tests was $P<0.05$ (two-sided).

## RESULTS

### *Seasonal change of non-breeding activity*

We monitored the non-breeding activity of a *B. j. formosus* population over five consecutive years. Toads began to appear on the ground surface from late April, and their non-breeding activities lasted until mid-November (Fig. 1). During this study, a total of 274 daily censuses were conducted, and a total of 700 toads were captured and released: 170 males, 201 females, 318 juveniles, and 11 sex unknowns. With the conversion of daily data into weekly data, we derived a total of 103 weekly observations during the study period. The weekly average of toads captured per day was 2.60 (SD=2.58): 0.68 (SD=1.00) for males, 0.83 (SD=1.01) for females, 1.05 (SD=1.93) for juveniles, and 0.04 (SD=0.22) for sex unknowns. Okuno (1984) reported that surface activity of adult toads were greatly suppressed during summer season, but we did not find any clear patterns of surface activity from late spring to late autumn, although activity was slightly reduced in August (Fig. 1). ACFs were estimated separately on a yearly basis using time-series data on the total number of toads. We could not detect any significant autocorrelations for all lags (1–11) except for lag 1 in 2010, which is an indication that the potential problems brought about by the temporal autocorrelation may not be severe.

*Relationships between non-breeding activity and meteorological variables and lunar cycle*

We generated RF models explaining the variation in surface activity levels of respective sex categories; 33–40% of the total variance was explained by each RF model. RMSEs were estimated to be 2.39 for total toads, 0.86 for males, 0.82 for females, and 1.52 for juveniles. CVI values showed that of the seven environmental variables monitored, temperature had the greatest effect on the surface activity of total toads (Fig. 2). Wind and atmospheric pressure variables also showed significant effects on surface activity, but effects of other meteorological variables (sunshine, rain and humidity) were not detected (Fig. 2). In contrast to the observations with breeding activity (Kusano et al., 2015; Kusano, 2021), lunar phase did not have any effects on surface activity during the non-breeding season regardless of sex categories (Fig. 2). The CVI values also showed that the importance of different predictors varied for different sex categories (Fig. 2). In particular, factors affecting surface activity differed markedly between sexually mature toads and juveniles (Fig. 2). For juveniles, the variable which contributed the most to the variation in surface activity was temperature, but for males and females, the most important variables were humidity, sunshine, and/or rain (Fig. 2).

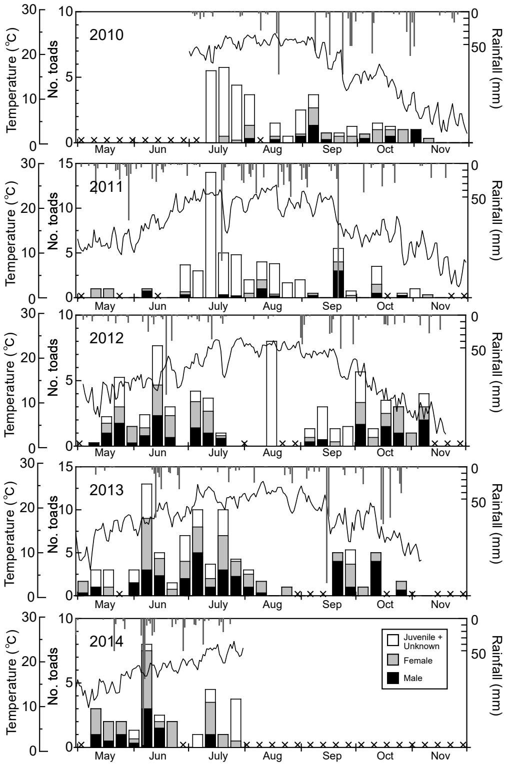


FIG. 1. Seasonal change of non-breeding activities of *Bufo japonicus formosus* from 2010 to 2014. Vertical thick bars indicate the weekly mean of daily number of toads captured. Meteorological conditions are also shown. Solid lines and narrow gray bars extending from the upper axis indicate the daily minimum temperature and daily precipitation, respectively. Crosses in the lower axis indicate the weeks when we could not conduct a census.

The predicted response patterns of surface activity to the respective environmental variables are shown in Fig. 3. For total toads and juveniles, temperature was the most important variable (Fig. 2), with the effect on surface activity of the toads being clearly positive (Fig. 3). As temperature increased, toads became more active, especially when the daily minimum temperature was >15°C. In the case of males, humidity and rain showed non-linear

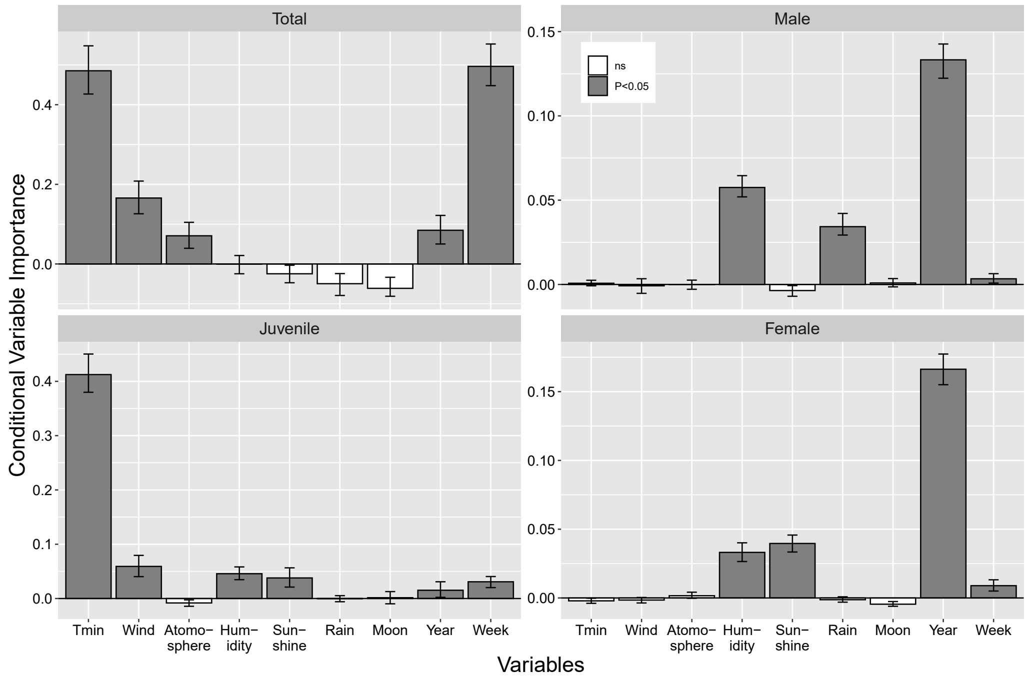


FIG. 2. Degree of contribution of each candidate predictor variable to the Random Forests models of non-breeding activity. The vertical axis shows the Conditional Variable Importance of the variables (see Strobl et al., 2008). The vertical lines show the 95% confidence intervals.

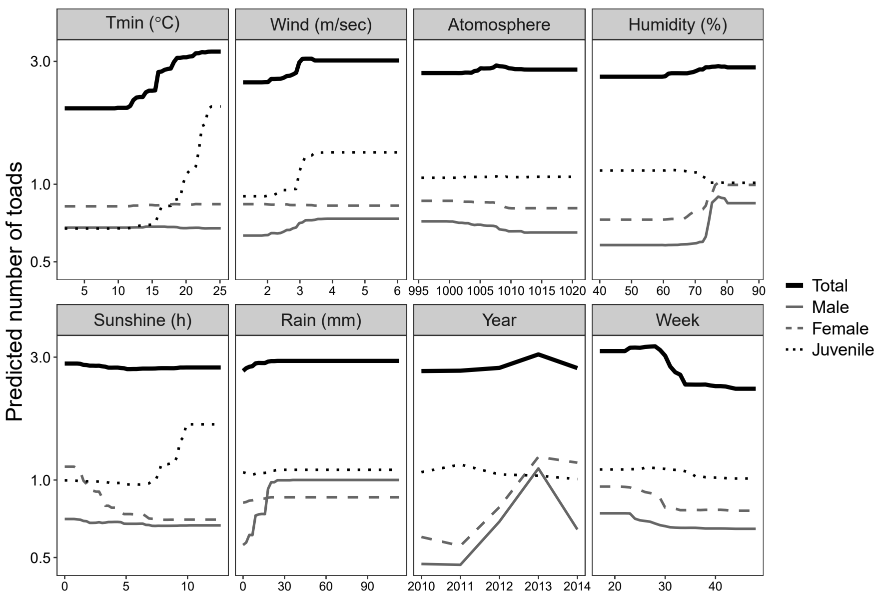


FIG. 3. Partial dependence of the number of toads captured on respective predictor variables based on Random Forests models (see Greenwell, 2017). Predicted values of the number of toads captured are plotted on a logarithmic scale.

effects. Surface activity was enhanced following a step-like trajectory when the relative humidity became >75%, and when precipitation for 48 h amounted to >30 mm. In the case of females, shorter sunshine (<7 h) and higher humidity (>70%) promoted surface activity (Fig. 3).

## Discussion

*Effect of lunar cycle on toad activity and seasonal difference*

We found that lunar cycle had no effect on surface activity of the toads during the non-breeding season, although its effect is notable during the breeding season: breeding activity became more intense when the lunar phase approached new moon, but weakened once the lunar phase approached full moon, even after taking into account the effects of the weather conditions (Kusano et al., 2015; Kusano, 2021). Why do toads respond to the moon differently in different seasons? The primary reasons for changes in amphibian behavior in response to lunar cycle are considered to be predator avoidance and temporal synchronization of breeding (Grant et al., 2009; Vignoli and Luiselli, 2013; Kusano et al., 2015). If predator avoidance is relevant to this behavior, we cannot explain why toads do not respond to the moon during the non-breeding season. If temporal synchronization of breeding is a main reason for lunar-mediated response during the breeding season, lack of the response during the non-breeding season is reasonable. Reproductive synchronization may enhance reproductive success of breeding individuals by maximizing the pool of available breeding adults and by reducing predation risk through predator dilution/satiation not only for breeding adults themselves, but also for their offspring (Ims, 1990). In *B. j. formosus*, a large number of adults aggregate at the breeding ponds every year from the neighboring summer home ranges. Since their breeding activities lasted only for a week during some years (Kusano et al., 2015), the timing of appearance of the adults in the breeding pond may be very important for successful breeding. In this context, the lunar phase may be used as an environmental cue to adjust the timing of reproduction. The result of the current study may strongly support the hypothesis of temporal synchronization of breeding.

*Effects of meteorological variables on toad activity and differences in sexes and age*

Amphibian activity is greatly influenced by weather conditions such as temperature and rainfall (Okuno, 1985b; Kusano and Fukuyama, 1989; Todd and Winne, 2006). However, the differential effects of environmental variables have not yet been compared in detail across sexes, and different seasons. In the current study, we surveyed the non-breeding activity in a population where breeding activity has already been analyzed and reported (Kusano et al., 2015). During the non-breeding season, both male and female toads become active on wet rainy days, but females become active when it is cloudy even without rainfall. The sexually mature adults do not seem to respond to temperature at all during this period, or for that matter during the breeding season (Kusano et al., 2015). Only for juveniles was temperature-dependent activity clearly detected in the present study. Although we do not have any evidence at present, large body size of the adult toads may be relevant to this finding: as might be expected, small juveniles may not be as tolerant of cold temperature as adults.

The surface activity of adult toads was affected by humidity regardless of sex, but the response to precipitation varied across sexes. Only males responded to precipitation directly; 48 hour periods with precipitation <20 mm suppressed the surface activity of males. This sexual difference in response to precipitation may be related to the sexual difference in microhabitat use, which Okamiya and Kusano (2018) demonstrated using a fluorescent powder tracking technique. They found that both sexes of toads preferred areas covered with grasses and mosses and a closed canopy, but male were more likely to use areas with an open canopy. Foraging in areas with an open canopy increases the risk of dehydration. Males are also generally smaller than females (Kusano et al., 2010), and smaller bodies have a lower capacity to hold water and thus run an increased risk of dehydration (Bartelt et al., 2004; Kuramoto, 2005). The higher risk of dehydration for males may help to explain to the stronger influence of precipitation on their behaviour.

A total of seven species of amphibians reproduce at the study site (Kusano et al., 2006, 2015; Kusano and Inoue, 2008); six of these species rarely emerge from the forested areas (see Okochi, 2001). Almost all amphibian species have disappeared from highly disturbed urban areas of Tokyo, but *B. j. formosus* has persisted even in these areas, although populations are in decline (Fukuyama and Kusano, 2013). These results have also been found in other amphibian species that continue to persist in disturbed habitats (Price et al., 2011; Smallbone et al., 2011; Scheffers and Paszkowski, 2012). This may be because of the ability to utilize a wide range of habitats, from disturbed open habitats to natural forested habitats (Warren and Büttner, 2008; Canessa et al., 2013; Fukuyama and Kusano, 2013). Understanding how such disturbance tolerant species spend their life within remnant landscapes may provide some important knowledge on understanding how animals adapt to the urbanized environments. This could also provide useful information for conservation of urban ecosystems. The further information on the life history and ecology is vital in terms of conserving the populations of common species such as *B. j. formosus*.

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