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Diet Composition of the Invasive American Bullfrog (*Lithobates catesbeianus*) in Onuma Quasi-National Park, Hokkaido, Japan

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Abstract: An invasive alien species American bullfrog (hereafter, 'bullfrog') is found in freshwater lakes in Onuma Quasi-National Park, Hokkaido, Japan. Bullfrog commonly feeds on red swamp crayfish in many areas. However, red swamp crayfish has not been confirmed in Komuna Lake, Onuma Quasi-National Park. The purpose of this study is to examine the trend of predation on native biomes in areas without crayfish presence. We detected the dietary composition of bullfrogs. The stomach contents of 469 individuals were analyzed and classified. The stomach contents of adult frogs accounted for 67.4% of volume were vertebrates including Actinopterygii and Amphibia such as Japanese crucian carp, topmouth gudgeon, bullfrog juveniles, bullfrog tadpoles and Japanese common toad. Further, aquatic animals were preyed more than terrestrial animals in volume (60.0%) and frequency (90.0%). From these results, bullfrogs in Onuma Quasi-National Park used most conspicuous alien aquatic species such as alien fish and frogs of the same species as food resources and further revealed that these alien aquatic species function as a substitute food for the crayfish. While the majority of bullfrog food resources are alien aquatic species, several rare aquatic animals were also preyed on. In the future, it will be necessary to investigate the predation pressure of bullfrogs on local biodiversity.

Key words: Alien aquatic animals; Bullfrog; Japan; Predation

Introduction

The American bullfrog (*Lithobates catesbeianus*; hereafter, 'bullfrog') is native to the eastern United States, but was introduced worldwide for food and then expanded its distribution (Lever, 2003). Bullfrogs are known as voracious predators, and the species is listed among the IUCN's "100 of the World's Worst Invasive Alien Species" (Lowe et al., 2000).

There are many studies on the feeding habits of bullfrogs (e.g., Barrasso et al., 2009), and previous studies indicated that an important food for bullfrogs was the red swamp crayfish (*Procambarus clarkia*) (e.g., Carpenter et al., 2002). Several additional studies have shown the impact of bullfrogs on aquatic animals (e.g., Carpenter et al., 2002; Hirai, 2004a; Wang et al., 2008; Wu et al., 2005).

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In Japan, bullfrogs were introduced in 1918 (Matsui and Maeda, 2018). Previous studies on food habits of bullfrogs in Japan were conducted only in areas where the red swamp crayfish was established (e.g., Hirai, 2004a; Dontchev and Matsui, 2016). There are almost no studies of bullfrog diet in areas where the red swamp crayfish is not present.

In Hokkaido, the northernmost island of Japan, the red swamp crayfish is limited only to some warm waters, such as hot spring drainages (Nakata et al., 2005). Although there are no records of red swamp crayfish presence in the Onuma Quasi-National Park, the invasive bullfrog is established there. This study of bullfrog feeding habits in Onuma Quasai-National Park will confirm the aquatic prey consumed by bullfrogs in the absence of crayfish. The purpose of this study is to examine the impact of predation on native fauna in areas where crayfish are not established, based on the dietary composition of bullfrogs.

Materials and Methods

Study Site

Bullfrogs were captured in Konuma Lake in Onuma Quasi-National Park (hereafter, 'OQNP'; 41°59' N, 140°39' E, Altitude 131.6 m), in Nanae Town, Hokkaido. OQNP was designated a Ramsar site in 2012 for its rich waterfowl diversity.

There are two invasive frogs in OQNP, the Japanese common toad (*Bufo japonicus formosus*) and the bullfrog. The exact year that bullfrogs were introduced to the park is unclear. OQNP also has two native frogs, the Ezo brown frog (*Rana pirica*) and the Japanese tree frog (*Dryophytes japonicus*).

There are two major lakes in OQNP, Onuna and Konuma Lakes. These lakes are known for freshwater fishing and fish farming, and there are many invasive fishes, such as Japanese crucian carp (*Carassius cuvieri*) introduced from Honshu. In addition, topmouth gudgeon (*Pseudorasbora parva*) was unintentionally introduced, along with other invasive fish. The red swamp crayfish was not present as of 2014 in Onuma or Konuma Lakes.

Bullfrog Capture

In 2012–2014, between June and October, both adult and juvenile bullfrogs were captured using scoop nets and spring wire baskets; no bait was used for the spring wire baskets (Diameter 330 mm x length 600 mm). Ten to 20 spring wire baskets were set evenly around the walking path in Konuma Lake. All wire baskets were set in the evening, 5–10 m from the lakeshore, at a depth of about 40–80 cm, and were collected in the morning of the following day. Also, bullfrogs were collected on the lakeside by scoop nets between 20:00 and 24:00 h by three to five persons.

Snout-vent length (SVL) of all captured individuals was measured with a vernier caliper to a precision of 0.1 mm. Based on Maeda and Matsui (1999), sex and maturity were confirmed based on secondary sexual characteristics and SVL. Individuals smaller than the adult male minimum size (SVL= 109.6 mm) were categorized as juveniles. The stomach contents were obtained by forced regurgitation, after which the frogs were euthanized by deep freezing. Stomach contents were preserved in 70% ethanol.

Stomach Content Analysis

Stomach contents were sorted to taxonomic class, and Crustacea and Insecta were classified by order. Crustacea are composed of Decapoda, Amphipoda, and Oniscoidea. In Insecta, Formicidae were classified separately from other Hymenoptera. The larvae of Insecta were collectively referred to as Insecta larvae.

In order to understand predation trends, prey items were also classified by two habitat types (terrestrial and aquatic). Gastropoda were separated into land snails and shellfish. Crustacea were separated into aquatic shrimp and Gammaridea, and terrestrial woodlouse. We also separated Insecta into larvae and adults, and the larvae of dragonflies, flies, and

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beetles were classified as aquatic insect larvae. Our study also separated amphibians into bullfrogs and toads. The bullfrog's habitat is near water (Hirai, 2004a), and therefore we classified bullfrog juveniles and both bullfrog and toad larvae as aquatic animals. On the other hand, juvenile and adult toads are classified as terrestrial animals because they inhabit on the dry land (Matsui and Maeda, 2018).

In addition, stomach contents were, when possible, identified to the species level.

To determine the composition of prey species in the diet, we calculated the occurrence of each prey taxon as a percentage of total prey, by number (N%) and by volume (V %). We also calculated the frequency with which each prey taxon was found among those stomachs that contained prey (F%). Volumes of prey items were calculated using the formula of Magnusson et al. (2003), based on body length and width of prey.

Results

Of 494 bullfrogs captured, 25 were excluded due to empty stomachs; stomach contents from 469 individuals (32 males, 28 females, 409 juveniles) were obtained, from which 3,659 individual prey items were extracted. The mean (±SD) SVL of bullfrogs was 76.1±29.6 (range=25.8–203.8) mm, 138.9± 19.2 (range=109.6–174.5) mm for males, 146.3±21.8 (range=111.6–203.8) mm for females, and 66.3±14.2 (range=25.8–109.4) mm for juveniles.

Extracted prey items were, when possible, identified to the species level; a total of 64 species were successfully identified. Of those, 17 were aquatic species. Mollusks included *Radix auricularia japonica* and *Gyraulus spirillus*. Shrimp, *P. paucidens*, were consumed. Six species of beetles were eaten, including *Rhantus suturalis*, *R. erraticus*, and *Acilius japonicus*. True bugs included *Notonecta triguttata*, *Appasus japonicus* and *A. major*. *Appasus major* is listed in the Red Data Book for Hokkaido.

Although bullfrogs preyed on some rare aquatic animals, they also preyed on three alien aquatic species, including bullfrogs, topmouth gudgeon, and Japanese crucian carp. Bullfrogs also preyed on the Japanese common toad, a terrestrial species that is listed as invasive species in the Hokkaido Prefecture Biodiversity Conservation Act. Other terrestrial prey included three species of snails, two species of dragonflies, two species of grasshoppers, four species of earwigs, nine species of true bugs, a species of butterfly, 15 species of beetles, and ten species of bees and ants.

Prey Composition of Adult Bullfrogs

Prey composition is listed in Table 1. Insecta included the highest percentage of prey by number and frequency (N%: 63.4%, F%: 88.3%) followed by Crustacea by number (17.4%), and Coleoptera by frequency (38.3%). In volume, Amphibia (31.9%), Insecta (29.9%), and Actinopterygii (28.8%) accounted for the largest proportions. Crustacea were mainly comprised of Oniscoidea (N%: 16.2%, V%: 0.3%, F%: 18.3%); Oniscoidea were preyed upon to a greater extent than Decapoda (N%: 0.5%, V %: 0.2%, F%: 3.3%). Oniscoidea were composed only of woodlice. Among the Insecta, Formicidae comprised the highest percentage by number (16.2%), Odonata by volume (13.1%), and Coleoptera by frequency (38.3%). Amphibia included one Japanese common toad (SVL=65 mm), eight juvenile bullfrogs (range=45.3–62.4 mm), and numerous bullfrog tadpoles (range=23–57 mm). Actinopterygii comprised topmouth gudgeon (range=24–68 mm) and Japanese crucian carp (range=25–72.2 mm). Mammalia included a rat. Decapoda were composed common prawn (*Palaemon paucidens*).

Of the recorded prey, 11.4% by number and 67.4% by volume were vertebrates. Actinopterygii and Amphibia were alien species, including Japanese crucian carp, topmouth gudgeon, juvenile and larval bullfrogs, and the Japanese common toad. All vertebrates were

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Table 1. Diet composition (in %) of adult and juvenile bullfrogs (396 prey items from 60 adults [volume=998,006.0 mm^3] and 3,263 from 409 juveniles [volume=1,029,570.7 mm^3]). In the table, "—" means no detection.

Taxon	Volume		Number		Frequency	
	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
Mammalia	6.7	—	0.3	—	1.7	—
Amphibia	31.9	4.6	4.0	0.6	18.3	2.4
Actinopterygii	28.8	16.5	7.1	1.7	30.0	8.3
Insecta	29.9	50.2	63.4	53.8	88.3	93.2
Odonata	13.1	8.5	6.6	1.5	18.3	9.5
Plecoptera	<0.1	—	0.3	—	1.7	—
Orthoptera	3.5	3.2	5.3	1.0	13.3	6.1
Dermaptera	0.1	2.6	0.5	3.8	3.3	23.0
Hemiptera	2.5	14.9	8.1	12.0	30.0	41.8
Neuroptera	—	<0.1	—	<0.1	—	0.2
Coleoptera	4.0	7.7	10.1	10.9	38.3	48.2
Diptera	<0.1	1.0	0.5	3.0	3.3	18.1
Lepidoptera	2.2	4.9	1.0	0.5	6.7	3.2
Trichoptera	0.4	1.3	0.8	0.6	5.0	3.9
Hymenoptera	0.6	1.7	4.8	3.3	15.0	16.6
Formicidae	<0.1	0.5	16.2	10.8	11.7	40.6
Insecta larvae	3.5	3.9	9.3	6.4	31.7	29.3
Crustacea	0.5	17.2	17.4	29.9	20.0	56.7
Decapoda	0.2	5.2	0.5	0.4	3.3	2.4
Amphipoda	<0.1	1.2	0.8	7.6	5.0	22.7
Oniscoidea	0.3	10.8	16.2	21.9	18.3	48.4
Gastropoda	0.8	6.2	1.3	3.1	6.7	17.6
Oligochaeta	0.5	1.0	0.5	0.2	1.7	1.5
Hirudinea	—	0.4	—	0.2	—	1.0
Arachnida	0.2	0.8	3.5	7.1	15.0	35.5
Myriapoda	0.7	3.2	2.0	2.8	11.7	14.7
Entognatha	<0.1	<0.1	0.5	0.6	3.3	1.5
Terrestrial animals	40.0	54.8	79.0	77.6	65.0	64.8
Aquatic animals	60.0	45.2	21.0	22.4	90.0	98.5

alien species, except for the unidentified species of rat.

By our classification of prey, terrestrial animals were consumed more frequently than aquatic animals by number (79.0%). On the other hand, aquatic animals comprised a higher proportion of prey than terrestrial species by volume (60.0%) and frequency (90.0%). Alien aquatic animals such as Japanese crucian carp, topmouth gudgeon, bullfrog juveniles, bullfrog tadpoles accounted for an especially high proportion of stomach contents by volume.

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Prey Composition of Juvenile Bullfrogs

Insecta showed the highest percentage of prey number, volume, and frequency (N%: 53.8%, V%: 50.2%, F%: 93.2%), followed by Crustacea (N%: 29.9%, V%: 17.2%, F%: 56.7%). Similar to adult bullfrogs, Crustacea mainly consisted of Oniscoidea (N%: 21.9%, V%: 10.8%, F%: 48.4%), which were was preyed upon to a greater extent than Decapoda (N%: 0.4%, V%: 5.2%, F%: 2.4%). Among the Insecta consumed, Hemiptera accounted for the highest percentage by number and volume (N%: 12.0%, V%: 14.9%), whereas Coleoptera were the highest by frequency (48.2%). In juveniles, 2.3% by number and 21.1% by volume were vertebrates.

According to our classification of prey, terrestrial species were consumed more than aquatic species by number (77.6%) and volume (54.8%). On the other hand, by frequency aquatic animals (98.5%) were preyed more than terrestrial animals (64.8%).

Discussion

In this study, 67.4% of bullfrog stomach contents, by volume, consisted of vertebrates, including Actinopterygii and Amphibia. Those prey alien species such as Japanese crucian carp, topmouth gudgeon, bullfrog larvae and juveniles, and the Japanese common toad. Furthermore, aquatic animals were preyed upon by adult bullfrogs more than terrestrial animals, by volume (60.0%) and frequency (90.0%)

The red swamp crayfish is an important prey item of bullfrogs in the USA (e.g., Carpenter et al., 2002). In previous studies, bullfrogs with a high V% or N% of red swamp crayfish showed a high ratio of predation on aquatic species (Tyler and Hoestenbach, 1979; Wu et al., 2005; Wylie et al., 2003). On the other hand, some studies reported low levels of predation on red swamp crayfish predation, associated with a lower level of predation on aquatic animals (Bruggers, 1973; Hothem et al., 2009).

In Japan, Hirai (2004a) pointed out that adult bullfrogs rely heavily on red swamp crayfish. Similarly, Dontchev and Matsui (2016) noted that bullfrogs prey on red swamp crayfish and crabs, and thus consume aquatic animals more than terrestrial species.

In this study, we found that adult bullfrogs preyed on aquatic animals more than terrestrial ones by volume (60.0%) and frequency (90.0%), even though they did not consume crayfish. In a study by Dontchev and Matsui (2016), the crayfish was the most conspicuous benthic animal in the study area, and the dietary composition of the bullfrog largely reflected the benthic fauna in the study area. In this study, most of the V% of aquatic animals consisted of Japanese crucian carp, topmouth gudgeon, bullfrog juveniles and tadpoles, which are alien species in Hokkaido. Our results indicate that bullfrogs in Onuma Quasi-National Park use the most conspicuous alien aquatic species, such as alien fish and frogs, as prey. This is similar to the findings of Hirai (2004a), and further reveals that these other alien aquatic species function substitute for crayfish as prey.

While the majority of bullfrog food resources are alien aquatic species, several rare aquatic animals were also consumed. Predation of bullfrogs on rare aquatic insects has become a major ecological problem in Japan (e.g., Hirai, 2004b; Hirai, 2005). In the future, it will be necessary to investigate further the predation pressure of bullfrogs on local biodiversity.

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This study was conducted in accordance with the Invasive Alien Species Act; all permits were obtained through the Hokkaido Prefectural Environmental Office (Permit #12000077). All field studies were conducted in accordance with national laws.

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