Analyzing the stomach contents of invasive American bullfrogs (*Lithobates catesbeiana)* in the West Kootenay Region of British Columbia

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Data collection done in cooperation with Becca Merenyi

Samples provided by Khaylish Fraser, Central Kootenay Invasive Species Society

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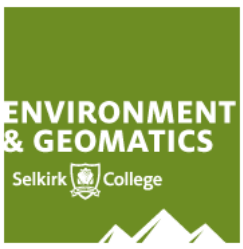

1. Abstract

What are American bullfrogs (*Lithobates canesbeiana*) at Lomond Lake in British Columbia eating? American bullfrogs are large, aquatic frogs that eat almost anything in front of them. After they were introduced to the province of BC, they began to spread and displace native amphibians. In some instances, they prey directly on the native amphibians. This research was done to gain an understanding of the diets of American bullfrogs through literature, to determine the diet trends and key prey species of bullfrogs at Lomond Lake, and to determine if the Lomond Lake population was preying on native amphibians. Bullfrog specimens from Lomond Lake were dissected and their stomach contents analyzed and organized in Microsoft Excel. Of the 34 bullfrogs dissected, 78% of the stomachs with contents contained insects. Almost 90% of the total stomach contents were insects. Other contents included arachnids, mammals, and vegetation. No native amphibians were identified within the stomach contents. This may be because of a lack of native amphibians at Lomond Lake, the availability of other more suitable prey options, or due to the small size of our specimens. Dissecting a larger number of Lomond Lake bullfrogs may help to further define the trends in their diets and help to confirm that our analyses are relevant to the whole population. Conducting similar research on the diets of amphibians native to Lomond Lake may deepen our understanding of why the bullfrogs do not appear to be preying on them currently and assess their risk of displacement and predation in the future.

2. Acknowledgements

My research partner, Becca Merenyi, a second-year Recreation, Fish and Wildlife student at Selkirk College, completed the data collection portion of this research with me. Doris Hausleitner, M.Sc, R.P. Bio, was my faculty advisor. She teaches two second year RFW courses at Selkirk College. Brenda Beckwith, PhD, assisted with the structure and editing of my applied research project and report. She teaches the Applied Research Project (RFW 271) course within which this work was completed. Valerie Huff assisted with the data analysis in Microsoft Excel. Khaylish Fraser of the Central Kootenay Invasive Species Society donated the American bullfrogs used for our research to Selkirk College.

3. Introduction

American bullfrogs (*Lithobates catesbeiana*) are one of the world's 100 most invasive species (Anthony 2013). They are brown or green aquatic frogs with golden eyes and are known for eating "anything they can fit in their mouths," from insects to snakes to mammals (Factsheet 7 n.d.). They are distinguishable from other frogs because of their distinct tympanum (ear) and their lack of dorsolateral folds. The males have yellow throat patches and the females have whitish-cream coloured throats. The females are larger than the males (Factsheet 7 n.d.).

A threat to other amphibians in British Columbia (BC), American bullfrogs were introduced to the province in the early 20th Century to be farmed for meat due to their large size of up to 20 centimeters, not including legs (Factsheet 7 n.d.). Since this time, they have displaced other amphibians by taking over their habitat and, in some instances, they prey directly on native amphibians (Anthony 2013), including species at risk (Crestonwildlife c2011). For example, the northern leopard frog, red-listed in BC and endangered federally, inhabits only two areas in BC (Crestonwildlife c2011). One of these areas is the Creston Valley Wildlife Management Area in the West Kootenay, a wetland that has experienced an invasion of American bullfrogs (Crestonwildlife c2011).

Information on the impacts of American bullfrogs in the West Kootenay region is limited currently and management efforts are somewhat constrained due to funding. Although some analysis of the bullfrogs' stomach contents has been done, it has not been the primary effort in their surveillance and management (Vogel et al. 2017). At present, bullfrogs are being managed only in the Creston and Nelway areas in the West Kootenay region where surveillance has been ongoing since 2014 (K. Fraser, pers. comm., 2019 November 12). This management has included passive and active acoustic and visual surveillance, capture and euthanization, and public outreach and education to date (K. Fraser, pers. comm., 2019 November 12). A bullfrog risk assessment is being developed by the American Bullfrog Action Team (ABAT) for the Kootenay-Boundary region to aid in future management decisions (K. Fraser, pers. comm., 2019 Nov 12). ABAT conducts the American bullfrog surveillance and eradication program to gather more scientific information on American bullfrogs in the region. The Central Kootenay Invasive Species Society (CKISS), an ABAT partner, has donated samples from their bullfrog euthanasia efforts at Lomond Lake near Nelway for students to use for research at Selkirk College.

I believe that with further research on the diet of these bullfrogs, better management practices can be created to minimize their potential effects on native amphibians. My research goal is to analyze the stomach contents of American bullfrogs from Lomond Lake to assess their diet, with an emphasis on the identification of native amphibian species. To meet my research goal, the specific objectives of this research are:

- To conduct a literature review to gain an understanding of how American bullfrogs have affected BC, their general habits, and common prey species.
- To determine the key species in the diet of American bullfrogs sampled from Lomond Lake through the analysis of stomach contents.
- To determine if American bullfrogs are eating native amphibians.
- To analyze and describe the trends in the diets of American bullfrogs, including male/female comparisons.

If American bullfrogs are consuming not only the prey of native amphibians, but the amphibians themselves, it is important to develop best management practices to conserve the native species. The results of my research will be sent to Khaylish Fraser, Aquatics Program Coordinator, at CKISS, to contribute to the ABAT bullfrog risk assessment.

4. Methods

4.1 Samples Background

Lomond Lake is located 1.3 km from Nelway, BC, and is accessed via Pend D'Oreille Road off of Highway 6. It is 27 km south of Salmo (Figure 1). CKISS captured, anesthetized, and euthanized, by freezing, American bullfrogs at Lomond Lake in 2016. Some specimens were given to Selkirk College by Khaylish Fraser to use for research. They have been kept frozen in storage in a freezer outside the maintenance building at Selkirk College and some specimens were used in 2016 and 2017 for student projects in the Recreation, Fish, and Wildlife Program.

Figure 1. Location of Lomond Lake, circled in pink, south of Salmo, BC.

4.2 Data Collection and Potential Analysis

I conducted this research with Becca Merenyi, a fellow Selkirk College student. Data collection and analysis occurred between December 1, 2019 and February 27, 2020 in a biology laboratory at the Selkirk College campus in Castlegar. We dissected 34 bullfrogs. We recorded length (mm), weight (g), and sex of each individual before dissection began. We identified yellow throat patches as male, white throat patches as female, and labelled those we were unsure of as "Unknown."

4.2.1 Dissection

Each frog was laid on its back and an opening was created with a scalpel on the underside of the body between the hind legs. Scissors were used to cut through the skin and muscle in the middle of the body perpendicular from the original opening up through the mandible. The liver and fat bodies were then removed to make the stomach visible and accessible (Hansler 2014). After using scissors to snip above and below the stomach, it was removed and sliced lengthwise around the outer curve with a scalpel to open it and empty the contents into a metal tray (Frog 2010). Anticipated contents included insects, invertebrates, and, depending on the size of the

bullfrogs, birds, amphibians, and small mammals. Once the dissections were complete, the frogs were disposed of in the garbage since they were not preserved with any chemicals. Our data for each frog was recorded and sorted in Microsoft Excel.

4.2.2 Analysis

The stomach contents were analyzed based on the sex (male and female) of the bullfrogs and the frequency of occurrence of the different items found in the stomachs. We identified trends in the diets of males versus females, as well as within the Lomond Lake bullfrog population in general. The most common species preyed upon by bullfrogs was expected to give insight into which native species, if any, are being threatened, by which group of bullfrogs (male, female) and to what extent. This could include the number of different species of insects, invertebrates, birds, or mammals being consumed, and what percentage each group made up of the whole diet. For example, insects might have included eight different species and made up 70% of all the identified species in the diet of adult male bullfrogs. If native amphibians were identified in the stomach contents it would confirm that the bullfrogs were threatening them more directly than in the competition for resources. The diet trends of our bullfrogs were compared with results from similar studies done on bullfrogs in other locations in order to assess the normalcy of the Lomond Lake population.

5. Results

We dissected 34 American bullfrogs, including 13 males, 13 females, and 8 whose sex we could not determine ("unknown"). Our largest frog was 140mm; the smallest was 54mm. Of the bullfrogs sampled, 21% had no stomach contents, made up of females and "unknown" bullfrogs. We did not find any native amphibian species in the stomachs.

Of the stomachs that had contents, most contained insects (78%), which made up almost 90% of the total contents (Table 1). The identified insect species included wasps (Formicidae family), bees (Apoidea family), dragonflies (Aeshnoidae family), beetles (Coleoptera order), grasshoppers (Acridoidea family), and moths (Lepidoptera order). In addition to insects, we observed spiders (Arachnida class), mice (Mammalia class), snails (Gastropoda class), and general vegetation.

Table 1. Frequency of occurrence of stomach contents in American bullfrogs from Lomond Lake.

	Number of Stomachs Containing	Frequency of Occurrence	Total Number Found in All Contents	% of All Stomach Contents
Insecta	21	77.8%	81	88.0%
Vegetation	5	18.5%	5	5.4%
Gastropoda	3	11.1%	4	4.3%
Mammalia	2	7.4%	2	2.2%
Arachnida	1	3.7%	1	1.1%

In general, we found the male bullfrogs to be larger both in length and weight than the females, and the bullfrogs in which we could not determine sex tended to be the smallest of our samples (Figure 2). In comparing males and females, we found that the males' stomachs more frequently contained vegetation, while females' stomachs contained the only arachnids and mammals identified in the study (Figure 3).

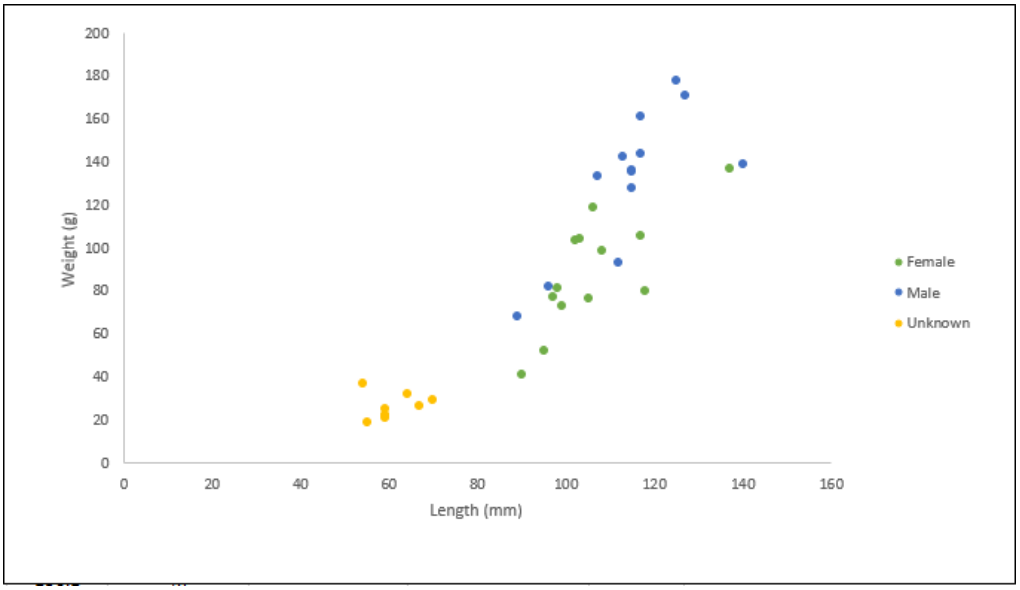

Figure 2. Weight and length distribution among American bullfrogs from Lomond Lake.

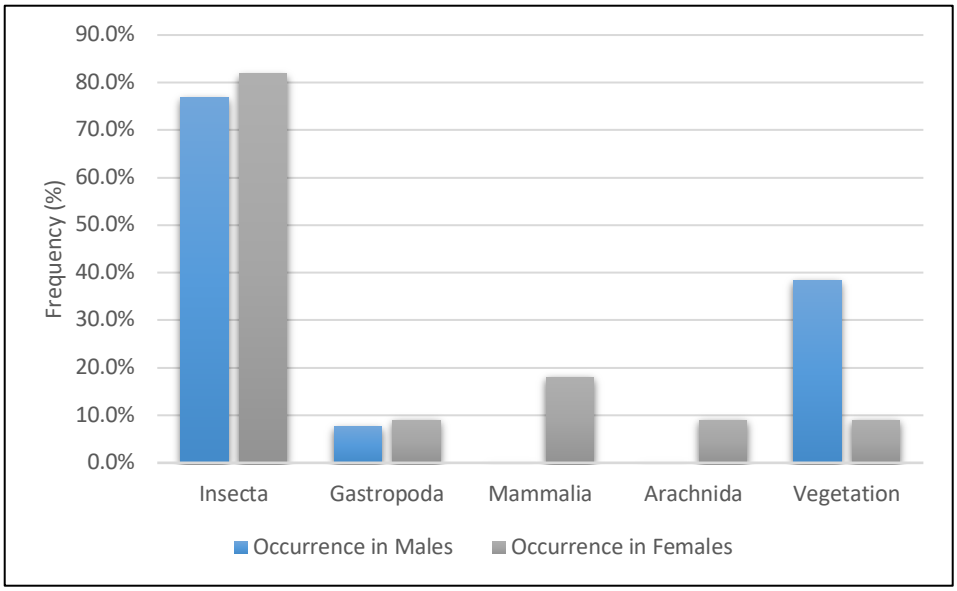

Figure 3. Frequency of occurrence of stomach contents in male and female American bullfrogs from Lomond Lake.

6. Discussion

The diet preference for American Bullfrogs in Lomond Lake consists largely of insects, a result mirrored by similar research from Vancouver Island, BC (Jancowski and Orchard 2013) and Argentina (Quiroga et al. 2015). Also consistent with Jancowski and Orchard (2013), we found a low percentage of arachnids in the stomach contents of our specimens, the male bullfrogs were larger than the females, and the smallest of the bullfrogs tended to have less in their stomachs. This may indicate that the Lomond Lake bullfrog population is growing and feeding as average bullfrogs would be. The range of contents found in the stomachs was within the ranges of these studies; although, not every species found in other studies, such as microbenthic invertebrates, was found in our Lomond Lake bullfrogs.

There may be a number of reasons why we did not observe amphibians in our bullfrog stomach contents. In contrast to the 200 mm length that American bullfrogs can grow to, even our largest (140 mm) was notably smaller. It could be that our specimens were not large enough to be consuming the amphibians that are native to Lomond Lake. It is also possible that there is enough other food for the bullfrogs to eat that they haven't yet taken an interest in eating other amphibians. Additionally, perhaps there are few native amphibians at Lomond Lake available to be eaten in the first place.

Because the male bullfrogs' stomachs did not contain either of the mice identified in the stomach contents, maybe it could be suggested that the males tend to eat less mammals than the females; however, I would not draw that conclusion unless I got the same results with a repeat sample of Lomond Lake bullfrogs. With further research and a larger sample size, perhaps more reliable comparisons of male versus female diets could be made.

6.1 Limitations

If we had had more students involved in our dissections, we could have analyzed more bullfrogs which would have helped to define our trends further. With only 34 stomachs emptied, it is possible that our contents did not define the average trends of the whole Lomond Lake population. Since our bullfrogs were smaller and likely younger than some populations, our findings may not be indicative of the real risk to native amphibians at Lomond Lake. If remaining bullfrogs at the lake are left to keep growing, it is possible that their diets will change, and native amphibians will be preyed on by these larger bullfrogs. If I were to do this research again, I would try harder to involve more people so that we could have more data to analyze.

6.2 Recommendations

I would recommend research be done on the native amphibians currently populating Lomond Lake. The species present, their diets, and their population sizes could be helpful in determining why they are not being preyed on by the bullfrogs and may give insight into their level of risk of displacement and predation. If possible, obtaining bullfrogs from the eradication efforts by ABAT in the Creston area and analyzing their stomach contents would be a good next step. That research could be compared with the Lomond Lake bullfrogs and together the diet information could be used to more widely describe the bullfrogs in the West Kootenay region. In addition, because the endangered Northern-leopard frog resides in the Creston Valley Wildlife Management Area (Crestonwildlife c2011), information on the diet trends of the Creston bullfrogs could be key in managing this at-risk species.

Finally, we observed pink colouring on several of our bullfrogs' thighs and stomachs, which our faculty advisor, Doris Hausleitner, previously taught us is a sign of chytrid fungus – a fungus that has significant negative effects on amphibians. Studying the remaining Lomond Lake bullfrogs to identify how many of them may have chytrid fungus and comparing the diets of those with

and without the fungus, could yield some interesting information that may also have management implications for ABAT.

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