Bombardier beetles repel invasive bullfrogs

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Invasive non-native predators negatively affect native species; however, some native species can survive the predation pressures of invasive species by using pre-existing antipredator strategies or evolving defenses against invasive predators. The American bullfrog *Lithobates catesbeianus* (Anura: Ranidae) has been intentionally introduced to many countries and regions, and has impacted native animals through direct predation. Bombardier beetles (Coleoptera: Carabidae: Brachininae: Brachinini) discharge chemicals at a temperature of approximately 100°C from the tip of the abdomen when they are attacked by predators. This "bombing" can successfully repel predators. However, adults of a native bombardier beetle *Pheropsophus* (*Stenaptinus*) *occipitalis jessoensis* have been reportedly found in the gut contents of the introduced bullfrog *L. catesbeianus* in Japan. These records suggest that the invasive bullfrog *L. catesbeianus* attacks the native bombardier beetle *P. occipitalis jessoensis* under field conditions in Japan; however, the effectiveness of the bombing defense against invasive bullfrogs is unclear. To test the effectiveness of the bombing defense against bullfrogs, we investigated the behavioral responses of *L. catesbeianus* juveniles to *P. occipitalis jessoensis* adults under laboratory conditions. Contrary to previous gut content results, almost all the bullfrogs (96.3%) rejected bombardier beetles before swallowing them; 81.5% rejected the beetles after being bombed, and 7.4% stopped attacking the beetles before being bombed. Only 3.7% successfully swallowed and digested the beetle. All of the beetles collected from bullfrog-invaded sites could deter bullfrogs, suggesting that the pre-existing defenses of bombardier beetles played an essential role in repelling bullfrogs. When treated beetles that were unable to discharge hot chemicals were provided, 77.8% of bullfrogs successfully swallowed and digested the treated beetles. These results indicate that bombing is important for the successful defense of *P. occipitalis jessoensis* against invasive bullfrogs. Although invasive bullfrogs have reportedly impacted native insect species, *P. occipitalis jessoensis* has an existing defense mechanism strong enough to repel the invasive predators.

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ABSTRACT

Invasive non-native predators negatively affect native species; however, some native species can survive the predation pressures of invasive species by using pre-existing antipredator strategies or evolving defenses against invasive predators. The American bullfrog *Lithobates catesbeianus* (Anura: Ranidae) has been intentionally introduced to many countries and regions, and has impacted native animals through direct predation. Bombardier beetles (Coleoptera: Carabidae: Brachininae: Brachinini) discharge chemicals at a temperature of approximately 100°C from the tip of the abdomen when they are attacked by predators. This "bombing" can successfully repel predators. However, adults of a native bombardier beetle *Pheropsophus* (*Stenaptinus*) *occipitalis jessoensis* have been reportedly found in the gut contents of the introduced bullfrog *L. catesbeianus* in Japan. These records suggest that the invasive bullfrog *L. catesbeianus* attacks the native bombardier beetle *P. occipitalis jessoensis* under field conditions in Japan; however, the effectiveness of the bombing defense against invasive bullfrogs is unclear. To test the effectiveness of the bombing defense against bullfrogs, we investigated the behavioral responses of *L. catesbeianus* juveniles to *P. occipitalis jessoensis* adults under laboratory conditions. Contrary to previous gut content results, almost all the bullfrogs (96.3%) rejected bombardier beetles before swallowing them; 81.5% rejected the beetles after being bombed, and 7.4% stopped attacking the beetles before being bombed. Only 3.7% successfully swallowed and digested the beetle. All of the beetles collected from bullfrog-invaded sites could deter bullfrogs, suggesting that the pre-existing defenses of bombardier beetles played an essential role in repelling bullfrogs. When treated beetles that were unable to discharge hot chemicals were provided, 77.8% of bullfrogs successfully swallowed and digested the treated beetles. These results indicate that bombing is important for the successful defense of *P. occipitalis jessoensis* against invasive bullfrogs. Although invasive bullfrogs have reportedly impacted native insect

species, *P. occipitalis jessoensis* has an existing defense mechanism strong enough to repel the invasive predators.

Subjects: Animal Behavior, Ecology, Entomology, Evolutionary Studies, Zoology

Keywords: Bombardier beetles, Brachinini, Carabidae, Chemical defences, Introduced predators, Invasive alien species

INTRODUCTION

Invasive non-native species negatively impact native biota (Doherty et al., 2016; Sugiura, 2016; David et al., 2017). In particular, invasive predators affect native communities and ecosystems through cascading effects (Goldschmidt, Witte & Wanink, 1993; O'Dowd, Green & Lake, 2003; Kenis et al., 2009; David et al., 2017; Rogers et al., 2017; McGruddy et al., 2021). Because native prey species do not share a co-evolutionary history with invasive predators (Fritts & Rodda, 1998; Strauss, Lau & Carroll, 2006; Carthey & Banks, 2014), many native species suffer predation by invasive species (Goldschmidt, Witte & Wanink, 1993; Sugiura, 2016; Doherty et al., 2016). However, some native species have survived the predation pressures of invasive species by using pre-existing antipredator strategies (Davis, Epp & Gabor, 2012; Carthey & Banks, 2014) or evolving defenses against invasive predators (Vermeij, 1982; Strauss, Lau & Carroll, 2006). Pre-existing antipredator defenses can play an important role in repelling invasive predators that have similar ecological traits to native predators (Carthey & Banks, 2014; Melotto et al., 2021). However, pre-existing defenses have received less attention than the evolution of anti-predator defenses in terms of native species' tolerance to invasive predators (Strauss, Lau & Carroll, 2006). Investigating the effectiveness of the pre-existing defenses of native species against invasive predators would enable a better understanding of how to mitigate the impacts of invasive predators on native species.

The American bullfrog *Lithobates catesbeianus* (Shaw) (Anura: Ranidae) has been intentionally introduced for various purposes to many countries and regions (western North America, South America, East and Southeast Asia, and Western Europe) from eastern North America (Ficetola et al., 2007; Ficetola, Thuiller & Miaud, 2007; Giovanelli, Haddad & Alexandrino, 2008; Bissattini & Vignoli, 2017; Groffen et al., 2019; Johovic et al., 2020). Eggs are laid in still water such as ponds (Govindarajulu, Price & Anholt, 2006). The larvae feed on algae, diatoms, cyanobacteria, protists, and tiny invertebrates in water (Kupferberg, 1997; Pryor, 2003; Ruibal & Laufer, 2012). Postmetamorphic juveniles and adults prey on various animals (including aquatic and terrestrial species) in and near water (Hirai, 2004; Govindarajulu, Price & Anholt, 2006; Dontchev & Matsui, 2016; Flynn, Kreofsky & Sepulveda, 2017; Laufer et al., 2021; Sarashina & Yoshida, 2021). Because bullfrog adults commonly reach a size (snout–vent length) of 180–200 mm (Werner, Wellborn & McPeek, 1995), they are able to swallow small vertebrates (e.g., fish, mammals, reptiles, and frogs) as well as invertebrates (Raney & Ingram, 1941; Stewart & Sandison, 1972; Bruneau & Magnin, 1980; Clarkson & DeVos, 1986; Govindarajulu, Price & Anholt, 2006; Flynn, Kreofsky & Sepulveda, 2017; Oda et al., 2019). Consequently, invasive bullfrogs have impacted native communities in invaded habitats (Kats & Ferrer, 2003; Li et al., 2011; Adriaens, Devisscher & Louette, 2013; Gobel, Laufer & Cortizas, 2019). Therefore, *L. catesbeianus* has been listed as one of the 100 ‘‘world’s worst invaders’’ (Lowe et al., 2000). Many studies have investigated the gut or stomach contents of adult and juvenile bullfrogs in native (Raney & Ingram, 1941; Korschgen & Moyle, 1955; Fulk & Whitaker, 1968; Stewart & Sandison, 1972; Bruneau & Magnin, 1980; Werner, Wellborn & McPeek, 1995) and invaded (Clarkson & DeVos, 1986; Balfour & Morey, 1999; Krupa, 2002; Hirai, 2004, 2005; Wu et al., 2005; Hirai & Inatani, 2008; Mori, 2008; Silva et al., 2009; Barrasso et al., 2009; Leivas, Leivas & Moura, 2012; Boelter et al., 2012; Jancowski & Orchard, 2013; Ortíz-Serrato, Ruiz-Campos & Valdez-Villavicencio, 2014; Quiroga et al., 2015; Liu et al., 2015; Dontchev & Matsui 2016; Flynn, Kreofsky & Sepulveda, 2017; Vrcibradic et al. 2017; Park et al. 2018; Bissattini, Buono & Vignoli, 2018, 2019; Oda et al., 2019; Matsumoto, Suwabe & Karube, 2020; Laufer et al., 2021; Nakamura & Tominaga, 2021) ranges, with the results indicating that introduced bullfrogs frequently attack native animal species in invaded areas.

However, few studies have directly observed how invasive bullfrogs can attack and swallow native prey. Investigating the attack behavior of bullfrogs would help to assess which native species suffer from bullfrog predation.

Adult bombardier beetles (Coleoptera: Carabidae: Brachininae: Brachinini) discharge toxic chemicals at a temperature of approximately 100°C (i.e., bombing) from the tip of abdomen when they are attacked by predators (Aneshansley et al., 1969; Dean, 1979; Eisner, Eisner & Siegler, 2005; Arndt et al., 2015; Sugiura, 2018, 2021). The hot chemicals can effectively protect the beetles from predators such as arthropods (Eisner, 1958; Eisner & Meinwald, 1966; Eisner & Dean, 1976; Eisner et al., 2006; Sugiura, 2021), amphibians (Eisner & Meinwald, 1966; Dean, 1980; Sugiura & Sato, 2018; Sugiura, 2018), reptiles (Bonacci et al., 2008), and birds (Kojima & Yamamoto, 2020). The bombardier beetle *Pheropsophus* (*Stenaptinus*) *occipitalis jessoensis* Morawitz (formerly called *Pheropsophus jessoensis*; Sugiura, 2018; Sugiura & Sato, 2018; Sugiura, 2021), which is commonly found in grassland, farmland, and forest edge environments in Japan, Korea, China, and Vietnam (Habu & Sadanaga, 1965; Yahiro et al., 1992; Ishitani & Yano, 1994; Fujisawa, Lee & Ishii, 2012; Ohwaki, Kaneko & Ikeda, 2015; Fedorenko, 2021), has been frequently studied to investigate the effectiveness of bombing as an anti-predator defense (Sugiura, 2018; Sugiura & Sato, 2018; Kojima & Yamamoto, 2020; Sugiura, 2021). When adults of *Ph. occipitalis jessoensis* are disturbed, they eject quinones (1,4-benzoquinone and 2-methyl-1,4-benzoquinone) and water (vapor) at a temperature of 100°C from the tip of the abdomen (Video S1; Kanehisa & Murase, 1977; Kanehisa, 1996). *Pheropsophus occipitalis jessoensis* can successfully deter birds (Kojima & Yamamoto, 2020), frogs (Sugiura, 2018), and praying mantises (Sugiura, 2021). However, adults of the bombardier beetle *Ph. occipitalis jessoensis* have been reportedly found in the stomach contents of field-collected bullfrogs in central Japan (Mori, 2008; Matsumoto, Suwabe & Karube, 2020). These records suggest that the invasive bullfrog *L. catesbeianus* attacks the native bombardier beetle *Ph. occipitalis jessoensis* under field conditions in Japan, but the bombing defense of *Ph. occipitalis jessoensis* against invasive bullfrogs remains unexplored. To test the effectiveness of the bombing defense against bullfrogs, we investigated how *L. catesbeianus* juveniles respond to *Ph. occipitalis jessoensis* adults under laboratory conditions. In addition, the responses of bullfrogs to *Ph. occipitalis jessoensis* collected from bullfrog-invaded sites were compared with those of beetles collected from non-invaded sites to investigate whether native bombardier beetles that coexist with invasive bullfrogs exhibit a stronger defense than beetles that do not coexist with bullfrogs.

MATERIALS AND METHODS

Study species

To investigate how bullfrogs respond to bombardier beetles under laboratory conditions, we used juveniles of the invasive bullfrog *L. catesbeianus* and adults of the bombardier beetle *Ph. occipitalis jessoensis*. The American bullfrog *L. catesbeianus* was intentionally introduced to Japan in 1918 (Matsui & Maeda, 2018). The introduced bullfrogs are commonly found in and near ponds, lakes, and paddy fields in Japan (Matsui & Maeda, 2018). The bombardier species *Ph. occipitalis jessoensis* is commonly found in grassland and farmland around the ponds, lakes, and paddy fields that bullfrogs have invaded in Japan. Bullfrog juveniles are much more abundant than the adults in the invaded areas in Japan (Sato & Nishihara, 2017; Matsumoto, Suwabe & Karube, 2020). Terrestrial arthropods, including beetles, are frequently identified in the gut or stomach contents of bullfrog juveniles (Hirai, 2004; Dontchev & Matsui, 2016; Sato & Nishihara, 2017; Matsui & Maeda, 2018; Sarashina & Yoshida, 2021; Nakamura & Tominaga, 2021). We found *Ph. occipitalis jessoensis* adults, *L. catesbeianus* juveniles, and native pond frogs *Pelophylax nigromaculatus* (Hallowell) (Anura: Ranidae) in the same grassland in Hyogo, central Japan, on the same date (Fig. 1). Therefore, bullfrog juveniles may frequently encounter adults of *Ph. occipitalis jessoensis* in grassland around ponds in Japan.

Fifty-four juvenile bullfrogs (snout–vent length: 42.2–59.6 mm) were collected from grassland around a pond in Hyogo, Japan, between August and October 2021. The bombardier beetle *Ph. occipitalis jessoensis* is frequently found in this sampling site. The snout–vent length and body weight of each bullfrog were measured to the nearest 0.01 mm and 0.1 mg, using an electronic slide caliper (CD-S15C, Mitutoyo, Kanagawa, Japan) and an electronic balance (CPA64, Sartorius Japan K.K., Tokyo, Japan), respectively. Juvenile bullfrogs were maintained separately in small plastic cages (120 × 85 × 130 mm, length × width × height) in the laboratory at 25°C (cf. Sugiura 2018, 2020b). Live mealworm larvae, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), were provided as food (cf. Sugiura, 2018, 2020b). Bullfrogs were starved for 24 h before the experiments to standardize their hunger level (cf. Sugiura, 2018, 2020b). Individual bullfrogs were not used repeatedly (cf. Sugiura, 2018, 2020b). Introduced bullfrogs have been designated as an "invasive alien species" in Japan. Therefore, transportation, laboratory keeping, and behavioral experiments of bullfrogs were performed with permission from the Kinki Regional Environmental Office of the Ministry of the Environment, Government of Japan (Number: 20000085).

Fifty-four adults of the bombardier beetle *Ph. occipitalis jessoensis* (body length: 15.2–20.2 mm) were collected from grasslands and farmlands in Hyogo (three sites), Shiga (one site), Kyoto (one site), and Shimane (one site), central Japan, in July–September 2020 and May–October 2021. Thirty-nine and 15 beetles were collected from bullfrog-invaded sites (three sites in Hyogo and one in Shimane) and non-invaded sites (one site in Kyoto and one in Shiga), respectively. Body length and body weight were measured to the nearest 0.01 mm and 0.1 mg using the electronic slide caliper and the electronic balance, respectively. Bombardier beetles were maintained separately in small plastic cases (diameter: 85 mm; height: 25 mm) in the laboratory at 25°C (cf. Sugiura, 2018; Sugiura & Sato, 2018; Sugiura, 2021). Dead larvae of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) were provided as food (cf. Sugiura, 2018; Sugiura & Sato, 2018; Sugiura, 2021). Individual beetles were not used repeatedly (cf. Sugiura, 2018; Sugiura & Sato, 2018; Sugiura, 2021).

Experiments

Following the method of Sugiura (2018, 2020b), we investigated how bullfrogs can attack bombardier beetles in our laboratory (25°C) between September and November 2021. Bullfrogs that ate mealworms >1 day before experiments were used. First, we placed a bullfrog in a plastic cage (120 × 85 × 130 mm, length × width × height). Then, we placed an adult beetle in the cage. We recorded the behavior of the bullfrog and the beetle using a digital camera (iPhone 12 Pro Max; Apple Inc., Cupertino, CA, USA) at 240 frames per second. When a bullfrog rejected a beetle, we played back the footage of the recorded behavior to investigate whether the rejection was due to bombing. When bombing sounds were heard or ejected vapor was seen, we considered that bombing forced the bullfrog to reject the beetle. When a bullfrog swallowed a beetle, we investigated whether it vomited the beetle (cf. Sugiura, 2018; Sugiura & Sato, 2018). Bullfrogs that did not vomit were considered to have digested the beetle. When a bullfrog did not swallow a beetle, we provided a mealworm as a palatable prey to the bullfrog several minutes after beetle rejection to determine whether this rejection was due to satiation (cf. Sugiura, 2018, 2020b). In total, 27 bullfrogs and 27 beetles were used in this experiment.

To test the role of hot chemical ejection by bombardier beetles in deterring bullfrogs, we provided bullfrogs with treated *Ph. occipitalis jessoensis* that were unable to eject hot chemicals (thereafter, treated beetles; cf. Sugiura & Sato, 2018; Sugiura, 2021). Following the method of

Sugiura & Sato (2018) and Sugiura (2021), we used forceps to repeatedly stimulate an adult *Ph. occipitalis jessoensis*. This treatment caused the beetle to release all the chemicals. We then used the same procedure as for the control beetles to observe whether a bullfrog successfully attacked the treated beetle in a transparent plastic case (length × width × height, 120 × 85 × 130 mm). In total, 27 bullfrogs and 27 beetles were used in the experiments. The sample size was determined based on the previous study (Sugiura, 2018).

All experiments were undertaken in accordance with the Kobe University Animal Experimentation Regulations (Kobe University's Animal Care and Use Committee, 30–01). No bullfrogs were injured during the feeding experiments. Because the release of bullfrogs into the wild is banned in Japan, the bullfrogs used in this study were euthanized by CO^2 asphyxiation after all experiments were conducted.

Data analysis

Fisher's exact test was used to compare the rejection rate of control beetles with that of treated beetles by bullfrogs; the rejection rate of bombardier beetles collected from invaded sites with that of beetles from non-invaded sites; and the rejection rate of control beetles by bullfrogs with that by native pond frogs. Data from Sugiura (2018) were also used as the rejection rate by the native pond frog *Pe. nigromaculatus*. Welch's *t*-test was used to compare the body size of bullfrogs and bombardier beetles between control and treated experiments. All the tests were performed at the 0.05 significance level. All analyses were performed using R ver. 3.5.2 (R Core Team, 2018).

RESULTS

All bullfrogs (n = 27) opened their mouths to attack bombardier beetles (control beetles);

however, 26 bullfrogs (96.3%) rejected bombardier beetles. The rejection was not due to satiation because 25 (96.2%) of the bullfrogs that rejected beetles ate mealworms immediately after the rejection. Only one bullfrog (3.7%) successfully swallowed and digested the beetle (Table 1). The swallowed beetle might not have bombed because no bombing sound was heard. Two bullfrogs (7.4%) rejected the beetles before being bombed; one bullfrog (3.7%) stopped attacking the beetle immediately after the tongue touched it, and one bullfrog (3.7%) spat out the beetle < 1 s after taking it into the mouth (Table 1). Two bullfrogs (7.4%) were bombed before taking the beetles into their mouths and immediately stopped the attack (Table 1). Twenty-two bullfrogs (81.5%) were bombed within 5 s of taking the beetles into their mouths and then spat them out within 2 s after being bombed (Video S2; Fig. 2; Table 1). The collection sites of bombardier beetles did not influence the rejection rates by bullfrogs (Fisher's exact test, $P = 1.0$); 94.4% of the beetles ($n = 18$) collected from bullfrog-invaded sites and 100% of the beetles ($n = 9$) from non-invaded sites were rejected by bullfrogs. The behavioral responses of bullfrogs to bombardier beetles were compared with those of the native pond frog species *Pe. nigromaculatus* (Fig. 3). The rate of swallowing and rejection of beetles did not significantly differ between the two species (Fisher's exact test, $P = 1.0$), but the rate of rejection before bombing significantly differed between the two species ($P = 0.000005$).

When treated beetles that were unable to bomb were provided, all bullfrogs ($n = 27$) attacked the beetles. Twenty-one bullfrogs (77.8%) successfully swallowed and digested treated beetles, while six bullfrogs (22.2%) spat out treated beetles within 7 s of taking them into their mouths (Table 1). All of the bullfrogs that rejected treated beetles ($n = 6$) ate mealworms after the rejection. The rejection rate of treated beetles by bullfrogs (22.2%) differed significantly from that of control beetles (96.3%; Table 1; Fisher's exact test, $P = 0.00000002$). These findings illustrated the importance of bombing for the successful defense of bombardier beetles against bullfrogs.

The body lengths and weights of treated beetles were not significantly different from those of control beetles (Table 2; t-test, $P = 0.16–0.86$). The snout–vent lengths and weights of bullfrogs that attacked control beetles were not significantly different from those of bullfrogs that attacked treated beetles (Table 2; t-test, $P = 0.50–0.66$).

DISCUSSION

The American bullfrog *L*. *catesbeianus* can eat any animals smaller than itself (Adriaens, Devisscher & Louette, 2013). Consequently, introduced bullfrogs have negatively affected native arthropods and amphibians through direct predation in invaded areas (Kats & Ferrer, 2003; Li et al., 2011; Adriaens, Devisscher & Louette, 2013; Gobel, Laufer & Cortizas, 2019; Groffen et al., 2019; Nakamura & Tominaga, 2021). Although the native bombardier beetle *Ph*. *occipitalis jessoensis* has reportedly been identified in the stomach contents of introduced bullfrogs in Japan (Mori, 2008; Matsumoto, Suwabe & Karube, 2020), our laboratory experiments showed that almost all bullfrogs rejected *Ph*. *occipitalis jessoensis* before swallowing them (Table 1). Therefore, *Ph*. *occipitalis jessoensis* can successfully repel invasive bullfrogs using a chemical weapon. To our knowledge, this is the first study to demonstrate the successful defense of a native insect species against invasive bullfrogs.

Some native species can evolve a tolerance to or defense against invasive predators (Strauss, Lau & Carroll, 2006). The high predation pressures by invasive bullfrogs may lead to the evolution of defenses in native prey species. However, all adults of *Ph*. *occipitalis jessoensis* collected from non-bullfrog-invaded sites could successfully defend against bullfrogs, suggesting that the pre-existing defense of *Ph*. *occipitalis jessoensis* was strong enough to repel bullfrogs. Like invasive bullfrogs, the native pond frog *Pe*. *nigromaculatus* has been shown to frequently reject *Ph. occipitalis jessoensis* under laboratory conditions (Fig. 3; Sugiura, 2018). Because both the native frog and the introduced bullfrog are frequently found in the same habitats in Japan (Fig. 1; Kambayashi et al., 2016; Sato, 2016; Tawa & Sagawa, 2017), the defenses of *Ph. occipitalis jessoensis* that originally functioned against native frogs could play an important role in repelling invasive bullfrogs.

Sugiura (2018) showed that 67.9% of *Pe. nigromaculatus* rejected *Ph. occipitalis jessoensis* before being bombed (Fig. 3). The native frog species stopped attacking beetles immediately after their tongues contacted the beetles, indicating that this frog species may avoid being bombed by detecting chemicals on the surface of the beetle (Sugiura, 2018). The present study showed that only 7.4% of bullfrogs rejected *Ph. occipitalis jessoensis* before being bombed (Fig. 3). Therefore, bombing by *Ph. occipitalis jessoensis* is much more important for a successful defense against invasive bullfrogs than against native frogs. Unlike native frogs, bullfrogs may not use their tongue to detect a deterrent chemical or the physical characteristics of *Ph. occipitalis jessoensis*. Alternatively, introduced bullfrogs may be unlikely to avoid being bombed by *Ph. occipitalis jessoensis* because the bullfrogs do not share a co-evolutionary history with *Ph. occipitalis jessoensis*. These hypotheses should be tested by investigating the responses of bullfrogs to the American bombardier beetle species that shares a co-evolutionary history with bullfrogs in the native habitat of North America (approximately 50 species recorded from the United States of America; Anichtchenko et al., 2022).

Adults of *Ph. occipitalis jessoensis* were found in the stomach contents of introduced bullfrogs in Japan (Mori, 2008; Matsumoto, Suwabe & Karube, 2020), although our results showed that almost all bullfrogs failed to eat adult *Ph. occipitalis jessoensis*. This inconsistency may be caused by the different body sizes of the bullfrogs used in the present and previous studies. Matsumoto, Suwabe & Karube (2020) found an adult *Ph. occipitalis jessoensis* in the stomach content of a juvenile bullfrog (snout–vent length: 83 mm) that was larger than the juveniles used in our experiments (snout–vent length: 43.4–59.6 mm). This suggests that *Ph. occipitalis jessoensis* may fail to defend itself against bullfrog adults and large juveniles. The importance of predator size for the successful defense of *Ph. occipitalis jessoensis* was suggested by Sugiura and Sato (2018) who showed that adult and large juvenile toads could more frequently eat adult *Ph. occipitalis jessoensis* than the small juveniles. However, juvenile bullfrogs of the size used in this study are much more abundant than the adults and large juveniles in invaded areas in Japan (Sato & Nishihara, 2017; Matsumoto, Suwabe & Karube, 2020). Therefore, unlike other native insect species, the native bombardier beetle *Ph. occipitalis jessoensis* may not suffer predation by invasive bullfrogs.

CONCLUSIONS

Bombardier beetles have chemical weapons to deter various types of predators (Eisner, Eisner & Siegler, 2005; Sugiura, 2020a). The native bombardier beetle *Ph. occipitalis jessoensis* can defend itself against the native pond frog *Pe. nigromaculatus* (Sugiura, 2018) and other native predators (Sugiura & Sato, 2018; Kojima & Yamamoto, 2020; Sugiura, 2021). Therefore, *Ph. occipitalis jessoensis* uses its pre-existing defense to defend against invasive bullfrogs, which occupy a similar niche to that of native pond frogs.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

• Shinji Sugiura conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

• Tomoki Date performed the experiments, prepared figures and/or tables, reviewed drafts of the paper, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

The insect and frog species investigated in this study were not protected species. Our study was not performed in a protected area or national park. No permissions were needed to collect non-protected insect and frog species in non-protected areas in Japan. Introduced bullfrogs has been designated as one of "invasive alien species" in Japan. Therefore, transportation, laboratory keeping, and behavioral experiments of bullfrogs were performed with permission from the Kinki Regional Environmental Office of the Ministry of the Environment Government of Japan (Number: 20000085).

Data Availability

The following information was supplied regarding data availability:

The raw data are available at figshare: Sugiura S, Date T. (2021): Data from: Bombardier beetles repel invasive bullfrogs. figshare. Dataset. https://figshare.com/s/4fe406cf2d835905a4f6

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/.

REFERENCES

Adriaens T, Devisscher S, Louette G. 2013. *Risk analysis report of non-native organisms in Belgium: risk analysis of American bullfrog Lithobates catesbeianus (Shaw)*. Brussel: Instituut voor Natuur- en Bosonderzoek.

Aneshansley DT, Eisner T, Widom JM, Widom B. 1969. Biochemistry at 100°C: explosive secretory discharge of bombardier beetles (*Brachinus*). *Science* **165(3888)**:61–63 DOI 10.1126/science.165.3888.61.

Anichtchenko A, Choi JB, Facchini S, Marrero J, Panin R, Potanin D, Roguet D, Solodovnikov I, Will KW. 2022. Carabidae of the World. Available at https://carabidae.org/ taxa/licinini-bonelli (accessed 23 February 2022).

Arndt EM, Moore W, Lee WK, Ortiz C. 2015. Mechanistic origins of bombardier beetle (Brachinini) explosion-induced defensive spray pulsation. *Science* **348(6234)**:563–567 DOI 10.1126/science.1261166.

Balfour PS, Morey SR. 1999. Prey selection by juvenile bullfrogs in a constructed vernal pool complex. *Transactions of the Western Section of the Wildlife Society* **35**:34–40.

Barrasso DA, Cajade R, Nenda S, Baloriani G, Herrera R. 2009. Introduction of the American bullfrog *Lithobates catesbeianus* (Anura: Ranidae) in natural and modified environments: an increasing conservation problem in Argentina. *South American Journal of Herpetology* **4(1)**:69–75 DOI 10.2994/057.004.0109.

Bissattini AM, Vignoli L. 2017. Let's eat out, there's crayfish for dinner: American bullfrog niche shifts inside and outside native ranges and the effect of introduced crayfish. *Biological Invasions* **19**:2633–2646 DOI 10.1007/s10530-017-1473-6.

Bissattini AM, Buono V, Vignoli L. 2018. Field data and worldwide literature review reveal that alien crayfish mitigate the predation impact of the American bullfrog on native amphibians. *Aquatic Conservation: Marine and Freshwater Ecosystems* **28(6)**:1465–1475 DOI 10.1002/aqc.2978.

Bissattini AM, Buono V, Vignoli L. 2019. Disentangling the trophic interactions between American bullfrogs and native anurans: Complications resulting from post-metamorphic ontogenetic niche shifts. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29(2)**:270–281 DOI 10.1002/aqc.3023.

Boelter RA, Kaefer IL, Both C, Cechin S. 2012. Invasive bullfrogs as predators in a Neotropical assemblage: What frog species do they eat? *Animal Biology* **62(4)**:397–408 DOI 10.1163/157075612X634111.

Bonacci T, Aloise G, Brandmayr P, Brandmayr TZ, Capula M. 2008. Testing the predatory behaviour of *Podarcis sicula* (Reptilia: Lacertidae) towards aposematic and non-aposematic preys. *Amphibia-Reptilia* **29(3)**: 449–453 DOI 10.1163/156853808785111986.

Bruneau M, Magnin E. 1980. Croissance, nutrition et reproduction de souaouarons *Rana catesbeiana* Shaw (Amphibia Anura) des Laurentides au nord de Montréal. *Canadian Journal of Zoology* **58(2)**:175–183 DOI 10.1139/z80-019.

Carthey AJR, Banks PB. 2014. Naïveté in novel ecological interactions: lessons from theory and experimental evidence. *Biological Reviews* **89(4)**:932–949 DOI 10.1111/brv.12087.

Clarkson RW, DeVos JCJ. 1986. The bullfrog, *Rana catesbeiana* Shaw, in the lower Colorado River, Arizona-California. *Journal of Herpetology* **20(1)**:42–49.

David P, Thébault E, Anneville O, Duyck PF, Chapuis E. 2017. Impacts of invasive species on food webs: a review of empirical data. *Advances in Ecological Research* **56**:1–60 DOI 10.1016/bs.aecr.2016.10.001.

Davis DR, Epp KJ, Gabor CR. 2012. Predator generalization decreases the effect of introduced predators in the San Marcos salamander, *Eurycea nana*. *Ethology* **118(12)**:1191–1197 DOI 10.1111/eth.12025.

Dean J. 1979. Defensive reaction time of bombardier beetles: an investigation of the speed of a chemical defense. *Journal of Chemical Ecology* **5(5)**:691–701 DOI 10.1007/BF00986554.

Dean J. 1980. Encounters between bombardier beetles and two species of toads (*Bufo americanus*, *B. marinus*): speed of prey-capture does not determine success. *Journal of Comparative Physiology* **135(1)**:41–50 DOI 10.1007/BF00660180.

Doherty TS, Glen AS, Nimmo DG, Ritchie EG, Dickman CR. 2016. Invasive predators and global biodiversity loss. *Proceeding of the National Academy of Sciences USA* **113(40)**:11261–11265 DOI 10.1073/pnas.1602480113.

Dontchev K, Matsui M. 2016. Food habits of the American bullfrog *Lithobates catesbeianus* in the city of Kyoto, central Japan. *Current Herpetology* **35(2)**:93–100 DOI 10.5358/hsj.35.93.

Eisner T. 1958. The protective role of the spray mechanism of the bombardier beetle, *Brachynus ballistarius* Lec. *Journal of Insect Physiology* **2(3)**:215–220.

Eisner T, Dean J. 1976. Ploy and counterploy in predator–prey interactions: orb-weaving spiders versus bombardier beetles. *Proceeding of the National Academy of Sciences USA* **73(4)**:1365–1367 DOI 10.1073/pnas.96.17.9705.

Eisner T, Meinwald J. 1966. Defensive secretions of arthropods. *Science* **153(3742)**:1341–1350. DOI 10.1126/science.153.3742.1341.

Eisner T, Eisner M, Siegler M. 2005. *Secret weapons: defenses of insects, spiders, scorpions, and other many-legged creatures*. Cambridge: The Belknap Press of the Harvard University Press.

Eisner T, Aneshansley DJ, del Campo ML, Eisner M, Frank JH, Deyrup M. 2006. Effect of bombardier beetle spray on a wolf spider: repellency and leg autotomy. *Chemoecology* **16(4)**:185–189 DOI 10.1007/s00049-006-0346-8.

Fedorenko DN. 2021. *Stenaptinus* (Coleoptera: Carabidae: Brachininae) of Vietnam. Note 3. *Russian Entomology Journal* **30(3)**:252–263 DOI 10.15298/rusentj.30.3.02.

Ficetola GF, Coïc C, Detaint M, Berroneau M, Lorvelec O, Miaud C. 2007. Pattern of distribution of the American bullfrog *Rana catesbeiana* in Europe. *Biological Invasions* **9(7)**:767–772 DOI 10.1007/s10530-006-9080-y.

Ficetola GF, Thuiller W, Miaud C. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species — the American bullfrog. *Diversity and Distributions* **13(4)**:476–485 DOI 10.1111/j.1472-4642.2007.00377.x.

Flynn LM, Kreofsky TM, Sepulveda AJ. 2017. Introduced American bullfrog distribution and diets in Grand Teton National Park. *Northwest Science* **91(3)**:244–256 DOI

10.3955/046.091.0305.

Fritts TH, Rodda GH. 1998. The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annual Review of Ecology and Systematics* **29**:113–140.

Fujisawa T, Lee CM, Ishii M. 2012. Species diversity of ground beetle assemblages in the distinctive landscapes of the Yodo River flowing through northern Osaka Prefecture, central Japan. *Japanese Journal of Environmental Entomology and Zoology* **23(2)**:89–100 DOI 10.11257/jjeez.23.89.

Fulk FD, Whitaker JO Jr. 1968. The food of *Rana catebeiana* in three habitats in Owen Country, Indiana. *Proceedings of the Indiana Academy of Science* **78**:491–496.

Giovanelli JGR, Haddad CFB, Alexandrino J. 2008. Predicting the potential distribution of the alien invasive American bullfrog (*Lithobates catesbeianus*) in Brazil. *Biological Invasions* **10(5)**:585–590 DOI 10.1007/s10530-007-9154-5.

Gobel N, Laufer G, Cortizas S. 2019. Changes in aquatic communities recently invaded by a top predator: evidence of American bullfrogs in Aceguá, Uruguay. *Aquatic Sciences* **81(1)**:8 DOI 10.1007/s00027-018-0604-1.

Goldschmidt T, Witte F, Wanink J. 1993. Cascading effects of the introduced Nile perch on the detritivorous/phytoplanktivorous species in the sublittoral areas of Lake Victoria. *Conservation Biology* **7(3)**:686–700 DOI 10.1046/j.1523-1739.1993.07030686.x.

Govindarajulu P, Price WS, Anholt BR. 2006. Introduced bullfrogs (*Rana catesbeiana*) in western Canada: Has their ecology diverged? *Journal of Herpetology* **40(2)**:249–260.

Groffen J, Kong S, Jang Y, Borzée A. 2019. The invasive American bullfrog (*Lithobates catesbeianus*) in the Republic of Korea: history and recommendations for population control. *Management of Biological Invasions* **10(3)**:517–535 DOI

10.3391/mbi.2019.10.3.08.
Habu A, Sadanaga K. 1965. Illustrations for identification of larvae of the Carabidae found in cultivated fields and paddy-fields (III). *Bulletin of the National Institute of Agricultural Sciences, Series C: Plant Pathology and Entomology* **19**:81–216 (in Japanese with English summary).
Hirai T. 2004. Diet composition of introduced bullfrog, *Rana catesbeiana*, in the Mizorogaike Pond of Kyoto, Japan. *Ecological Research* **19(4)**:375–380 DOI 10.1111/j.1440-1703.2004.00647.x.
Hirai T. 2005. On the giant water bug, *Lethocerus deyrolli*, found in stomach contents of a bullfrog, *Rana catesbeiana*. *Bulletin of Kansai Organization for Nature Conservation* **27(1)**:57–58. (in Japanese with English summary).
Hirai T, Inatani Y. 2008. Predation by *Rana catesbeiana* on an adult male of *R. porosa brevipoda*. *Bulletin of the Herpetological Society of Japan* **2008(1)**:6–7. (in Japanese).
Ishitani M, Yano K. 1994. Species composition and seasonal activities of ground beetles (Coleoptera) in a fig orchard. *Japanese Journal of Entomology* **62(1)**:201–210.
Jancowski K, Orchard SA. 2013. Stomach contents from invasive American bullfrogs *Rana catesbeiana* (= *Lithobates catesbeianus*) on southern Vancouver Island, British Columbia, Canada. *NeoBiota* **16**:17–37 DOI 10.3897/neobiota.16.3806.
Johovic I, Gama M, Banha F, Tricarico E, Anastácio PM. 2020. A potential threat to amphibians in the European Natura 2000 network: Forecasting the distribution of the American bullfrog *Lithobates catesbeianus*. *Biological Conservation* **245**:108551 DOI 10.1016/j.biocon.2020.108551.
Kambayashi C, Uto T, Shioji T, Kurabayashi A, Shimizu N. 2016. Amphibian fauna in the Higashi-Hiroshima Campus, Hiroshima University. *Bulletin of the Hiroshima University*

Museum **8**:17–29. (in Japanese with English Abstract).

Kanehisa K. 1996. Secretion of defensive substance by Carabidae and Brachinidae. *Bulletin of the Research Institute for Bioresources, Okayama University* **4(1)**:9–23 (in Japanese with English summary).

Kanehisa K, Murase M. 1977. Comparative study of the pygidial defensive systems of carabid beetles. *Applied Entomology and Zoology* **12(3)**:225–235 DOI 10.1303/aez.12.225.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9(2)**:99–110 DOI 10.1046/j.1472-4642.2003.00013.x.

Kenis M, Auger-Rozenberg MA, Roques A, Timms L, Péré C, Cock MJW, Settele J, Augustin S, Lopez-Vaamonde C. 2009. Ecological effects of invasive alien insects. *Biological Invasions* **11(1)**:21–45 DOI 10.1007/s10530-008-9318-y.

Kojima W, Yamamoto R. 2020. Defense of bombardier beetles against avian predators. *The Science of Nature* **107(4)**:36 DOI 10.1007/s00114-020-01692-z.

Korschgen LJ, Moyle DL. 1955. Food habits of the bullfrog in central Missouri farm ponds. *The American Midland Naturalist* **54(2)**:332–341.

Krupa JJ. 2002. Temporal shift in diet in a population of American bullfrog (*Rana catesbeiana*) in Carlsbad Caverns National Park. *The Southwestern Naturalist* **47(3)**:461–467.

Kupferberg SJ. 1997. The role of larval diet in anuran metamorphosis. *American Zoologist* **37(2)**:146–159 DOI 10.1093/icb/37.2.146.

Laufer G, Gobel N, Berazategui M, Zarucki M, Cortizas S, Soutullo A, Martinez-Debat C, de Sá RO. 2021. American bullfrog (*Lithobates catesbeianus*) diet in Uruguay compared with other invasive populations in Southern South America. *North-Western Journal of Zoology* **17(1)**:e211502.

Leivas PT, Leivas FWT, Moura MO. 2012. Diet and trophic niche of *Lithobates catesbeianus* (Amphibia: Anura). *Zoologia* **29(5)**:405–412 DOI 10.1590/S1984-46702012000500003.

Li Y, Ke Z, Wang Y, Blackburn TM. 2011. Frog community responses to recent American bullfrog invasions. *Current Zoology* **57(1)**:83–92 DOI 10.1093/czoolo/57.1.83.

Liu X, Luo Y, Chen J, Guo Y, Bai C, Li Y. 2015. Diet and prey selection of the invasive American bullfrog (*Lithobates catesbeianus*) in southwestern China. *Asian Herpetological Research* **6(1)**:34–44 DOI: 10.16373/j.cnki.ahr.140044.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. *100 of the world's worst invasive alien species: a selection from the global invasive species database*. Auckland: IUCN/SSC Invasive Species Specialist Group (ISSG).

Matsui M, Maeda N. 2018. *Encyclopedia of Japanese frogs*. Tokyo: Bun-ichi Sogo Shuppan.

Matsumoto R, Suwabe S, Karube H. 2020. Diet of *Xenopus laevis* and *Lithobates catesbeianus* trapped in Nakaogino Area, Atsugi, Kanagawa Prefecture, Japan. *Bulletin of the Kanagawa Prefectural Museum (Natural Science)* **49**:85–99. (in Japanese with English abstract).

McGruddy RA, Howse MWF, Haywood J, Ward CJI, Staufer TB, Hayek-Williams M, Toft RJ, Lester PJ. 2021. Invasive paper wasps have strong cascading effects on the host plant of monarch butterflies. *Ecological Entomology* **46(2)**:459–469 DOI 10.1111/een.12992.

Melotto A, Ficetola GF, Alari E, Romagnoli S, Manenti R. 2021. Visual recognition and coevolutionary history drive responses of amphibians to an invasive predator. *Behavioral Ecology* **32(6)**:1352–1362 DOI 10.1093/beheco/arab101.

Mori I. 2008. Predation by introduced bullfrog *Rana catesbeiana* on a breeding male of *Rhacophorus schlegelii* and the other animals. *Bulletin of the Okayama Prefecture Nature Conservation Center* **16**:61–62 (in Japanese).

Nakamura Y, Tominaga A. 2021. Diet of the American bullfrog *Lithobates catesbeianus* naturalized on Okinawajima, Ryukyu Archipelago, Japan. *Current Herpetology* **40(1)**:40–53 DOI 10.5358/hsj.40.40.

Oda FH, Guerra V, Grou E, de Lima LD, Proença HC, Gambale PG, Takemoto RM, Teixeira CP, Campião KM, Ortega JCG. 2019. Native anuran species as prey of invasive American bullfrog *Lithobates catesbeianus* in Brazil: a review with new predation records. *Amphibian & Reptile Conservation* **13(2)**:217–226.

O'Dowd DJ, Green PT, Lake PS. 2003. Invasional 'meltdown' on an oceanic island. *Ecology Letters* **6(9)**: 812-817 DOI 10.1046/j.1461-0248.2003.00512.x.

Ohwaki A, Kaneko Y, Ikeda H. 2015. Seasonal variability in the response of ground beetles (Coleoptera: Carabidae) to a forest edge in a heterogeneous agricultural landscape in Japan. *European Journal of Entomology* **112(1)**:135–144 DOI 10.14411/eje.2015.022.

Ortíz-Serrato L, Ruiz-Campos G, Valdez-Villavicencio JH. 2014. Diet of the exotic American bullfrog, *Lithobates catesbeianus* in a stream of northwestern Baja California, Mexico. *Western North American Naturalist* **74(1)**:116–122 DOI 10.3398/064.074.0112.

Park CD, Lee CW, Lim JC, Yang BG, Lee JH. 2018. A study on the diet items of American bullfrog (*Lithobates catesbeianus*) in Ga-hang wetland, Korea. *Korean Journal of Environment and Ecology* **32(1)**:55–65 DOI 10.13047/KJEE.2018.32.1.55 (in Korean with English abstract).

Pryor GS. 2003. Growth rates and digestive abilities of bullfrog tadpoles (*Rana catesbeiana*) fed algal diets. *Journal of Herpetology* **37(3)**:560–566 DOI 10.1670/153-02N.

Quiroga LB, Moreno MD, Cataldo AA, Aragón-Traverso JH, Pantano MV, Olivares JPS, Sanabria EA. 2015. Diet composition of an invasive population of *Lithobates catesbeianus* (American Bullfrog) from Argentina. *Journal of Natural History* **49(27–28)**:1703–1716 DOI 10.1080/00222933.2015.1005711.

R Core Team. 2018. *R, a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Raney EC, Ingram WM. 1941. Growth of tagged frogs (*Rana catesbeiana* Shaw and *Rana clamitans* Daudin) under natural conditions. *The American Midland Naturalist* **26(1)**:201–206.

Rogers HS, Buhle ER, HilleRisLambers J, Fricke EC, Miller RH, Tewksbury JJ. 2017. Effects of an invasive predator cascade to plants via mutualism disruption. *Nature Communications* **8**:14557 DOI 10.1038/ncomms14557.

Ruibal M, Laufer G. 2012. Bullfrog *Lithobates catesbeianus* (Amphibia: Ranidae) tadpole diet: description and analysis for three invasive populations in Uruguay. *Amphibia-Reptilia* **33(3–4)**:355–363 DOI 10.1163/15685381-00002838.

Sarashina M, Yoshida T. 2021. Diet composition of the invasive American bullfrog (*Lithobates catesbeianus*) in Onuma Quasi-National Park, Hokkaido, Japan. *Current Herpetology* **40(1)**:77–82 DOI 10.5358/hsj.40.77.

Sato R, Nishihara S. 2017. Impacts of invasive bullfrogs and the control strategy. In: Takahashi K ed. *Recovering fish*. Tokyo: Kouseisha, 68–80.

Sato T. 2016. Reptiles and amphibians distributed on the periphery of an army cemetery in Nara City. *Annual Bulletin of Oyasato Institute for the Study of Religion, Tenri University* **22**:49–74. (in Japanese with English summary).

Silva ET, Reis EP, Feio RN, Filho OPR. 2009. Diet of the invasive frog *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae) in Viçosa, Minas Gerais state, Brazil. *South American Journal of Herpetology* **4(3)**:286–294 DOI 10.2994/057.004.0312.

Stewart MM, Sandison P. 1972. Comparative food habits of sympatric mink frogs, bullfrogs, and green frogs. *Journal of Herpetology* **6(3/4)**:241–244.

Strauss SY, Lau JA, Carroll SP. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters* **9(3)**:357–374. DOI 10.1111/j.1461-0248.2005.00874.x.

Sugiura S. 2016. Impacts of introduced species on the biota of an oceanic archipelago: the relative importance of competitive and trophic interactions. *Ecological Research* **31(2)**:155–164 DOI 10.1007/s11284-016-1336-0.

Sugiura S. 2018. Anti-predator defences of a bombardier beetle: is bombing essential for successful escape from frogs? *PeerJ* **6**:e5942 DOI 10.7717/peerj.5942.

Sugiura S. 2020a. Predators as drivers of insect defenses. *Entomological Science* **23(3)**:316–337 DOI 10.1111/ens.12423.

Sugiura S. 2020b. Active escape of prey from predator vent via the digestive tract. *Current Biology* **30(15)**:R867–R868 DOI 10.1016/j.cub.2020.06.026.

Sugiura S. 2021. Beetle bombing always deters praying mantises. *PeerJ* **9**:e11657 DOI 10.7717/peerj.11657.

Sugiura S, Sato T. 2018. Successful escape of bombardier beetles from predator digestive systems. *Biology Letters* **14(2)**:20170647 DOI 10.1098/rsbl.2017.0647.

Tawa K, Sagawa S. 2017. Breeding habitats of frogs in paddy field and fallow field biotopes in Shounji (Toyooka City, Hyogo Prefecture). *Yaseihukki* **5**:29–38. (in Japanese with English Abstract).

Vermeij GJ. 1982. Phenotypic evolution in a poorly dispersing snail after arrival of a predator. *Nature* **299(5881)**:349–350 DOI 10.1038/299349a0.

Vrcibradic D, Diaz A, Cosendey BN, Nascimento BB, Borges-Júnior VNT. 2017. *Trichodactylus dentatus* (Crustacea, Decapoda, Trichodactylidae) and other prey of a large adult of the exotic American bullfrog, *Lithobates catesbeianus* (Ranidae), caught in a disturbed habitat in southeastern Brazil. *Herpetology Notes* **10**:375–378.

Werner EE, Wellborn GA, McPeek MA. 1995. Diet composition in postmetamorphic bullfrogs and green frogs: implications for interspecific predation and competition. *Journal of Herpetology* **29(4)**:600–607. .

Wu Z, Li Y, Wang Y, Adams MJ. 2005. Diet of introduced bullfrogs (*Rana catesbeiana*): predation on and diet overlap with native frogs on Daishan Island, China. *Journal of Herpetology* **39(4)**:668–674.

Yahiro K, Fujimoto T, Tokuda M, Yano K. 1992. Species composition and seasonal abundance of ground beetles (Coleoptera) in paddy fields. *Japanese Journal of Entomology* **60(4)**:805–813.

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Figure legend

Figure 1 A bombardier beetle, an invasive bullfrog, and a native frog. (A) An adult bombardier beetle *Pheropsophus occipitalis jessoensis*. (B) A juvenile bullfrog *Lithobates catesbeianus*. (C) An adult pond frog *Pelophylax nigromaculatus*. These photographs were taken at the same site and microhabitat on the same date. Photo credit: Shinji Sugiura.

Figure 2 Temporal sequence of the bullfrog *Lithobates catesbeianus* rejecting a control adult *Pheropsophus occipitalis jessoensis*. (A) 0 ms. (B) 375 ms. (C) 900 ms. (D) 2,200 ms. (E) 2,575 ms. (F) 2,625 ms. (G) 2,650 ms (H) 3,475 ms. The bullfrog spat out the beetle after taking it into its mouth. Bombing by the beetle was audible and the ejected vapor (E) was observed just before the bullfrog spat out the beetle (see Video S2). Credit: Shinji Sugiura and Tomoki Date.

Figure 3 Behavioral responses of the invasive bullfrog *Lithobates catesbeianus* and the native pond frog *Pelophylax nigromaculatus* to adults of the bombardier beetle *Pheropsophus occipitalis jessoensis*. Swallow: bullfrogs or frogs successfully swallowed control beetles. Reject before bombed: bullfrogs or frogs stopped attacking control beetles before being bombed. Reject after bombed: bullfrogs or frogs rejected control beetles after being bombed. The graph showing data for *Pe*. *nigromaculatus* was taken from Sugiura (2018). Photo credit: Shinji Sugiura.

Supplemental Information

Video S1 An adult *Pheropsophus occipitalis jessoensis* discharging hot chemicals. The beetle discharged quinones and water vapor in response to the forceps. This video is from Sugiura (2018). Video credit: Shinji Sugiura.

Video S2 A juvenile bullfrog *Lithobates catesbeianus* rejecting a control adult of the bombardier beetle *Pheropsophus occipitalis jessoensis*. The bullfrog took the beetle into its mouth, but spat out the beetle immediately after being bombed by the beetle. Video credit: Shinji Sugiura and Tomoki Date.

Table 1(on next page)

Table 1

Responses of the bullfrog *Lithobates catesbeianus* to control and treated adults of the bombardier beetle *Pheropsophus occipitalis jessoensis*.

Table 1 Responses of the bullfrog *Lithobates catesbeianus* to control and treated adults of the bombardier beetle *Pheropsophus occipitalis jessoensis*.

Frog response [a]	Frog behavior [b]	Control beetles [c] % (*n*)	Treated beetles [c] % (*n*)
Eat	Swallow	3.7 (1)	77.8 (21)
Reject (subtotal)		96.3 (26)	22.2 (6)
Reject before bombed	Stop attack	3.7 (1)	0.0 (0)
	Spit out	3.7 (1)	22.2 (6)
Reject after bombed	Stop attack	7.4 (2)	–
	Spit out	81.5 (22)	–
Total		100.0 (27)	100.0 (27)

Notes:

[a] Eat: bullfrogs successfully ate beetles. Reject before bombed: bullfrogs rejected beetles before or without being bombed. Reject after bombed: bullfrogs rejected beetles after being bombed.

[b] Swallow: bullfrogs successfully swallowed beetles. Stop attack: bullfrogs stopped attacking beetles before taking them into their mouths. Spit out: bullfrogs spat out beetles after taking them into their mouths.

[c] Control beetles and treated beetles are the *Pheropsophus occipitalis jessoensis* that were able and unable to discharge hot chemicals, respectively.

Table 2(on next page)

Table 2

Sizes of the bombardier beetle *Pheropsophus occipitalis jessoensis* and the bullfrog *Lithobates catesbeianus* used in this study.

Table 2 Sizes of the bombardier beetle *Pheropsophus occipitalis jessoensis* and the bullfrog *Lithobates catesbeianus* used in this study.

Species	Boy size	Treatment		Statistical comparison	
		Control beetles $n = 27$	Treated beetle $n = 27$	t value	P value
Bombardier beetle	Body length (mm) [a]	17.6 ± 0.2 (15.2–20.2)	17.6 ± 0.2 (15.5–19.6)	0.25	0.80
	Boy weight (mg) [a]	265.8 ± 12.4 (149.1–411.3)	241.7 ± 11.4 (146.5–376.2)	1.43	0.16
			268.9 ± 12.4 (164.4–409.9) [b]	(−0.18) [c]	(0.86) [c]
Bullfrog	Snout–vent length (mm) [a]	48.2 ± 0.8 (43.5–59.6)	47.8 ± 0.7 (42.2–57.3)	0.44	0.66
	Body weight (mg) [a]	9206.6 ± 554.7 (6136.9–18257.1)	8720.9 ± 458.1 (5575.6–16763.8)	0.68	0.50

Notes:

[a] Values are the mean ± standard error (range: minimum–maximum).

[b] Body weight of bombardier beetles before treatment.

[c] Statistical result of a comparison between treated beetles (before treatment) and control beetles.

Figure 1

Figure 1

A bombardier beetle, an invasive bullfrog, and a native frog. (A) An adult bombardier beetle *Pheropsophus occipitalis jessoensis*. (B) A juvenile bullfrog *Lithobates catesbeianus*. (C) An adult pond frog *Pelophylax nigromaculatus*. These photographs were taken at the same site and microhabitat on the same date. Photo credit: Shinji Sugiura.

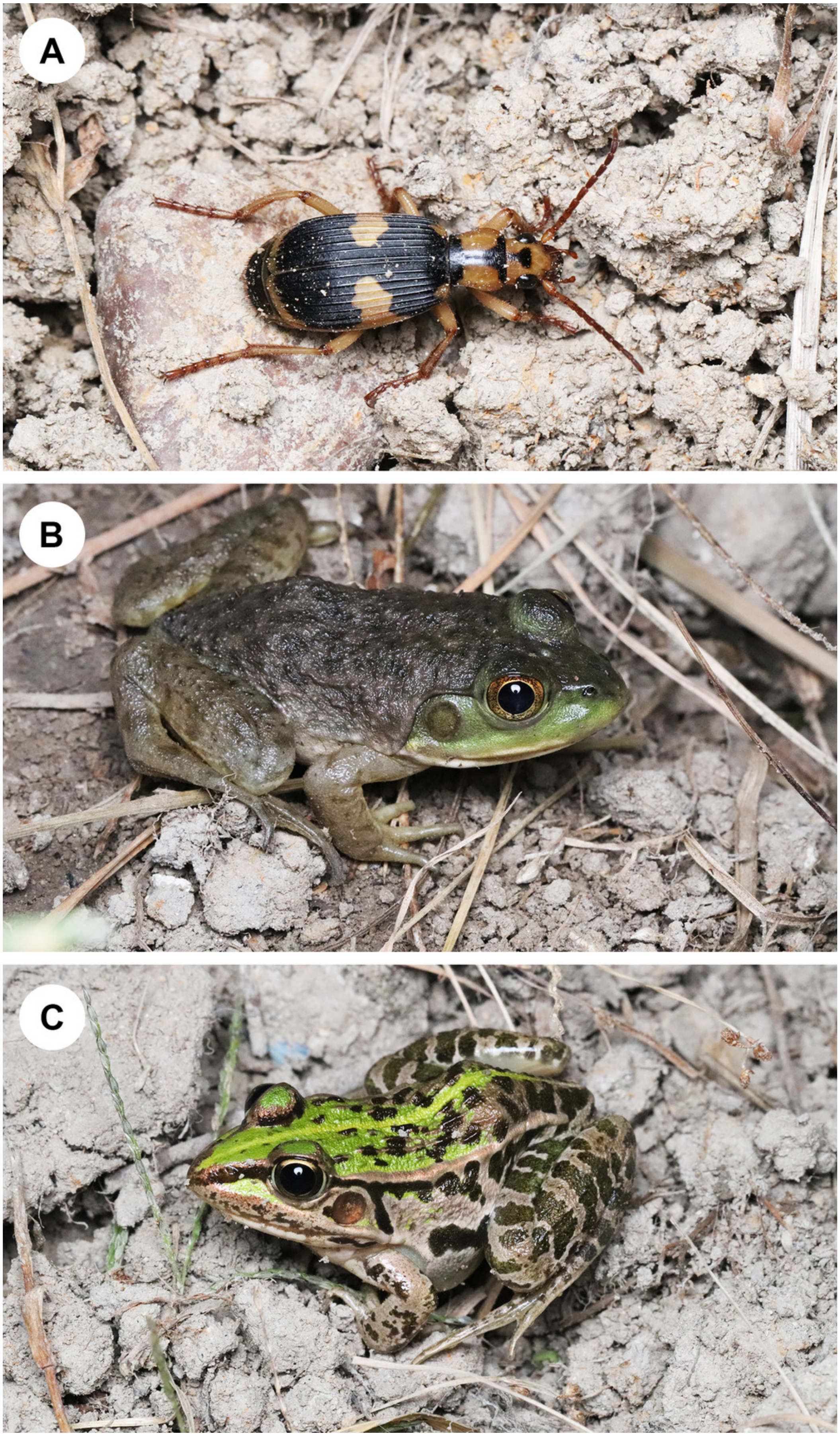
A
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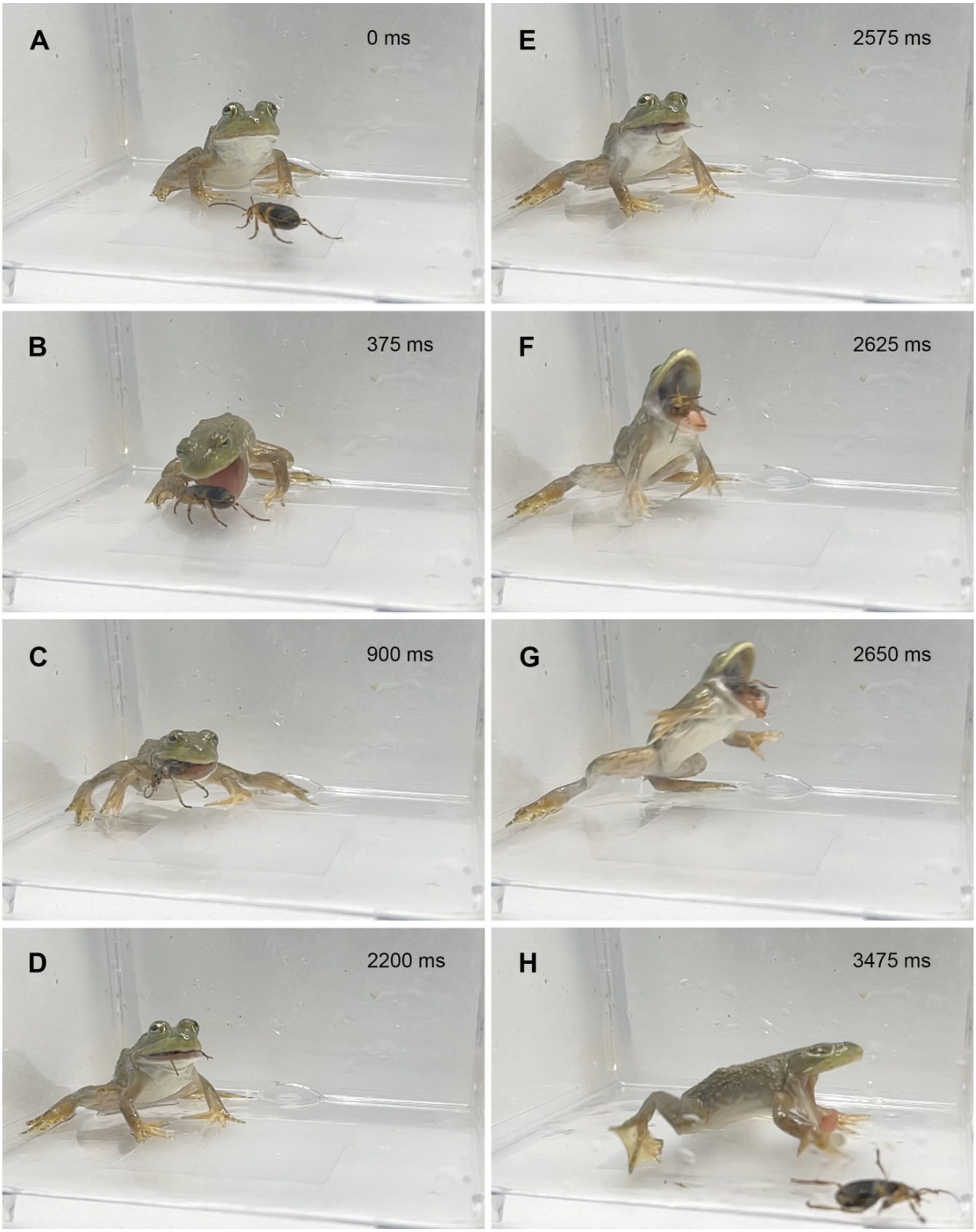
Figure 2

Figure 2

Temporal sequence of the bullfrog *Lithobates catesbeianus* rejecting a control adult *Pheropsophus occipitalis jessoensis*. (A) 0 ms. (B) 375 ms. (C) 900 ms. (D) 2,200 ms. (E) 2,575 ms. (F) 2,625 ms. (G) 2,650 ms (H) 3,475 ms. The bullfrog spat out the beetle after taking it into its mouth. Bombing by the beetle was audible and the ejected vapor (E) was observed just before the bullfrog spat out the beetle (see Video S2). Credit: Shinji Sugiura and Tomoki Date.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0 ms
B
375 ms
C
900 ms
D
2200 ms
E
2575 ms
F
2625 ms
G
2650 ms
H
3475 ms

Figure 3

Figure 3

Behavioral responses of the invasive bullfrog *Lithobates catesbeianus* and the native pond frog *Pelophylax nigromaculatus* to adults of the bombardier beetle *Pheropsophus occipitalis jessoensis*. Swallow: bullfrogs or frogs successfully swallowed control beetles. Reject before bombed: bullfrogs or frogs stopped attacking control beetles before being bombed. Reject after bombed: bullfrogs or frogs rejected control beetles after being bombed. The graph showing data for *Pe*. *nigromaculatus* was taken from Sugiura (2018). Photo credit: Shinji Sugiura.

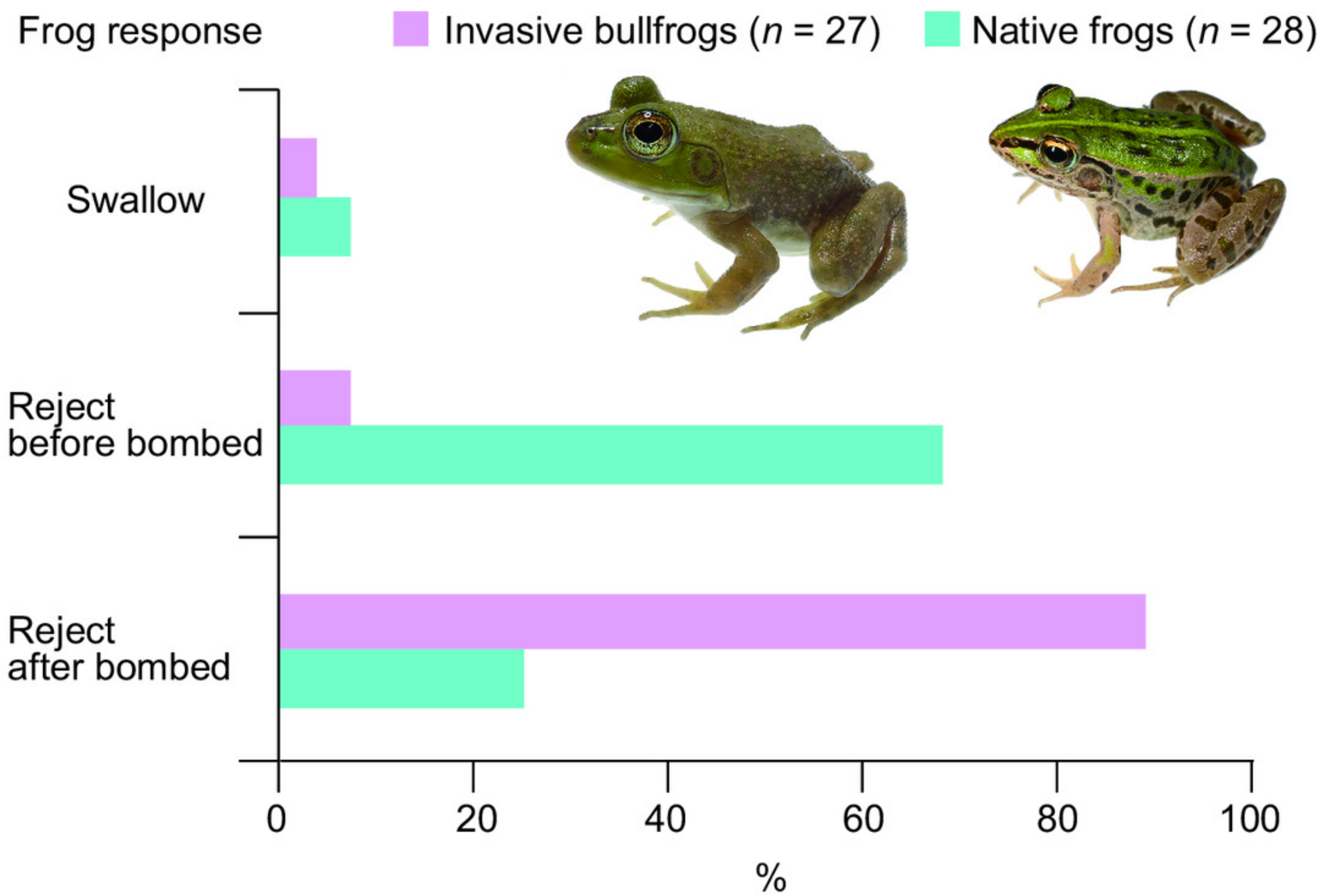
Frog response
Invasive bullfrogs (n = 27)
Native frogs (n = 28)
Swallow
Reject before bombed
Reject after bombed
0
20
40
60
80
100
%

Figure 3