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Source: Current Herpetology, 39(1) : 38-46

Published By: The Herpetological Society of Japan

URL: <https://doi.org/10.5358/hsj.39.38>

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Relative Abundance Differences of Two Invasive Toad Species on Minami-daito and Kita-daito Islands, Japan

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Abstract: Biological invasions are major threats to global biodiversity, and island ecosystems are especially vulnerable. Many previous studies have reported the presence of invasive species on islands, but relatively few studies have quantified the population status of such species. Furthermore, multiple invasive species are often introduced to the same region or island. In such case, quantifying the relative abundance of each species would provide us with significant insight into their population dynamics and potential species interactions. *Rhinella marina* and *Bufo gargarizans miyakonis* were introduced to both Minami-daito and Kita-daito Islands before 1945. Although some studies have reported their invasion and establishment, few studies have quantified their relative abundance. To reveal their relative abundance, we set 5 km line transects and counted the number of each species from 11 to 31 July 2019. We applied generalized linear models to examine the relationship between the number of individuals observed with species and environmental factors. We also applied these models to examine the relationship between the number of individuals observed with island and environmental factors. We found the relative abundance of *R. marina* was significantly higher than that of *B. g. miyakonis* on Minami-daito Island. This is the opposite pattern of 30 years ago, suggesting the population dynamics of these invasive toads is unstable on Minami-daito Island. Although *B. g. miyakonis* was found on both islands, no individual of *R. marina* was found on Kita-daito Island. The population dynamics may depend on environmental differences between islands and ecological differences between species. Future studies are needed to reveal the potential factors determining the differences in relative abundance between islands and between species.

Key words: *Bufo gargarizans miyakonis*; Daito Islands; Invasive species; Relative abundance; *Rhinella marina*; Toad

INTRODUCTION

Human-induced disturbances cause global biodiversity loss, and one of the main drivers is biological invasions (Clavero and Garcia-

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Berthou, 2005). As human transport and commerce developed worldwide, various biota have migrated beyond their native distribution range following human activity (Hulme, 2009). Although most of the immigrants do not settle in the new range, some survive and breed excessively in the invaded area (Mack et al., 2000). These successful invaders can directly and indirectly interact with native species, and degrade native ecosystems (Lockwood et al., 2013). To conserve global biodiversity, it is essential to understand the population status of invasive species in each local area, especially unique ecosystems such as islands.

Island ecosystems have outstanding evolutionary distinctiveness, hence each insular biota is an essential element for global biodiversity (Banks and Dickman, 2007; Whittaker and Fernandez-Palacios, 2007). However, it is often the case that island ecosystems are especially vulnerable to invasive species because of the lack of co-evolutionary history with predators or toxic animals (Doherty et al., 2016). A previous study reported that island endemics accounted for 87% of extinct species (Doherty et al., 2016). The most impacted species are insular biota, indicating that understanding the population status of invasive species on islands should be a high priority.

Many previous studies have reported the presence or absence of invasive species on islands (Clavero and Garcia-Berthou, 2005; Lockwood et al., 2013). However, relatively few studies have evaluated population status after the invasion. The population that was introduced to an island can change dramatically because of environmental factors and species interactions (Kaji et al., 2009; Grant and Grant, 2006). Furthermore, it is common for multiple invasive species to be introduced to the same region, and they might have competitive interactions if they are closely related species or have a similar ecological niche. For example, introduced dingoes suppress introduced fox populations through competition for prey in Australia (Cupples et

al., 2011), and introduced rats suppress introduced mouse populations through competition in New Zealand (Ruscoe et al., 2011). In such cases, quantifying the relative abundance of each invasive species would provide a significant insight into population dynamics and potential competitive interactions of the invasive species.

The cane toad (*Rhinella marina*) and Miyako toad (*Bufo gargarizans miyakonis*) were introduced to both Minami-daito Island and Kita-daito Island (Fig. 1) before World War II, in a harbor that has no native amphibians (Matsui, 1975; Ota and Toyama, 1992; Ota, 1999; Ota et al., 2004; Maenosono and Toda, 2007; Kita-daito village, 2017; Kita-daito village, 2019; Minami-daito village, 1990; Matsui and Maeda, 2018). *Rhinella marina* is a highly problematic invasive species that is native to southern America, and has a body length of 89–155 mm (Shine, 2010; Matsui and Maeda, 2018). This species was introduced to many areas worldwide to control sugar cane pests, and causes negative impacts on native predators via strong toxin defenses (Kidera et al., 2008; Shine, 2010). For example, native predators such as snakes, crocodiles and marsupials that attempt to eat *R. marina* are often poisoned due to a lack of co-evolutionary history of exposure to the toad's bufotoxins (Phillips and Shine, 2006; Doody et al., 2009; Kidera and Ota, 2008; Shine, 2010). *Rhinella marina* inhabits open lands close to human activity and sugar cane fields, and probably breeds all year around (intensively from December to January) in still water such as artificial ponds (Matsui and Maeda, 2018). *Bufo gargarizans miyakonis* is native to Miyako Island, Japan and has a body length of 61–119 mm (Matsui and Maeda, 2018). This species was also introduced to several islands to control sugar cane pests, and potentially affects native fauna (Ota and Toyama, 1992; Ota, 1999; Ota et al., 2004; Maenosono and Toda, 2007; Matsui and Maeda, 2018). *Bufo gargarizans miyakonis* inhabits sugar cane fields and breeds from September to March in still water (Matsui and

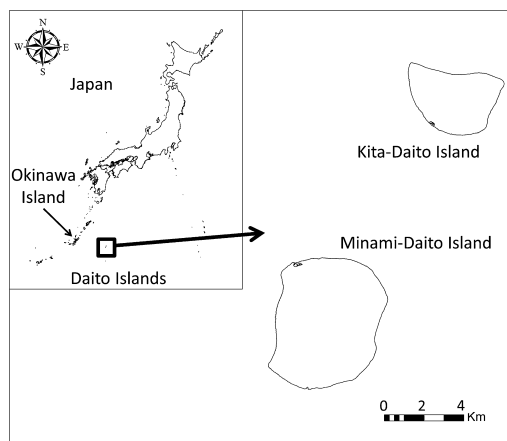


FIG. 1. A map showing the study locations, Daito Islands (Minami-daito and Kita-daito Islands) approximately 360 km east from Okinawa Island, Japan.

Maeda, 2018). Both toad species feed on various terrestrial animals such as snails, centipedes, cockroaches, beetles, grasshoppers and ants (Matsui and Maeda, 2018). The breeding environment of both species is similar and the breeding period is long, hence they sometimes breed in the same place at the same time (Matsui and Maeda, 2018). Considering these ecological similarities, these species may have competitive interactions, however few studies have quantified their relative abundance. Quantifying the relative abundance of each species would clarify which species would be more abundant in the area, which might result from competition and/or environmental factors.

MATERIALS AND METHODS

Study area

The Daito Islands are located approximately 360 km east of Okinawa Island, Japan and mainly consist of Minami-daito Island and Kita-daito Island with an area of 30 km² and 12 km², respectively (Fig. 1). These islands are uplifted coral atolls, and have never been connected to any other land mass, hence they harbor a unique fauna such as

Pteropus dasymallus daitoensis. These islands have a sub-tropical climate with an annual mean temperature of approximately 22°C and annual mean rainfall of approximately 1,700 mm. Most of the land area was covered by sugar cane fields and a small forest, and some relative large ponds are also present.

Field research

We set five line transects on Minami-daito Island and six line transects on Kita-daito Island along roads. Each transect was approximately 5 km in length. Parts of the transects were located beside still water that would be potential breeding sites for toads. We visually searched for toads during the night (1930–2200) from 11 to 31 of July 2019 by driving a motorbike at a constant speed of approximately 10–20 km/hr. When a toad was detected, we recorded the species and geographic location. We summed the number of individuals of each species in every 5 km line transect.

Statistical analysis

We applied generalized linear models (GLM) to examine the relationship between the number of individuals in a line transect with species (*R. marina* vs *B. g. miyakonis*) and various environmental factors on each island. The error distribution was identified as a Gaussian distribution. We used the number of individuals in each line transect as a response variable and species, temperature, humidity, and wind speed as explanatory variables.

We also applied generalized linear models to examine the relationship between the number of individuals in each line transect with island (Minami-daito Island vs Kita-daito Island) and various environmental factors for each species. The error distribution was identified as a Gaussian distribution. We used the number of individuals for each species in each line transect as a response variable and island, temperature, humidity, and wind speed as explanatory variables. We obtained the data on temperature, humidity and wind speed

TABLE 1. Results of the number of individuals of *Rhinella marina* (*R. m.*) and *Bufo gargarizans miyakonis* (*B. g. m.*) and environmental factors on Minami-daito (MD) and Kita-daito (KD) islands.

| Island | Route | Date | <i>R. m.</i> | <i>B. g. m.</i> | Temperature (°C) | Humidity (%) | Wind speed (m/s) |
|--------|-------|--------------|--------------|-----------------|------------------|--------------|------------------|
| MD | 1 | 18 Jul. 2019 | 93 | 0 | 29.2 | 89 | 7.2 |
| MD | 2 | 19 Jul. 2019 | 146 | 5 | 31.4 | 88 | 6.6 |
| MD | 3 | 20 Jul. 2019 | 117 | 7 | 31.3 | 90 | 5.5 |
| MD | 4 | 20 Jul. 2019 | 134 | 3 | 31.3 | 90 | 5.5 |
| MD | 5 | 21 Jul. 2019 | 116 | 0 | 31.8 | 85 | 3.9 |
| KD | 1 | 24 Jul. 2019 | 0 | 0 | 32.1 | 82 | 2.8 |
| KD | 2 | 24 Jul. 2019 | 0 | 0 | 32.1 | 82 | 2.8 |
| KD | 3 | 25 Jul. 2019 | 0 | 2 | 32.3 | 83 | 2.6 |
| KD | 4 | 25 Jul. 2019 | 0 | 3 | 32.3 | 83 | 2.6 |
| KD | 5 | 26 Jul. 2019 | 0 | 111 | 32.6 | 82 | 2.2 |
| KD | 6 | 27 Jul. 2019 | 0 | 23 | 31.4 | 85 | 3.8 |

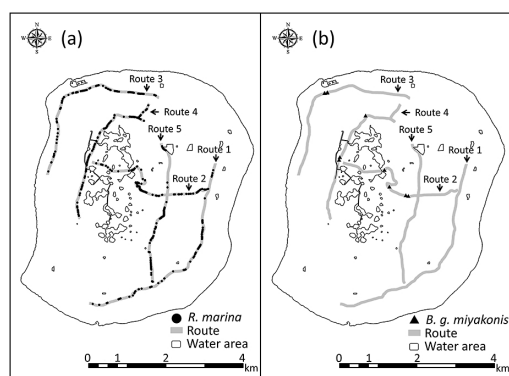


FIG. 2. Results of line transect surveys of (a) *Rhinella marina* (black points) and (b) *Bufo gargarizans miyakonis* (black triangles) on Minami-daito Island. Grey lines represent transect routes (Route 1–5) and thin lines represent water areas.

from the free climate data provided by Japan Meteorological Agency (<https://www.jma.go.jp/jma/index.html> last accessed on 15 Aug 2019). All statistical analyses were performed using R version 3.6.0 (R Development Core Team 2019).

RESULTS

The mean number of *R. marina* and *B. g. miyakonis* per 5 km line transect on Minami-

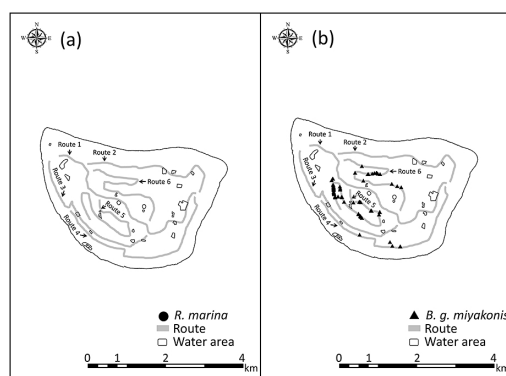


FIG. 3. Results of line transect surveys of (a) *Rhinella marina* (black points) and (b) *Bufo gargarizans miyakonis* (black triangles) on Kita-daito Island. Grey lines represent transect routes (Route 1–6) and thin lines represent water areas.

daito Island was 121.2 and 3.0, respectively (Fig. 2a, b, Table 1). The mean number of *R. marina* and *B. g. miyakonis* per 5 km line transect on Kita-daito Island was 0 and 23.2, respectively (Fig. 3a, b, Table 1).

The result of GLM analysis showed that the number of *R. marina* individuals per 5 km line transect was significantly greater than *B. g. miyakonis* on Minami-daito Island (coefficient estimate = 118.2, $p < 0.001$) (Fig. 4, Table 2a). In contrast, there was no significant

TABLE 2. Results of generalized linear models examining the relationship between the number of individuals per 5 km line transect with species (*Rhinella marina* vs *Bufo gargarizans miyakonis*), temperature, humidity, and wind speed on (a) Minami-daito Island and (b) Kita-daito Island. Significance is represented in bold letters.

| (a) | | | | | |
|-----------------------|----------------------|-----------|------------|---------|------------------|
| Response variable | Explanatory variable | Estimate | Std. Error | t value | p value |
| Number of individuals | (Intercept) | -557.1 | 283.789 | -1.963 | 0.107 |
| | Species | 118.2 | 7.682 | 15.387 | <0.001 |
| | Temperature | 15.6 | 6.415 | 2.436 | 0.059 |
| | Humidity | 0.3 | 2.560 | 0.120 | 0.909 |
| | Wind speed | 8.5 | 5.973 | 1.416 | 0.216 |
| (b) | | | | | |
| Response variable | Explanatory variable | Estimate | Std. Error | t value | p value |
| Number of individuals | (Intercept) | -146433.2 | 62241.070 | -2.353 | 0.051 |
| | Species | -23.2 | 15.150 | -1.529 | 0.170 |
| | Temperature | 4672.5 | 1994.460 | 2.343 | 0.052 |
| | Humidity | -173.0 | 78.740 | -2.197 | 0.064 |
| | Wind speed | 3801.3 | 1636.380 | 2.323 | 0.053 |

difference in the number individuals between *R. marina* and *B. g. miyakonis* on Kita-daito Island (Fig. 4, Table 2b). Temperature, humidity, and wind speed had no significant effect on the number of toads on either island (Table 2a, b).

The GLM analysis showed that the number of *R. marina* individuals on Minami-daito Island was significantly greater than on Kita-daito Island (coefficient estimate=105.3, $p<0.001$) (Fig. 4, Table 3a). The analysis also showed that temperature and wind speed significantly increased the number of individuals observed (temperature: coefficient estimate=27.5, $p<0.001$; wind speed: coefficient estimate=15.9, $p<0.01$) (Table 3a). There was no significant difference in the number of *B. g. miyakonis* between Minami-daito and Kita-daito Islands (Fig. 4, Table 3b). Temperature, humidity, and wind speed had no significant effect on the number of *B. g. miyakonis* when comparing Minami-daito and Kita-daito Islands (Table 3b).

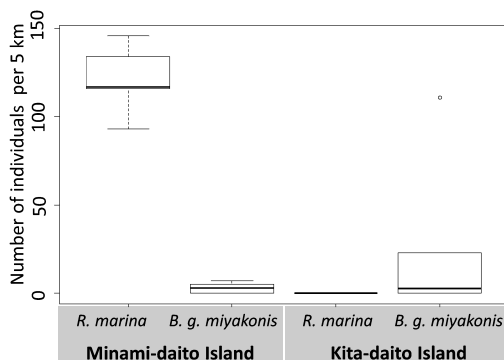


FIG. 4. Box plots showing the number of individuals of *Rhinella marina* and *Bufo gargarizans miyakonis* that were observed along 5 km line transects on Minami-daito and Kita-daito Islands. The top bar represents the maximum value, and the bottom bar represents the minimum value excluding outliers. The upper and bottom bars of the box represent the upper and lower quartiles, respectively. The central bar of the box represents the median.

TABLE 3. Results of generalized linear models examining the relationship between the number of individuals per 5 km line transect with island (Minami-daito Island vs Kita-daito Island), temperature, humidity, and wind speed of (a) *Rhinella marina* (b) *Bufo gargarizans miyakonis*. Significance is represented in bold letters.

| (a) | | | | | |
|-----------------------|----------------------|----------|------------|---------|------------------|
| Response variable | Explanatory variable | Estimate | Std. Error | t value | p value |
| Number of individuals | (Intercept) | -934.8 | 171.712 | -5.444 | <0.01 |
| | Island | 105.3 | 8.030 | 13.113 | <0.001 |
| | Temperature | 27.5 | 4.126 | 6.676 | <0.001 |
| | Humidity | 0.1 | 1.476 | 0.042 | 0.968 |
| | Wind speed | 15.9 | 3.820 | 4.164 | <0.01 |
| (b) | | | | | |
| Response variable | Explanatory variable | Estimate | Std. Error | t value | p value |
| Number of individuals | (Intercept) | -278.9 | 1213.034 | -0.230 | 0.826 |
| | Island | -11.6 | 56.725 | -0.205 | 0.844 |
| | Temperature | 6.9 | 29.144 | 0.238 | 0.820 |
| | Humidity | 1.0 | 10.427 | 0.099 | 0.925 |
| | Wind speed | -2.2 | 26.988 | -0.081 | 0.938 |

DISCUSSION

We found the relative abundance of *R. marina* was far greater than *B. g. miyakonis* on Minami-daito Island independent of environmental factors such as temperature and humidity. We also found the relative abundance of *R. marina* on Minami-daito Island was significantly greater than on Kita-daito Island independent of the environmental factors. In contrast, there was no significant difference in the relative abundance of *B. g. miyakonis* between Minami-daito Island and Kita daito-Island.

Our result of the field survey in 2019 on Minami-daito Island indicates that *R. marina* is likely to be more established than *B. g. miyakonis* on Minami-daito Island nowadays. However, interestingly, it was reported to be the opposite pattern 30 years ago: *B. g. miyakonis* was more abundant than *R. marina* on this island (Ota and Toyama, 1992). This temporal change of relative abundance of two invasive toads may suggest the instability of their populations on Minami-daito Island.

Several studies have reported that population size, especially on islands, changes significantly over several decades. For example, the population of Sika deer (*Cervus nippon*) introduced to Nakanoshima Island, Hokkaido, Japan changed dramatically by degraded vegetation (Kaji et al., 2009), and the island population of Darwin's Finch (*Geospiza fortis*) changed drastically by drought and competition (Grant and Grant, 2006). Although there is no report of significant environmental change on Minami-daito Island and no study of species interaction between *R. marina* and *B. g. miyakonis*, there might be competitive interactions between them because their habitat, prey items, and breeding environment are all similar. Their potential competitive interactions might also influence the relative abundance of *R. marina* and *B. g. miyakonis* on Minami-daito Island. Moreover, poaching for the pet trade could be one of the potential factors influencing the change in relative abundance of toads on Minami-daito Island.

Although there was no statistically signifi-

cant difference in the relative abundance between *R. marina* and *B. g. miyakonis* on Kita-daito Island, we found *B. g. miyakonis* easily, whereas we could not find any *R. marina* individuals on Kita-daito Island. This pattern is likely to be consistent with the previous study: *B. g. miyakonis* was more abundant than *R. marina* (Ota and Toyama, 1992).

Our results show that *R. marina* is abundant on Minami-daito Island whereas they are not abundant on Kita-daito Island. In contrast, *B. g. miyakonis* inhabit both Minami-daito Island and Kita-daito Island. Although the reason for the different pattern of relative abundance is not clear, environmental differences between these islands and ecological differences between these toads might be relevant. For example, the area of land mass and fresh water resource of Kita-daito Island is smaller than Minami daito-Island. Since the amount of water is different between these islands, water quality (e.g. water pollution by pesticide for sugar cane, salinity) might be different. Furthermore, these toad species may have a similar ecological niche and may use similar fresh water environment to breed, suggesting that they are potentially competing with one another as seen in other anurans (Komine et al., 2019). *Bufo g. miyakonis* is native to Miyako Island which is an uplifted atoll similar to the Daito Islands, hence *B. g. miyakonis* might have an evolved higher endurance against limited water resources on Kita-daito Island than *R. marina*. Revealing the potential competition and species differences of endurance against drought, water pollution and salinity would provide important insight to understand the patterns of abundance. Additionally, the relative abundance of invasive species may be related to their ecological impact (Watari et al., 2008; Komine et al., in press), hence further studies are needed to address the impact of these two invasive species on native ecosystems.

To enhance the validity of our conclusion, we should collect data in other seasons and

years in the next study. Furthermore, we have no data on the number of individuals of each toad species initially introduced, or their initial introduction points. Initial population size might cause low genetic diversity, which might lead to population instability.

ACKNOWLEDGMENTS

We are especially grateful to Dr. Hidetoshi Ota for providing important information on invasive toads on the Daito Islands. We are highly grateful to Seiro Hadana, Ryousuke Higa, and Morio Shiroma for providing valuable information on the fauna of Kita-daito Island. We thank the people of the Daito Islands for supporting our field research. We appreciate Komine S and Komine I giving us broad support in this research. This study was financially supported by the Fujiwara Natural History Foundation.

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Accepted: 23 December 2019