DISTRIBUTION OF NATIVE POND BREEDING AMPHIBIANS AND POTENTIAL THREAT MITIGATION ON AND ADJACENT TO BOUNDARY-SMITH CREEK WILDLIFE MANAGEMENT AREA

Idaho Department of Fish and Game


American Bullfrog, David Moskowitz Photo

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INTRODUCTION

Amphibians have experienced dramatic declines worldwide due to disease, habitat loss and degradation, climate change, and invasive species (Collins and Storfer 2003, Stuart et al. 2004). These declines are not limited to areas with high human populations, but have also occurred in seemingly pristine environments and protected areas (Adams et al. 2013). For example, the northern leopard frog (*Rana pipiens*) was once widely distributed across the western US and Canada but has apparently been extirpated from large portions of its historic range, including the Idaho panhandle (Lucid et al 2016). However, an isolated native population remains just 15 km across the border at British Columbia's Creston Valley Wildlife Management area (CVWMA).

Recent detections of bullfrogs (*Lithobates catesbeianus*) in the Kootenai River Valley near the Canadian border have raised concern about potential detrimental impacts on native amphibians, including the nearby leopard frog population and western toads (*Anaxyrus boreas*), a Species of Greatest Conservation Need (SGCN) in Idaho (IDFG 2017, Lucid 2015). Bullfrogs are native to the eastern United States, but have been introduced throughout the western United States and the world. Bullfrogs are considered one of the 100 most harmful invasive species and threaten native amphibians through predation, competition, and by serving as vectors for disease including the amphibian chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]) (Lowe et al. 2000, Adams and Pearl 2007).

Bullfrogs have proven challenging to eradicate due to their high fecundity, ability to disperse long distances, and density dependence in the larval and adult stages (Govindarajulu et al. 2005, Adams and Pearl 2007). These characteristics highlight the importance of preventing introductions from occurring in the first place and early detection of new invasions. Studies of the efficacy of direct removal indicate that removal actions should focus on post-metamorphic individuals and must eradicate a high proportion of the population to successfully reduce the population size (Govindarajulu et al. 2005, Orchard 2011). These actions are most effective in areas with small, isolated ponds where the probability of reinvasion via overland dispersal is low (Adams and Pearl 2007). Because we suspect that the Kootenai River Valley has only recently been invaded by bullfrogs and there are only a few permanent waterbodies suitable for their reproduction, it may be a good system in which to test these removal methods. With no action, we anticipate that bullfrogs will soon invade the CVWMA.

In 2017, Idaho Fish and Game initiated a project to gain a better understanding of the distribution of native pond-breeding amphibians and their threats including bullfrogs and disease in the Kootenai River Valley. Our objectives were to (1) map the distribution of native amphibians and non-native bullfrogs, (2) test and implement a potential bullfrog control method, and (3) test for the presence of the amphibian chytrid fungus (Bd) in the study area.

MATERIALS AND METHODS

Study Area

Our study was located in the portion of the Kootenai River Valley from Copeland Road to the Canadian border in northern Idaho (Map 1). This area is bordered by the Selkirk Mountains on the west and the Purcell Mountains on the east. The valley is primarily private agricultural land (wheat, canola, and cattle). We used Google Earth satellite imagery (imagery date: 11 July 2014) to identify all ponds and wetlands in the study area. For waterbodies located

on private land, we contacted landowners by phone or email to request permission to access their land.

The Kootenai Valley has undergone dramatic hydrologic changes since Libby Dam was constructed in 1975 and most natural wetlands are no longer functional. The majority of privately held wetlands are highly modified or constructed ponds connected to irrigation ditches. This is also true for the wetlands in the northern portion of the study area which occur within Boundary Smith Creek Wildlife Management Area (BSCWMA), a restored wetland complex owned and managed by IDFG. Four native pond-breeding amphibian species currently occur in the area: western toads (*Anaxyrus boreas)*, Columbia spotted frogs (*Rana luteiventris*), long toed salamanders (*Ambystoma macrodactylum*), and pacific tree frogs (*Pseudacris regilla*). Northern leopard frogs (*Rana pipiens)* historically occurred in the area.

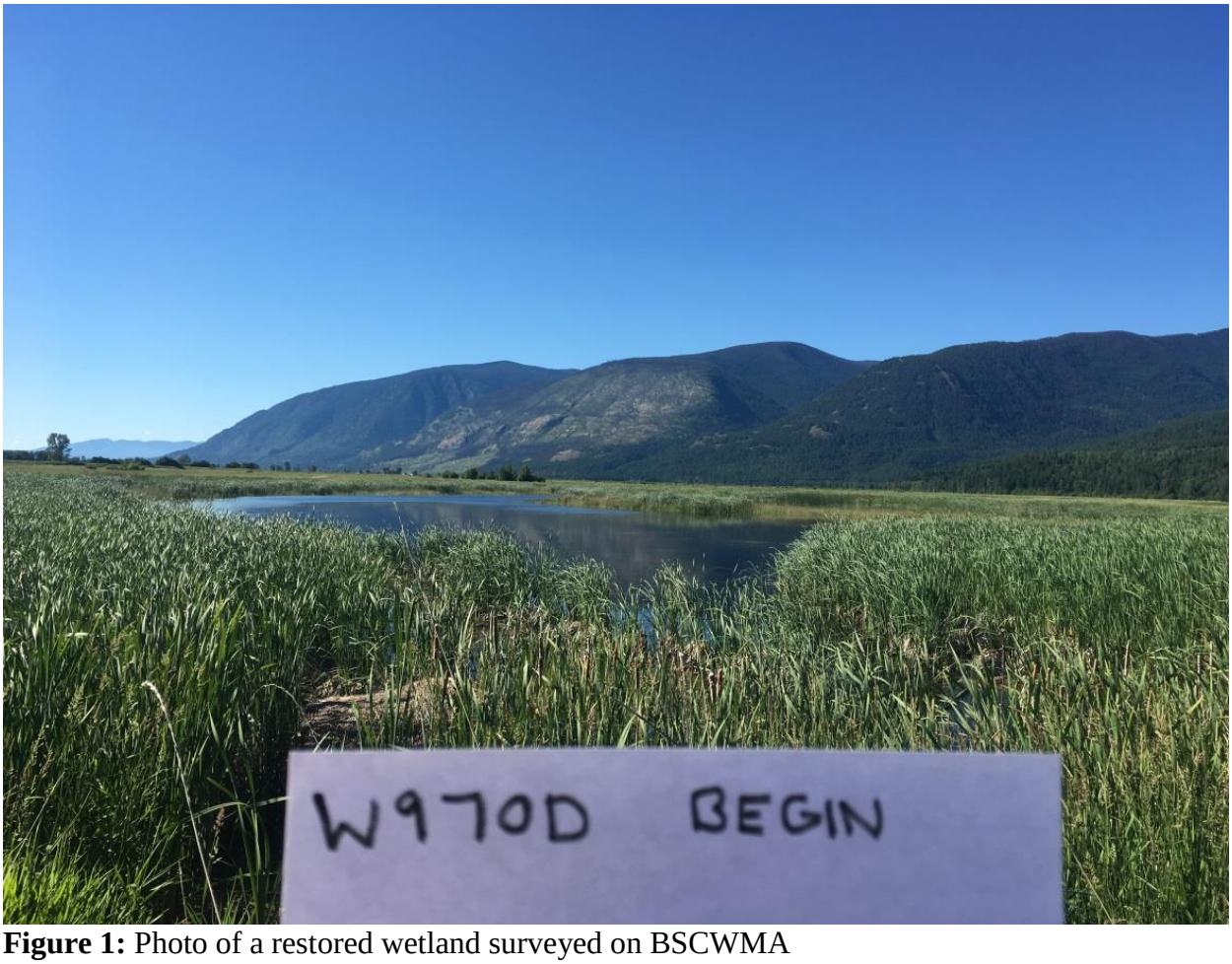
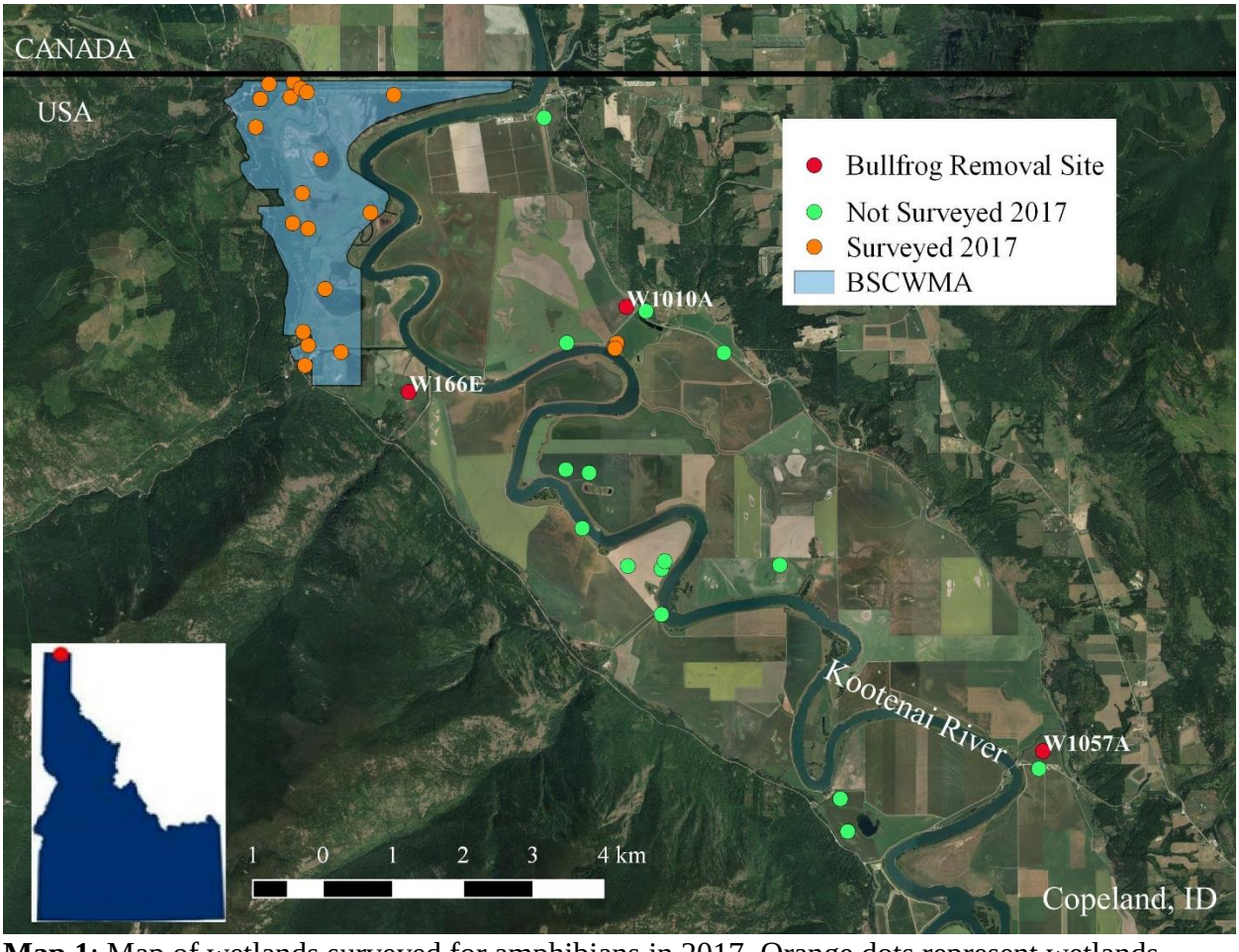


Figure 1: Photo of a restored wetland surveyed on BSCWMA

Amphibian Surveys

At each wetland that we were granted permission to access, we conducted a full perimeter dip net survey in late June or early July when amphibian larvae should be free swimming and easily detectable (Lucid et al 2016). We used 12" deep, 3/16" mesh dip nets (Delta Net and Twine, Greenville, MS) and sampled all microhabitats along the shoreline. We counted each amphibian species by life stage (egg, no legs, two legs, four legs and tail, or fully formed). Exact quantities were recorded for 0-10 individuals per section. If there were more than

10 individuals per section, quantities were estimated by order of magnitude to the nearest 10, 100, or 1000. We took photographs of one larval and one adult amphibian of each species observed at each wetland. We also took tissue samples from up to five individuals of each species. We characterized habitat characteristics for each wetland and recorded presence of other species of interest including painted turtles, garter snakes, fish, and invasive weeds (Appendices 1 and 2).



Map 1: Map of wetlands surveyed for amphibians in 2017. Orange dots represent wetlands surveyed for amphibians, red dots are wetlands where we conducted bullfrog removal, and green dots are wetlands on private land that we did not have permission to survey. Bullfrog removal sites were also surveyed for native amphibians.

Figure 2: IDFG intern Modeline Celestin conducts a dip net survey for amphibians

Bullfrog Removal

We conducted bullfrog removal in three focal wetlands by electrofrogging at night. The electrofrogger is a backpack electrofisher with specialized wand (Orchard 2012) designed specifically for frog capture. We worked in a team of two and used a canoe to access the targeted wetlands. Once in the wetland, we slowly paddled the shoreline. The person in the back paddled and steered while the person in the front used a high-powered headlamp (Fenix HL60R Rechargable Headlamp, Fenix Lighting, Lone Tree, CO) and handheld flashlight (Fenix RC11 Rechargable Flashlight, Fenix Lighting, Lone Tree, CO) to search for bullfrogs. Bullfrog eye shine indicated the presence of a bullfrog and the bright light caused the bullfrog to stay still (Orchard 2011).

When a bullfrog was spotted, we paddled slowly and quietly toward the frog and used the electrofrogger to shock the frog. Once the frog was immobilized, we scooped it up using a net attached to the bottom of the electrofrogger and stored it in a lidded bucket. We then continued around the perimeter of the wetland removing all fully formed bullfrogs observed. We continued

doing laps around the wetland until no more bullfrogs were observed, keeping track of which lap each frog was captured. At the end of the night we used digital calipers to measure snout to vent length (SVL) and determined the sex of each bullfrog captured and then euthanized frogs with several drops of clove oil diluted in water. We conducted bullfrog removal at least three times in each wetland over the course of the summer (Appendices 3 and 4).

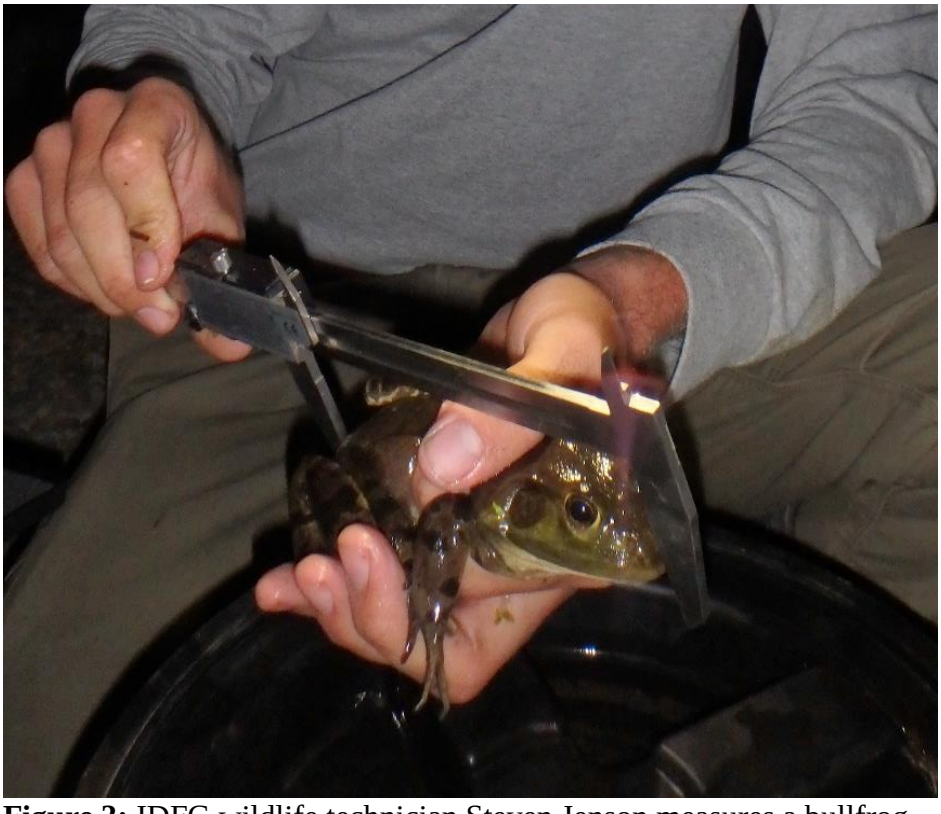


Figure 3: IDFG wildlife technician Steven Jenson measures a bullfrog

Disease testing

We tested the first ten adult bullfrogs encountered at each focal removal site for the amphibian chytrid fungus (Batrachochytrium dendrobatidis [Bd]), a widespread pathogen that is hypothesized to be the cause of mass mortality in some amphibian populations (Daszak et al. 2003). To avoid contaminating disease samples, we placed all tested bullfrogs in their own gallon Ziploc bag and used new vinyl gloves for each individual. We used fine tip swabs from Advantage Bundling/Medical Wire Co. (catalog number MW113) and gently swabbed the ventral surfaces of the skin approximately 20-30 times, targeting the pelvic patch, ventral thighs, and toe webbing. Swabs were stored in vials without ethanol and were frozen for storage. Swabs were analyzed at the Amphibian Disease Lab at the San Diego Zoo using PCR.

RESULTS

Amphibian Surveys

We identified a total of 38 wetlands in our study area and conducted amphibian surveys at 23 wetlands. Of the 21 wetlands located on private land, we obtained permission to survey a total of six. Of those that we didn't get permission to survey, we contacted landowners but were

refused permission to survey seven and we were unable to contact the owners of eight wetlands. We surveyed all distinct waterbodies on BSCWMA, a total of 17 wetlands (Map 1, Appendix 5).

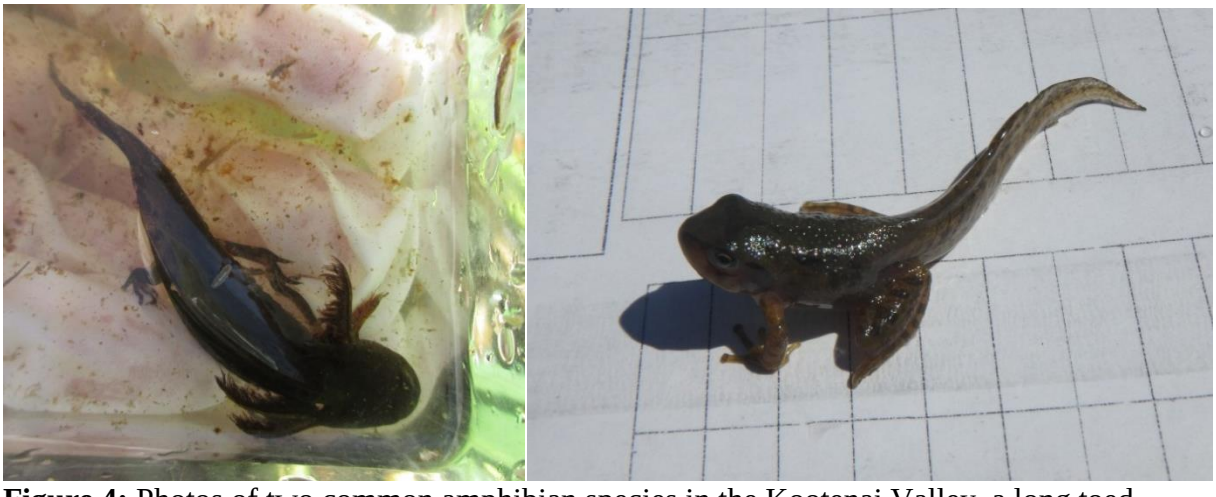
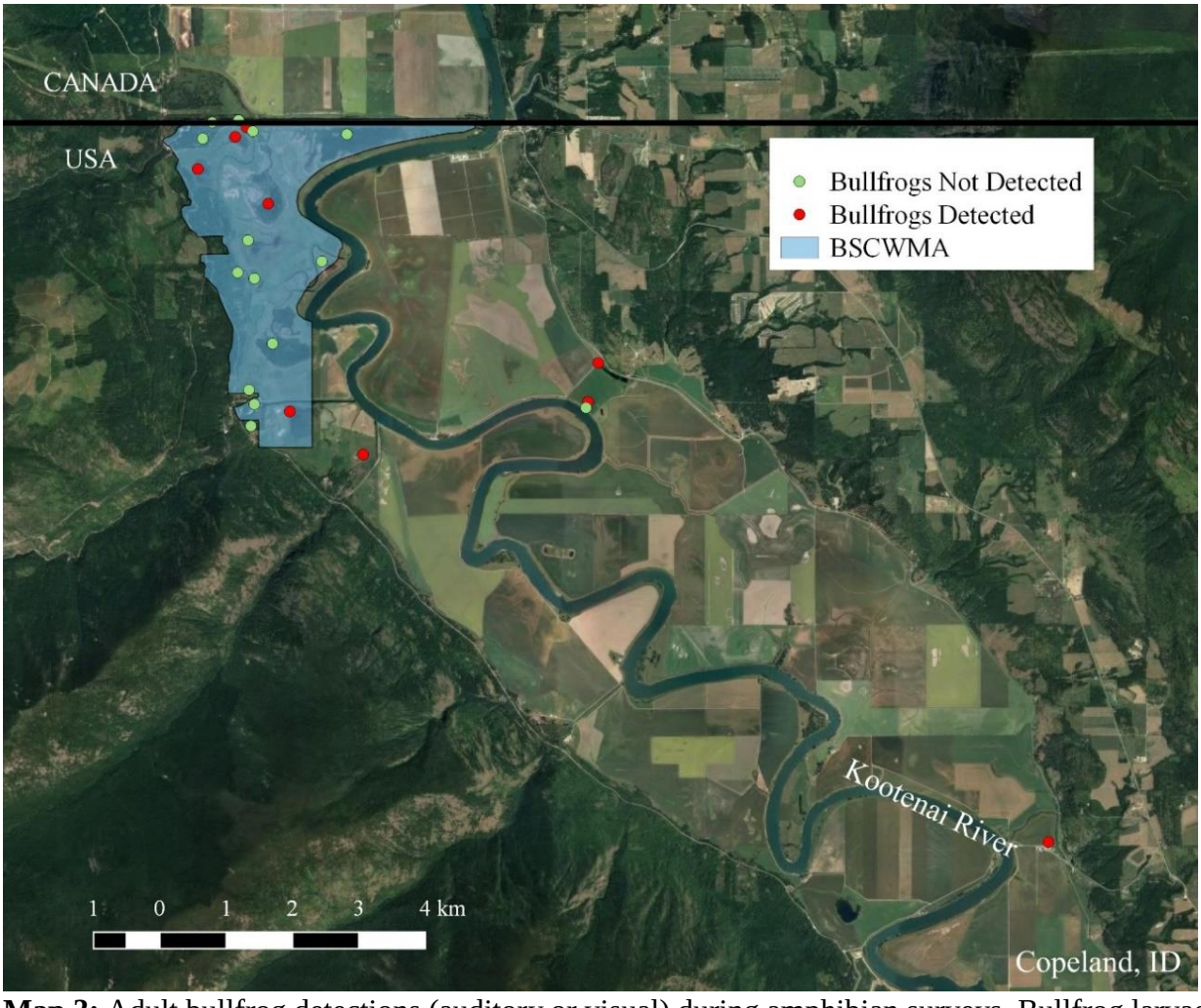


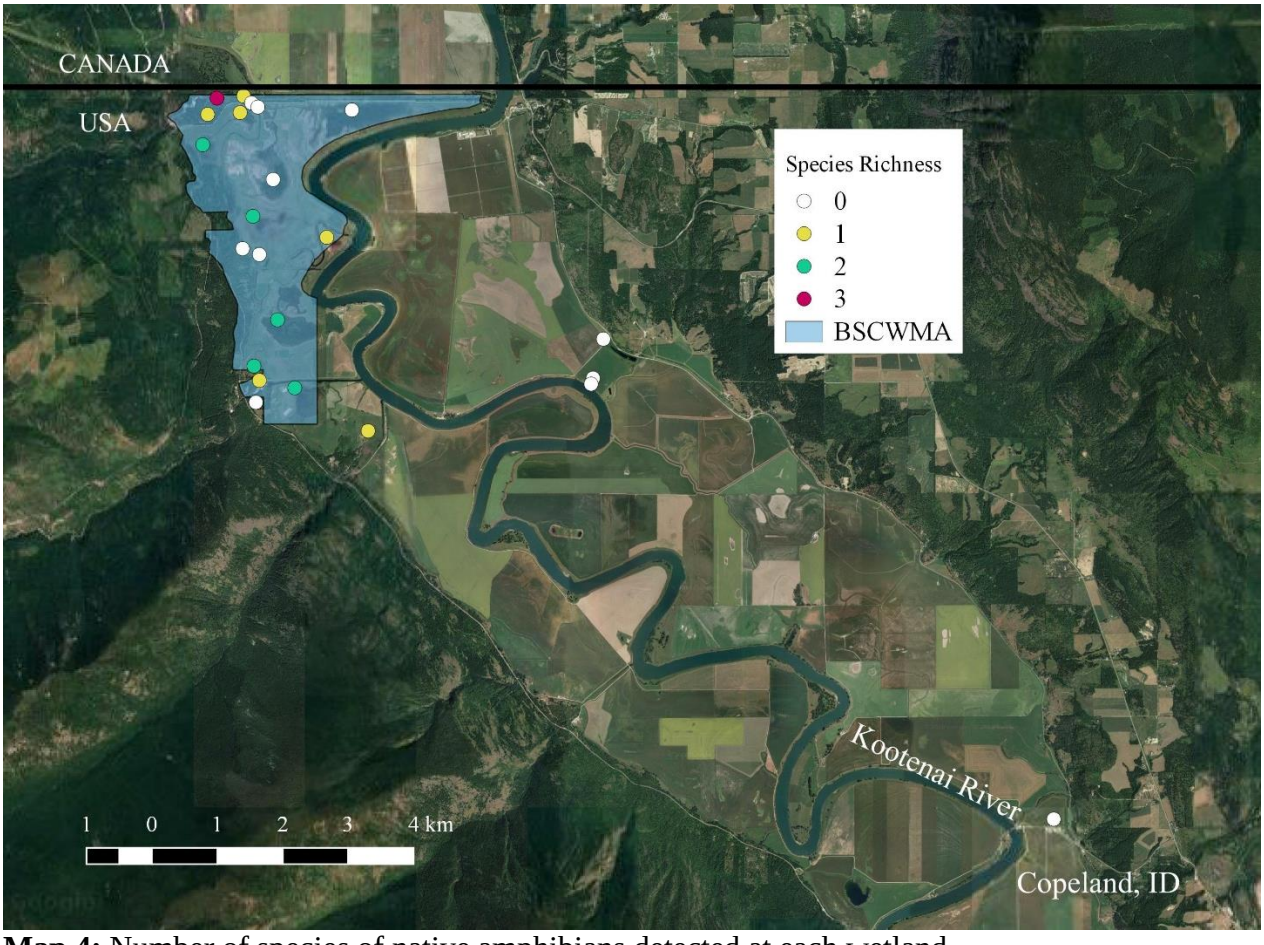
Figure 4: Photos of two common amphibian species in the Kootenai Valley, a long toed salamander larva on the left and a Pacific tree frog metamorph on the right.

We detected all four common, native, pond breeding amphibian species during our dip net surveys (Table 1, Map 2). Long-toed salamanders and Pacific tree frogs were detected most often, at ten and seven wetlands, respectively. Columbia spotted frogs and western toads were only detected at one wetland each. Native amphibian species richness ranged from zero to three amphibian species per wetland (mean = 0.83).Surprisingly, we did not detect bullfrog larvae at any wetland in the study area during our dip net surveys. However, we did detect bullfrog adults either visually or audibly in nine wetlands (Map 3). Because their calls carry long distances, it was often difficult to pinpoint the location of bullfrog calls, so some of these detections may have been from the same wetland. Bullfrogs were exclusively detected in wetlands with maximum depth greater than 1.5 m. Painted turtles and common garter snakes were detected in five wetlands each (Table 1).

Map 2: Native amphibian larvae detections from dip net surveys in 2017



Map 3: Adult bullfrog detections (auditory or visual) during amphibian surveys. Bullfrog larvae were not detected during dip net surveys.



Map 4: Number of species of native amphibians detected at each wetland.

Table 1: Species detected during wetland surveys in 2017. Species names are abbreviated; PT = Painted Turtle, GS = common garter snake, BULL = American bullfrog, CSF = Columbia spotted frog, LTS = long-toed salamander, TREE=Pacific tree frog, WT = western toad. Bullfrog site indicates whether or not a site was a focal bullfrog removal pond.

Wetland	Landowner	Bullfrog Site	PT	GS	BULL	CSF	LTS	TREE	WT
W166A	IDFG	No	0	0	0	0	0	1	1
W166B	IDFG	No	0	0	1	0	1	1	0
W166D	IDFG	No	0	0	0	0	1	0	0
W166F	IDFG	No	1	0	0	0	1	1	0
W970A	IDFG	No	0	1	1	0	1	0	0
W970B	IDFG	No	0	0	1	0	1	1	0
W970C	IDFG	No	0	0	0	0	0	0	0
W970D	IDFG	No	0	0	1	0	0	0	0
W970E	IDFG	No	0	0	0	0	1	1	0
W970F	IDFG	No	1	0	0	0	1	0	0
W970G	IDFG	No	0	0	0	0	1	0	0
W970I	IDFG	No	0	0	1	0	0	0	0
W970J	IDFG	No	0	0	0	1	1	1	0
W970K	IDFG	No	0	0	0	0	0	1	0
W970L	IDFG	No	0	1	0	0	0	0	0
W970M	IDFG	No	1	1	0	0	0	0	0
W970O	IDFG	No	0	0	0	0	0	0	0
W1010A	Private	Yes	1	1	1	0	0	0	0
W1010C	Private	No	0	0	1	0	0	0	0
W1010D	Private	No	0	0	0	0	0	0	0
W1057A	Private	Yes	1	1	1	0	0	0	0
W166C	Private	No	0	0	0	0	0	0	0
W166E	Private	Yes	0	0	1	0	1	0	0

Bullfrog Removal

We removed a total of 50 bullfrogs (8 male, 42 female) from three wetlands over nine visits. Bullfrogs ranged in size from 48 mm SVL to 166 mm SVL, with different size distributions at each wetland (Figure 5). W166E contained many smaller, juvenile frogs, while we only removed three large adult frogs from W1010A. W1057A contained frogs that spanned the entire size range.

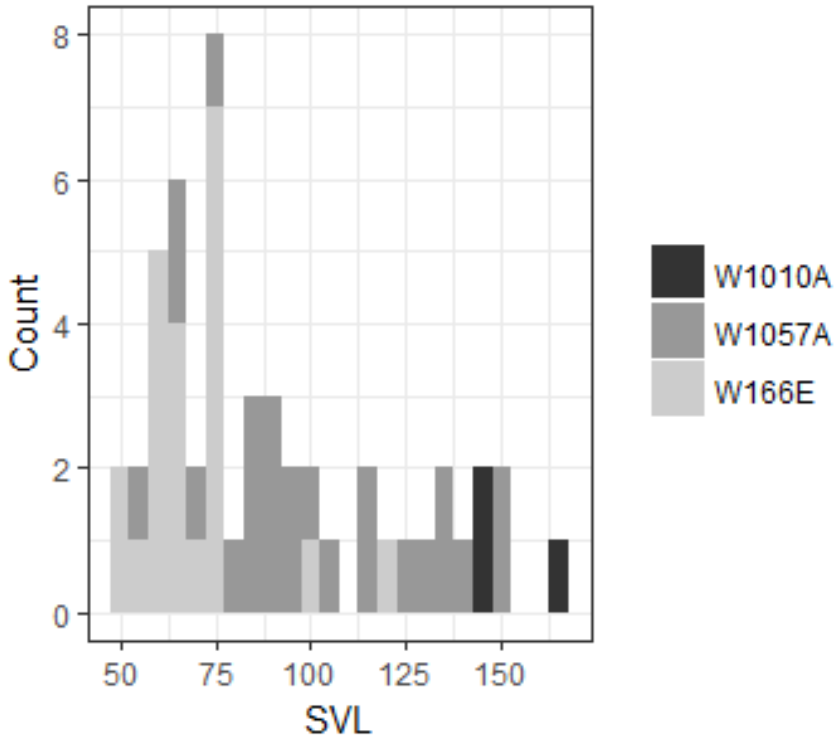


Figure 5: Size distribution of bullfrogs removed from each site.

Efficacy of bullfrog removal varied by site (Figure 6). While we were unable to estimate total abundance of bullfrogs and detection probability due to low numbers of captures at each site, habitat characteristics were important in our ability to locate and shock frogs. For example, at W166E, we removed 15 frogs on the first visit, 7 on the second visit, and 0 on the third visit, suggesting that electrofrogging was successful at reducing the population size considerably. W166E was a relatively small site, and we were able to access all parts of the wetland. In contrast, at W1057A, we removed 7 frogs on the first visit, 9 on the second, and 9 on the third. This site contained extensive woody debris, limiting our ability to access frogs and allowing them to escape more easily.

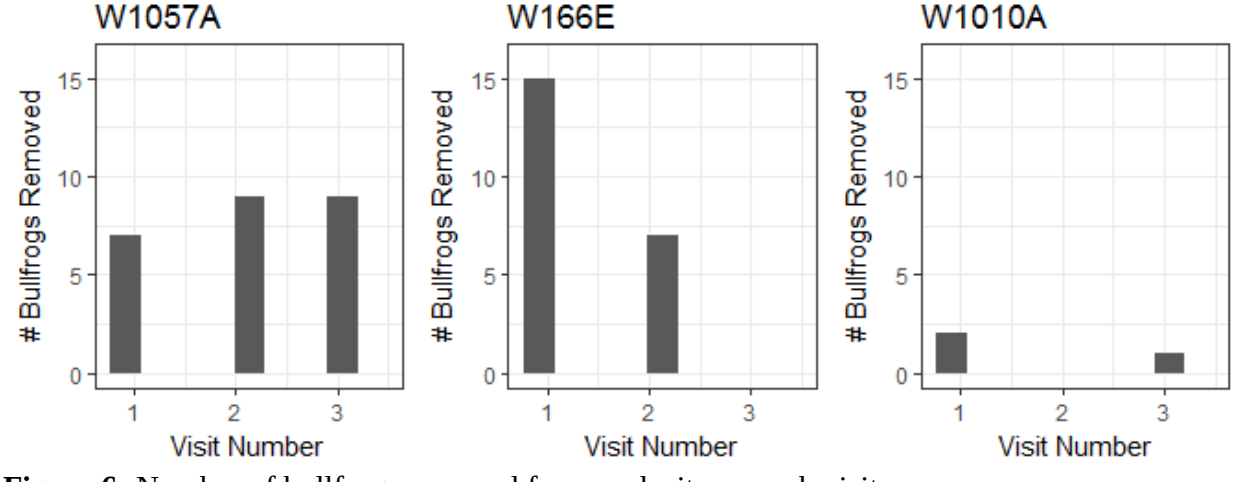


Figure 6: Number of bullfrogs removed from each site on each visit.

Disease

We tested 23 adult bullfrogs from three wetlands for Bd. Of these, 6 were determined to be Bd positive, 3 were equivocal, and the remaining 14 were negative. At least one bullfrog from each site came back as positive, indicating that Bd is likely widespread but occurring at low density in the Kootenai Valley (Table 2).

Table 2: Bd results for each bullfrog tested in 2017

Wetland ID	Sample ID	Species	Bd	Date Collected
W1010A	W1010AV1BULLBDA	American Bullfrog	Equivocal	6/22/2017
W1010A	W1010AV1BULLBDB	American Bullfrog	Positive	6/22/2017
W1010A	W1010AV3BULLBDC	American Bullfrog	Equivocal	7/19/2017
W1057A	W1057AV1BULLBDA	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDB	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDC	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDD	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDE	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDF	American Bullfrog	Negative	7/17/2017
W1057A	W1057AV1BULLBDG	American Bullfrog	Positive	7/17/2017
W1057A	W1057AV2BULLBDH	American Bullfrog	Negative	7/25/2017
W1057A	W1057AV2BULLBDI	American Bullfrog	Negative	7/25/2017
W1057A	W1057AV2BULLBDJ	American Bullfrog	Negative	7/25/2017
W166E	W166EV1BULLBDA	American Bullfrog	Positive	7/6/2017
W166E	W166EV1BULLBDB	American Bullfrog	Negative	7/6/2017
W166E	W166EV1BULLBDC	American Bullfrog	Equivocal	7/6/2017
W166E	W166EV1BULLBDD	American Bullfrog	Positive	7/6/2017
W166E	W166EV1BULLBDE	American Bullfrog	Positive	7/6/2017
W166E	W166EV1BULLBDF	American Bullfrog	Positive	7/6/2017
W166E	W166EV1BULLBDG	American Bullfrog	Negative	7/6/2017
W166E	W166EV1BULLBDH	American Bullfrog	Negative	7/6/2017
W166E	W166EV1BULLBDI	American Bullfrog	Negative	7/6/2017
W166E	W166EV1BULLBDJ	American Bullfrog	Negative	7/6/2017

DISCUSSION

We were surprised at the lack of detection of bullfrog reproduction (eggs, larvae, or metamorphs) at any of our surveyed wetlands, even though adults were heard or seen at nine wetlands. Although bullfrog larvae have been detected in the study area in recent years (Lucid 2015), this suggests support for our hypothesis that bullfrogs have only recently invaded the Kootenai Valley and have yet to become fully established. Additionally, our surveys were not designed to account for detection probability so these results likely underestimate the distribution of native amphibians and bullfrogs. Many of the wetlands that we surveyed were dominated by thick reed canary grass and cattails, which likely reduced our ability to detect amphibian larvae, especially when they occurred at low density. Doing night-time callback surveys for adults may be a better way to identify additional wetlands to target for bullfrog removal. Bullfrogs were

detected in the restored wetland cells on BSCWMA, but the large size and thick vegetation in these sites would make removal challenging. In colder climates, including northern Idaho, bullfrogs require permanent water to successfully reproduce because larvae are unable to complete metamorphosis in a single season, so removal efforts should focus on these waterbodies.

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Terry Anderson, a habitat biologist with the British Columbia Ministry of the Environment donated his time to drive all the way down from Nelson, BC to help us figure out how to use the electrofrogging equipment.

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APPENDIX 1: IDAHO PANHANDLE WETLAND SURVEY – PROTOCOL 2017

SURVEY

1) Approach wetland quietly and scan for turtles as you approach
2) Write the wetland number and 'begin' on the laminated card. Photograph wetland from aspect which best shows its character. Take waypoint and compass bearing of wetland photo.
3) While at the wetland keep an eye out for, and take note of, target non-amphibian species
4) If there are two observers available, survey the wetland in opposite directions so that each person surveys half the wetland.
5) Turn on GPS track before beginning survey.
6) Dip net and visually search along wetland shoreline and record each amphibian species and development stage you detect.
7) Estimate the number of each species you detect. If <10 individuals, count each one. If 10-100 individuals estimate to the nearest 10 (i.e. 20, 30, 40...). If there are more estimate '100s' or '1000s'. Don't just count what's in the net; count all the amphibians you see.
8) Collect 5 individual animals of each species detected in zip top bags partially filled with water (make sure adults have access to air for breathing). Preferentially collect adults, then fully formed metamorphs, then larval stage individuals.
9) **Photographs:** Photograph 1 adult and 1 larval stage individual from each species. Take 3 photographs of each animal selected for photography. Place animal in photo booth and take a dorsal, ventral, and lateral view photo. Label as directed below.
10) Collect a Bd and tissue sample from each adult or fully formed juvenile collected. Collect a tissue sample (but not Bd) sample from each collected larvae. Record SVL length of each fully formed animal sampled.
11) **Bd Samples**: Follow protocol outlined below.
12) **Tissue Samples:** Clip one digit (digit 3 or 5 is best) from hind foot of adult, collect whole small tadpoles, clip tail from large tadpole, or collect single egg. Place tissue sample into dry coin envelope (do not place in a vial) and seal the envelope. *Fill sample envelopes out completely (including life stage).* Between each sample wipe scissors with cotton (your shirt) then with a bleach wipe. Spread coin envelopes out to dry at room temperature then store at room temperature.
13) If bumblebees are encountered during survey spend 10 minutes attempting to capture bumblebee and put in zip lock bag. Photograph the dorsal side of the bumblebee in bag.
14) Draw a diagram of the wetland which includes relevant habitat: submerged logs, emergent vegetation, talus slopes, cliffs, inlet, and outlet. Record habitat covariates on datasheet.
15) Write the wetland number on laminated 'end' card and take a photo.
16) If additional wetlands are encountered beyond what's on the list: Photograph and waypoint the wetland as protocol dictates. If time permits, complete a survey of the wetland. If time does not permit, partially fill out a data sheet with a wetland name, photoID, and photo bearing.

Photo ID *Photo IDs and Sample IDs should correspond
Wetland: W, cell #, P: The photo of the wetland from wetland 867A: **W867AP**
Plant/Bee: W, cell #, P, letter: bee photo after pictures have been taken of two plants wetland 867A: **W867APC**
Amphibian: W, cell #, A, Sample ID, P, photo#: The third photograph of the fourth amphibian to be sampled at wetland 867A: **W867AADP3**
Sample ID
Tissue: W, cell #, A, letter: The fourth amphibian to be sampled at wetland 867: **W867AD**
BD: W, cell #, BD, letter: second frog swabbed at wetland 867: W867

HYGIENE, ANIMAL HANDLING, AND EQUIPMENT CLEANING

1) When you arrive at the wetland wash hands with biodegradable soap in a spot the soap will not run off into the water (like on the road by the truck) Do not apply additional sunscreen or bug spray unless you wash your hands again.
2) Handle adult amphibians with clean wet hands. Observe tadpoles and transport other amphibians in plastic zip top bags. Do not handle tadpoles directly unless collecting tissues. Discard bags after one use.
3) Clean mud, snails, and plants from equipment with stiff brush at site. Rinse in wetland.
4) At truck spray all equipment which touched wetland with Quat (.5 oz/ gallon) (preferred) or bleach (10%). Spread equipment out to dry in back of truck while traveling to next site.

BD SAMPLING

1) **Sample only the first 5 fully formed adults (preferred) or juveniles of each species at each wetland.**
2) Start the swabbing procedure as soon as possible after capture, without putting amphibians in a container together or in water that another amphibian has just been held in.
3) Wear a fresh vinyl glove for each amphibian handled to prevent transfer of chytid to the swab sample between amphibians or from stream water, etc.
4) Open the swab package and tube on a stable surface if working alone, or have another person handle them. Do not touch or get water onto swab tip or inside of tube during handling.
5) Pick up the amphibian from the top and try to minimize touching the animal's underside during handling.
6) Using a single swab, gently swab the ventral surfaces of the skin approximately 20-30 times. Target areas to include the pelvic patch (5 passes with the swab), ventral thighs (5 passes each side with the swab) and toe webbing (5 passes on each foot). It is not necessary to swab the dorsal skin surfaces.
7) Place swab inside empty tube without brushing it against the outside or rim of the tube. After swab tip is about half way inside tube, bend swab handle against rim of tube to snap it off.
8) Screw the cap on the tube firmly (but do not over tighten).
9) Label the side and top of the tube with sample ID. Place tube in coin envelope and fill envelope out completely (make sure to include species name, date collected, and sample ID).
10) To prevent spreading disease, dispose of swab stick and glove in a designated, sealed bag.
11) **Do not let sample get extremely hot (like in the cab of your truck). Put samples in freezer at Smith creek at the end of each day. Samples must be kept frozen.**

APPENDIX 2: IDAHO PANHANDLE WETLAND SURVEY – Datasheet 2017

Bullfrog Detected? (Y/N) _____
Stage (s):
How? (circle): Heard, seen, handled

Idaho Panhandle Wetland Survey – Data Sheet - 2017

**Create track of wetland survey. All waypoints should be in WGS84 (decimal degrees)

Wetland ID_____ MBI Cell:_____ Date (e.g. 15 June 2014):_____ Start Time:_____ Observer(s):_____
Visit number: _____ Track ID:_____(WetlandID, Date, T)
Directions to Wetland:

Landowner:_____ Phone #(if Private Individual): _____ Email:_____

Site is (circle one): Wet or Dry Search Type (circle one): Full Perimeter, Partial Perimeter (reason)_____

Photo Waypoint: _____ _____, Bearing: ____°Wetland Photo ID (W, cell#, P):_____

Weather (circle one): Sunny, Mostly Sunny, Partly Sunny, Overcast, Light Rain, Heavy Rain, Snow

Wetland Type (circle one): Natural Pond, Ephemeral Natural Pond, Constructed Pond, Modified Natural Pond, Lake, Stream , Channels Near Stream, Puddles, Emergent Wetland, Meadow, Forest-No Wetland, Not-Forested-No Wetland, Beaver Pond, Other: _____

Surrounding Vegetation Type: Forest, Cattle Grazing, Farming, Natural Grassland, Natural Riparian, WMA

Dominate Vegetation Type (circle one): sedges/rushes, cattails, willows, reed canary grass, bare ground, other _____

%open water (circle one) : <25%, 25-50%, 50-75%, >75%

Max. Depth (circle one): <0.5 M, 0.5 – 1.5 M, >1.5M

Wetland Diagram

Wetland Perimeter (m)_____

Amphibians Detected

Species	Stage	Abundance

Samples Collected

Tissue sample ID: W, Cell#, A, Letter

BD sample ID: W, Cell#, BD, Letter

*mm Fully formed only

Species	Stage	A or Juvy	Bd ID	Tissue ID	Photo Y/N

Abundance Categories
1, 2, 3, 4, 5, 6, 7, 8, 9
10s (estimate to nearest 10, 20, 30, 40...
100s
1000s

Stages
EGG: Eggs
NL: No Legs
BL: Beginning Legs
LEGS: 4 legs + Tail
AJ: Fully formed juvy or adult.
AJF: AJ Fledging - only use when mass of juvys is leaving pond. Usually late summer.

Species Codes:
Bullfrog: **BULL**
Columbia Spotted Frog: **CSF**
Long Toed Salamander: **LTS**
Tree/Chorus Frog: **TREE**
Western Toad: **WT**
Northern Leopard Frog: **NLF**
Tailed Frog: **TAIL**
ID Giant Salamander: **GIANT**

Observed - record number detected (0 if not detected) - do not take photo

Painted Turtle_____ Snapping Turtle_____

T. Garter Snake:_____ C. Garter Snake:_____
(red dorsal spots) (no red dorsal spots)

Hoary Marmot:____ Golden Mantled G.S._____

Yellow-bellied Marmot:_____ Pika:_____

Bumblebee Observed?(Y/N)_____
PhotoID: W, Cell#, P, letter

Photo ID

Fish Observed? (Y/N) _____

Fish Species (if known)
_____

Observed - Yes or No
do not take photo

Spotted Knapweed:_____

Devil's Club:_____

Whitebark Pine:_____

Tansy: _____

Sundew Observed - Yes or No
Sundew:_____
PhotoID: W, Cell#, P, letter

Latitude	Longitude	Photo ID

APPENDIX 3: BULLFROG REMOVAL PROTOCOL 2017

Conduct work only under the following conditions:

- After sunset, when it is fully dark
- When nighttime low air temperature is predicted to be ≥ 35 degrees F and there is no precipitation and winds are predicted to be calm.

Electroshocker Settings: 135 Volts 55Hz Frequency 20% Duty Cycle

Personnel

Two people should work together from a boat during each removal event. One person paddles the boat while the other operates the shocker.

1. Record waypoint directly from GPS (decimal degrees), start air/water temperature (Celsius), and start time (military).
2. Paddle around the perimeter of the wetland while both people shine bright lights ahead. When a fully formed bullfrog is observed paddle slowly toward it while maintaining the beam of the light pointed at the frog.
3. Shock the frog and remove from the water. Confirm species is bullfrog and return to water if it is not a bullfrog. Do not return bullfrogs to the water.
4. Continue perimeter search and process bullfrogs and end of first lap.
5. Place animal in 5 gallon bucket with screw top lid. Place first 10 animals in 1gal zip tops.
6. Swab the 1st 10 bullfrogs encountered at each focal site for Bd. For each swabbed frog, use a clean pair of gloves and thoroughly swab the belly, legs, and feet. Swabs should be labeled with site name, date, and species.

Example: First bullfrog swabbed for Bd during the first visit to wetland 1412A on July 18 2017	W1412AVIBULLBDA 18 July 2017


Male Femal

7. Identify frog as male (tympanic membrane much larger than eye) or female (tympanic membrane about same size as eye).
8. Use digital calipers to measure (mm) snout to vent length (SVL) from tip of animals nose to the opening of the cloaca.
9. Take a toe clip tissue sample from first 10 bullfrogs.
10. Repeat perimeter repeats of pond until no more fully formed bullfrogs are encountered. Record stop time (military) and total number of laps completed.
11. After exiting boat fill the 5 gallon bucket with enough water to cover bullfrogs and add several drops of clove oil. Replace screw top lid and leave bucket overnight.
12. In the morning drain water from euthanized bullfrogs. Dispose of carcasses in woods.

HYGIENE and EQUIPMENT CLEANING

1) Wash hands away from wetland with biodegradable soap before beginning work. Handle adult amphibians with clean wet hands or gloves.

2) After work is complete clean mud, snails, and plants from equipment with stiff brush at site. Rinse in wetland.

3) Spray all equipment that touched wetland with Quat solution (.25oz/gallon). Spread equipment out to dry in back of truck while travelling to next site or dry in sun the next day.

APPENDIX 4: BULLFROG REMOVAL DATASHEET 2017

Wetland ID_____ Cell: _____ Date (e.g. 15 June 2017):_____ Observer(s):_____
Visit Number _____
Wetland Waypoint: _____ _____ Wetland Name (e.g. 'Nancy's Pond'):
_____
Weather (circle one): Clear, Mostly Clear, Partly Cloudy, Overcast, Light Rain, Heavy Rain, Snow
Start Time: _____ Start Air Temperature: _____°C Start Water Temperature: _____°C
End Time: _____ Laps around perimeter _____
Bullfrog Captures

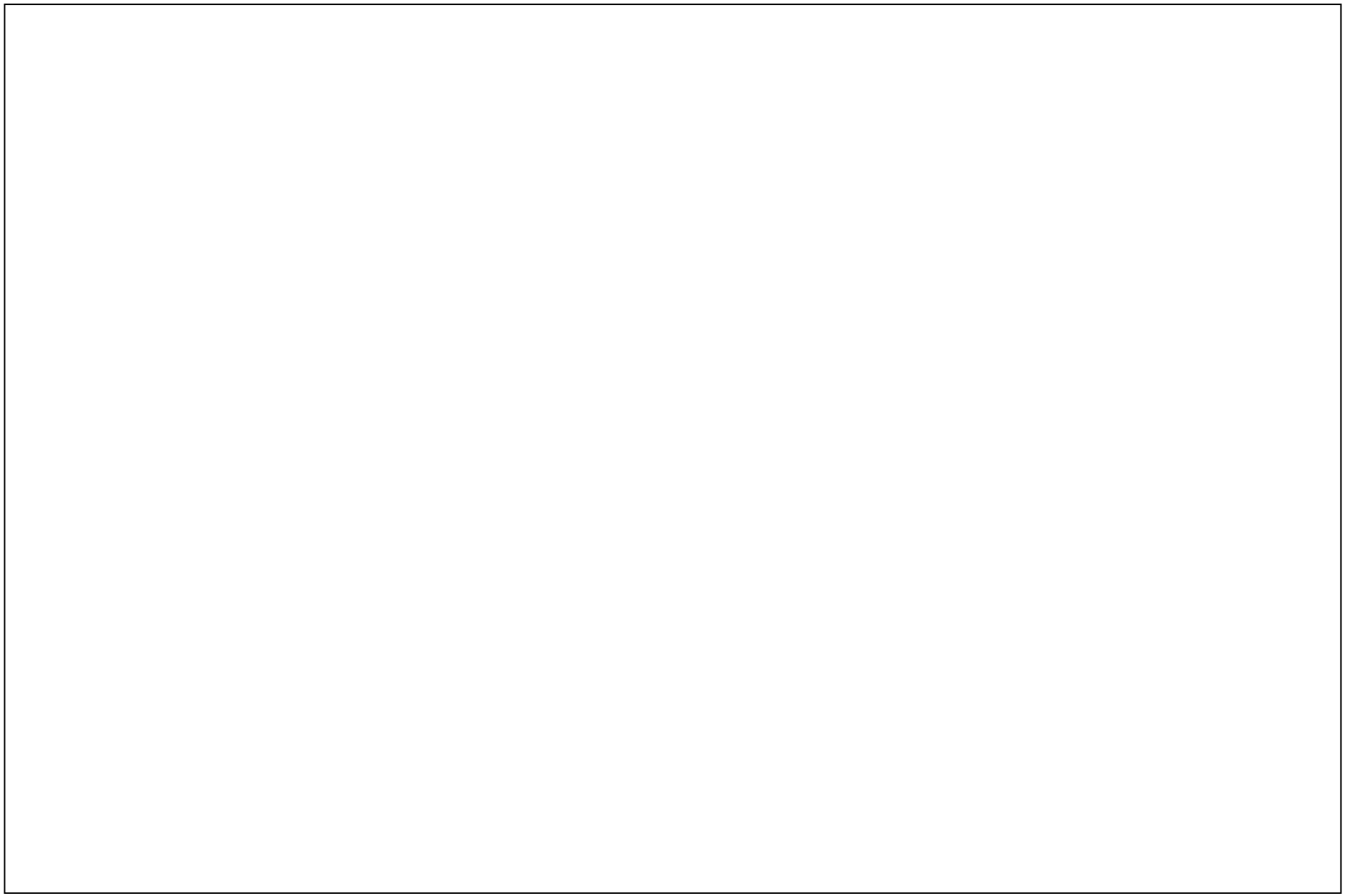
BF#	Gender	SVL (mm)	Bd? (y/n)	Tissue? (y/n)	Lap #	BF#	Gender	SVL (mm)	Bd? (y/n)	Tissue? (y/n)	Lap #
1						21					
2						22					
3						23					
4						24					
5						25					
6						26					
7						27					
8						28					
9						29					
10						30					
11						31					
12						32					
13						33					
14						34					
15						35					
16						36					
17						37					
18						38					
19						39					
20						40					

Total # Females: _____ Total # Males: _____

Total # Captured Bullfrogs (F+M): _____ Total # Missed Bullfrogs by Lap:
_____
Bd sample names (range) _____ Total # Bd Samples: _____
Tissue sample names (range) _____ Total# Tissue Samples: _____

HOW TO LABEL BD SWABS: Wetland ID, Visit number, species, BD, letter
Example: W1412V1BULLBDA- the first bullfrog sampled for bd at the first visit of wetland 1412

HOW TO LABEL Tissue Samples: Wetland ID, Visit number, species, letter
Example: W1412V1BULLA- the first bullfrog sampled for tissue at the first visit of wetland 1412



Draw diagram of pond including bullfrog capture (B) and miss (M) locations.

APPENDIX 5: Locations of wetlands in study area

Wetland	Longitude	Latitude	Bullfrog Site	Permission	Surveyed 2017
W1010A	-116.481	48.96724	Yes	Yes	Yes
W1010C	-116.483	48.96202	No	Yes	Yes
W1010D	-116.483	48.96125	No	Yes	Yes
W1057A	-116.389	48.90324	Yes	Yes	Yes
W166A	-116.547	48.96981	No	WMA	Yes
W166B	-116.543	48.96074	No	WMA	Yes
W166C	-116.551	48.95881	No	Yes	Yes
W166D	-116.551	48.96172	No	WMA	Yes
W166E	-116.528	48.955	Yes	Yes	Yes
W166F	-116.552	48.96364	No	WMA	Yes
W970A	-116.554	48.9974	No	WMA	Yes
W970B	-116.562	48.99311	No	WMA	Yes
W970C	-116.532	48.99774	No	WMA	Yes
W970D	-116.548	48.98849	No	WMA	Yes
W970E	-116.552	48.98359	No	WMA	Yes
W970F	-116.537	48.98079	No	WMA	Yes
W970G	-116.554	48.9996	No	WMA	Yes
W970I	-116.552	48.99868	No	WMA	Yes
W970J	-116.559	48.99931	No	WMA	Yes
W970K	-116.561	48.99716	No	WMA	Yes
W970L	-116.554	48.97928	No	WMA	Yes
W970M	-116.551	48.9785	No	WMA	Yes
W970O	-116.551	48.99814	No	WMA	Yes
W1009A	-116.48	48.92992	No	No	No
W1009B	-116.473	48.92949	No	No Contact	No
W1009C	-116.472	48.93061	No	No Contact	No
W1009D	-116.473	48.92295	No	No	No
W1009E	-116.447	48.93006	No	No Contact	No
W1009F	-116.434	48.89639	No	No	No
W1009G	-116.432	48.89167	No	No	No
W1010B	-116.476	48.96663	No	No Contact	No
W1010E	-116.494	48.96205	No	No	No
W1010F	-116.459	48.96062	No	No Contact	No
W1010G	-116.489	48.94333	No	No Contact	No
W1010H	-116.494	48.94381	No	No Contact	No
W1010I	-116.49	48.93537	No	No	No
W1057B	-116.39	48.90072	No	No	No
W970N	-116.499	48.99444	No	No Contact	No