Appendix III. Amphibians - Multi-species Baseline Initiative

Lucid, M.K., L. Robinson, and S.E. Ehlers. 2016. Multi-species Baseline Initiative project report. 2010-2014. Idaho Department of Fish and Game, Coeur d'Alene, Idaho, USA.

Appendix IIIa: Protocols and Datasheets
Appendix IIIb: Chytrid Fungus Detection Data
AppendixIIIc: Landowner Letter and Postcard
Appendix IIId: Environmental DNA vs Dipnetting Field Assessment
Appendix IIIe: Photographs of Examined Historic Record Museum Specimens of Rana pipiens and Rana sylvatica

Amphibian Survey Protocol

SURVEY

1) Locate wetland. If there is not a wetland at the point you were assigned conduct a 30-minute timed search for amphibians and complete the remainder of this protocol.
2) Approach wetland quietly and scan for turtles as you approach.
3) Write the wetland number and 'begin' on the laminated card. Photograph wetland from aspect which best shows its character. This point will also be the start of the transect. Record waypoint.
4) While at the wetland keep an eye out for, and take note of, target non-amphibian species.
5) Use a 50-meter section of rope to measure your first transect. You should survey the wetland in a clockwise fashion. If two observers are available, survey the transect at the same time with one observer going clockwise and the other going counter-clockwise. Only use the rope for the first transect and estimate the distance for the remaining transects.
6) Dip-net each 50-meter section and record each amphibian species and development stage you detect. Estimate abundance in each section. 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.
7) Repeat until entire shoreline has been surveyed or up to 500 meters.
8) Collect a tissue sample from and photograph the first **two** individuals of each common amphibian species you encounter. Photograph and collect tissue samples from **5** each of tiger salamander, n. leopard frog, or wood frog. Samples from adults are preferred (record life stage).
    a. 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. *Fill sample envelopes out completely (including life stage).* **Between each sample wipe scissors with cotton (your shirt) then with a bleach wipe.**
    b. Take three photographs of each animal sampled (tissue or Bd). Place animal in photo container and take a dorsal, ventral, and lateral view.
9) **If adult spotted frogs are present, swab the first 3 captured according to BD protocol (below).**
    a. Only collect tissue from 2 of the spotted frogs. There should be 2 tissue samples and 3 Bd samples if there are adult spotted frogs at wetland.
10) Draw a diagram of the wetland which includes relevant habitat: submerged logs, emergent vegetation, talus slopes, cliffs, inlet, outlet, and transect locations.
11) If bumblebees are encountered during survey spend 5 minutes attempting to photograph individuals. If *western* bumble bee is seen spend up to 15 minutes attempting to photograph.
12) Write the wetland number on laminated 'end' card.

Labeling

Photo ID

Wetland: W, cell #, P: The photo of the wetland from wetland 867: **W867P**
Tidbit: W, cell #, PTidbit: The photo of the tidbit from wetland 867: **W867PTidbit**
Plant/Bee: W, cell #, P, letter: bee photo after pictures have been taken of two plants wetland 867: **W867PC**
Amphibian: W, cell #, A, Sample ID, P_L: The fourth amphibian to be sampled at wetland 867 lateral view: **W867ADP_L**

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: W867BDB

Photo ID and Sample ID letters should correspond.

HYGIENE, ANIMAL HANDLING, AND EQUIPMENT CLEANING

1) When you arrive at the wetland use a plastic bag to get some water from wetland. Dig a small hole about 75 paces from wetland and wash hands with biodegradable soap over hole. Fill hole in. 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 lock 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 10% bleach. Spread equipment out to dry in back of truck while traveling to next site.
5) Soak rope in 10% bleach solution.

BD SAMPLING

Sample only the first three adult spotted frogs captured. Do not sample if spotted frogs not present.

1) 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.
2) Wear a fresh vinyl glove for each amphibian handled to prevent transfer of chytrid to the swab sample between amphibians or from stream water, etc.
3) 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.
4) Pick up the amphibian from the top and try to minimize touching the animal's underside during handling.
5) Swab the underside of the amphibian from just below throat to rear toe tips and back 15 times. Alternate legs each time. Apply some pressure while swabbing, but don't squash the animal. Do not contaminate the swab by setting down or touching something with it before or after you swab the animal.
6) Place swab inside 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.
7) Screw the cap on the tube firmly (but do not over tighten).
8) Label the side and top of the tube with sample ID. Place tube in coin envelope and *fill envelope out completely.*
9) To prevent spreading disease, dispose of swab stick and glove in a designated, sealed bag.
10) Do not let sample get extremely hot (like in the cab of your truck). Store at room temperature away from light when you return from the field.

WETLAND SURVEY DATASHEET

Wetland ID: _____ Cell: _____ Date (e.g. 15 June 2014):_____ Start Time: _____ Observer(s):_____

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: _____

Site is (circle one): Wet or Dry Search Type (circle one): Full Perimeter, 500 Meter Shoreline, 30 Minute T.S.

Wetland Waypoint: _____Lat:_____ Long: _____ Wetland Photo ID: (W, cell#, P) _____

TidBit: Yes or No TidBit Waypoint: _____ Lat: _____Long:_____ TidBit SN: _____

Wetland Diagram

Last Section (m): _____

Total Perimeter (m): _____

Tidbit Diagram

Tidbit Photo ID: _____

Tidbit Depth (cm):____

Distance to shore (cm):_____

Directions to plot:

Directions to tidbit:

Landowner name:

Landowner phone:

Observed- Yes or No –do not take photo

Spotted Knapweed: ____ Devil's Club: _____

Whitebark Pine: _____ Tansy: _____

Amphibians by 50m Section

Species	Stage	Abundance	Section

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.

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

Observed - Yes or No_____
Round Leaf Sundew: _____ Long Leaf Sundew: ____
Rare Moonwort: _____
Fill in table for each location species is observed.
Photo ID: W, Cell#, P, letter

Species	Latitude	Longitude	Photo ID

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

Painted Turtle_____ Snapping Turtle_____

T. Garter Snake: _____ C. Garter Snake: _____

Hoary Marmot: ____ Golden Mantled G.S._____

Yellow-bellied Marmot: _____ Pika: _____

Bumblebee Observed? (Y/N)_____

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

Species	Photo ID

Fish Observed? Fish Species (if known):

Y/N: _____ _____

Samples: Adult Spotted Frogs Present? _____
Sample ID: W, Cell#, A, Letter
Photo ID: W, Cell#, BD, Letter

Species	Stage	(T)issue or (Bd)	Sample ID	Photo ID

SUPPLIES

Item	Number/ survey	Supplier	Item #	Web page
12" deep, 3/16" mesh Dip Net	2/person	Forestry Suppliers	77609	www.forestry-suppliers.com
Waders				
Quart zip top bags	10	StockPkg	3630A	https://stockpkg.com/
Parachute cord5/32"	2			
4 ½" Stainless-steel surgical scissors	2	Carolina Biological Supply	622505	http://www.carolina.com/
Disposable latex gloves (powder-free)	6	eSafety Supplies	20010FIT	https://www.esafetysupplies.com
MW fine-tipped plastic DrySwab, peel	3	Lakewood Biochemical, Co.	Mw113	http://www.lakewoodbio.com/
Screw top tubes with ethanol	3			
Plastic aquarium-small (photos)	1			
Bleach **Spray bottle**				

Appendix IIIb. Chytrid detection and zoospore count for swabbed Columbia spotted frogs.

Animal ID	Date Collected	Latitude	Longitude
W007ABDA	8/31/2013	48.7101	-117.27100
W7ABDB	8/31/2013	48.7101	-117.27100
W38BDA	6/4/2014	***48.3044**	**-117.01311**
W38BDB	6/4/2014	**48.3044**	**-117.01311**
W38BDC	6/4/2014	**48.3044**	**-117.01311**
W41BDA	8/19/2013	48.4443	-117.01783
W41BDB	8/19/2013	48.4443	-117.01783
W41BDC	8/19/2013	48.4443	-117.01783
W47BBDA	7/18/2013	48.7015	-117.03170
W48ABDA	8/30/2013	48.7425	-117.06126
W48ABDB	8/30/2013	48.7425	-117.06126
W48BBDA	9/4/2013	48.7741	-117.04978
W48BBDB	9/4/2013	48.7741	-117.04978
W48BBDC	9/4/2013	48.7741	-117.04978
W48BV1BDA	5/30/2014	48.7741	-117.04978
W48BV1BDB	5/30/2014	48.7741	-117.04978
W48BV1BDC	5/30/2014	48.7741	-117.04978
W48BV2BDA	6/17/2014	48.7741	-117.04978
W48BV2BDB	6/17/2014	48.7741	-117.04978
W48BV2BDC	6/17/2014	48.7741	-117.04978
W48BV3BDA	7/11/2014	48.7741	-117.04978
W48BV3BDB	7/11/2014	48.7741	-117.04978
W48BV3BDC	7/11/2014	48.7741	-117.04978
W48BV4BDA	7/29/2014	48.7741	-117.04978
W48BV4BDB	7/29/2014	48.7741	-117.04978
W48BV4BDC	7/29/2014	48.7741	-117.04978
W48BV5BDA	8/13/2014	48.7741	-117.04978
W48BV5BDB	8/13/2014	48.7741	-117.04978
W48BV5BDC	8/13/2014	48.7741	-117.04978
W48BV6BDA	9/4/2014	48.7741	-117.04978
W49BDA	8/30/2013	48.7930	-117.04305
W49BDB	8/30/2013	48.7930	-117.04305
W56BDA	6/14/2013	48.3912	-116.96167
W56BDB	6/14/2013	48.3912	-116.96167
W56BDC	6/14/2013	48.3912	-116.96167
W59BDA	7/31/2013	48.5531	-116.98752
W67V6BDA	9/4/2014	48.8778	-117.00529
W67V7BDA	9/26/2014	48.8778	-117.00529
W78ABDA	8/16/2013	48.6690	-116.93479
W78ABDB	8/16/2013	48.6690	-116.93479
W95BDA	8/5/2013	48.9032	-116.85519
W98BDA	8/17/2013	48.2725	-116.76801
W98BDB	8/17/2013	48.2725	-116.76801
W98BDC	8/17/2013	48.2725	-116.76801

W99BDA	8/17/2013	48.2994	-116.74477
W109BDA	8/13/2013	48.7791	-116.77924
W109BDB	8/13/2013	48.7791	-116.77924
W109BDC	8/13/2013	48.7791	-116.77924
W111BDA	8/12/2013	48.8588	-116.77599
W111BDB	8/12/2013	48.8588	-116.77599
W113BDA	8/6/2013	48.9417	-116.78936
W113BDB	8/6/2013	48.9417	-116.78936
W113BDC	8/6/2013	48.9417	-116.78936
W115BDA	6/5/2013	48.3018	-116.72021
W115BDB	6/5/2013	48.3018	-116.72021
W120BDA	8/15/2013	48.5509	-116.69915
W123BDA	8/31/2013	48.6714	-116.69757
W123BDB	8/31/2013	48.6714	-116.69757
W123BDC	8/31/2013	48.6714	-116.69757
W135BDA	8/13/2013	48.6988	-116.65073
W135BDB	8/13/2013	48.6988	-116.65073
W135BDC	8/13/2013	48.6988	-116.65073
W136BDA	8/13/2013	48.7080	-116.64771
W137BDA	8/3/2013	48.7776	-116.67493
W140BDA	7/18/2013	48.9030	-116.63202
W140BDB	7/18/2013	48.9030	-116.63202
W140BDC	7/18/2013	48.9030	-116.63202
W144BDA	8/20/2013	48.5114	-116.57792
W145BDA	8/26/2013	48.5513	-116.55980
W148BDA	8/27/2013	48.6595	-116.59918
W148BDB	8/27/2013	48.6595	-116.59918
W148V3BDA	7/11/2014	48.6595	-116.59918
W148V3BDB	7/11/2014	48.6595	-116.59918
W148V3BDC	7/11/2014	48.6595	-116.59918
W148V4BDA	7/30/2014	48.6595	-116.59918
W148V4BDB	7/30/2014	48.6595	-116.59918
W148V4BDC	7/30/2014	48.6595	-116.59918
W148V5BDA	8/13/2014	48.6595	-116.59918
W148V5BDB	8/13/2014	48.6595	-116.59918
W148V5BDC	8/13/2014	48.6595	-116.59918
W149BDA	8/10/2013	48.7395	-116.61693
W149BDB	8/10/2013	48.7395	-116.61693
W149BDC	8/10/2013	48.7395	-116.61693
W158BDA	8/27/2013	48.6092	-116.52594
W158BDB	8/27/2013	48.6092	-116.52594
W158BDC	8/27/2013	48.6092	-116.52594
W167BDA	9/3/2013	48.6435	-116.42785
W167BDB	9/3/2013	48.6435	-116.42785
W167BDC	9/3/2013	48.6435	-116.42785
W169BDA	9/2/2013	**48.7417**	**-116.41412**
W169BDB	9/2/2013	**48.7417**	**-116.41412**
W169BDC	9/2/2013	**48.7417**	**-116.41412**

W172BDA	6/15/2014	**48.8750**	**-116.42736**
W172BDB	6/15/2014	**48.8750**	**-116.42736**
W172BDC	6/15/2014	**48.8750**	**-116.42736**
W642ABDA	7/2/2014	**47.5251**	**-117.00643**
W642ABDB	7/2/2014	**47.5251**	**-117.00643**
W642ABDC	7/2/2014	**47.5251**	**-117.00643**
W642BBDA	6/29/2014	**47.5347**	**-116.96253**
W642BBDB	6/29/2014	**47.5347**	**-116.96253**
W642BBDC	6/29/2014	**47.5347**	**-116.96253**
W653BDA	6/3/2014	**48.0333**	**-117.02763**
W653BDB	6/3/2014	**48.0333**	**-117.02763**
W653BDC	6/3/2014	**48.0333**	**-117.02763**
W680BDA	6/29/2014	**47.5059**	**-116.95135**
W680BDB	6/29/2014	**47.5059**	**-116.95135**
W680BDC	6/29/2014	**47.5059**	**-116.95135**
W691BDA	7/11/2014	**47.9948**	**-116.98153**
W691BDB	7/11/2014	**47.9948**	**-116.98153**
W694BDA	6/4/2014	**47.9948**	**-116.98153**
W694BDB	6/4/2014	**48.1384**	**-116.98236**
W694BDC	6/4/2014	**48.1384**	**-116.98236**
W714BDA	7/12/2014	**47.2296**	**-116.85555**
W714BDB	7/12/2014	**47.2296**	**-116.85555**
W714BDC	7/12/2014	**47.2296**	**-116.85555**
W715BDA	7/16/2014	**47.2765**	**-116.85729**
W724BDA	6/15/2014	**47.6654**	**-116.87930**
W724BDB	6/15/2014	**47.6654**	**-116.87930**
W724BDC	6/15/2014	**47.6654**	**-116.87930**
W731BDA	6/4/2014	47.9824	-116.89313
W731BDB	6/4/2014	47.9824	-116.89313
W731BDC	6/4/2014	47.9824	-116.89313
W73BDA	8/16/2013	48.4560	-116.89911
W754BDA	7/13/2014	**47.2473**	**-116.75713**
W763BDA	5/1/2014	**47.6202**	**-116.76163**
W763BDB	5/1/2014	**47.6202**	**-116.76163**
W763BDC	5/1/2014	**47.6202**	**-116.76163**
W777BDA	8/15/2013	48.4984	-116.82166
W777BDB	8/15/2013	48.4984	-116.82166
W777BDC	8/15/2013	48.4984	-116.82166
W795BDA	7/14/2014	**47.1582**	**-116.68570**
W795BDB	7/14/2014	**47.1582**	**-116.68570**
W795BDC	7/14/2014	**47.1582**	**-116.68570**
W796BDA	7/16/2014	**47.2114**	**-116.67783**
W797BBDA	7/13/2014	**47.2508**	**-116.73061**
W797BBDB	7/13/2014	**47.2508**	**-116.73061**
W797BBDC	7/13/2014	**47.2508**	**-116.73061**
W79BDA	8/16/2013	48.7323	-116.89452
W79BDB	8/16/2013	48.7323	-116.89452
W79BDC	8/16/2013	48.7323	-116.89452

W81BDA	8/3/2013	48.8283	-116.94142
W81BDB	8/3/2013	48.8283	-116.94142
W81BDC	8/3/2013	48.8283	-116.94142
W849BDA	6/15/2014	**47.8331**	**-116.68495**
W851BDA	6/15/2014	**47.8889**	**-116.66737**
W851BDB	6/15/2014	**47.8889**	**-116.66737**
W851BDC	6/15/2014	**47.8889**	**-116.66737**
W854BDA	8/24/2013	**48.0542**	**-116.70102**
W854BDB	8/24/2013	**48.0542**	**-116.70102**
W854BDC	8/24/2013	**48.0542**	**-116.70102**
W878BDA	6/29/2014	**47.5397**	**-116.57151**
W878BDB	6/29/2014	**47.5397**	**-116.57151**
W881BDA	6/13/2014	47.6915	-116.60610
W883BDA	5/26/2013	47.7709	-116.58474
W883BDB	5/26/2013	47.7709	-116.58474
W883BDC	5/26/2013	47.7709	-116.58474
W890BDA	6/3/2014	**48.0846**	**-116.58562**
W890BDB	6/3/2014	**48.0846**	**-116.58562**
W891BDA	8/14/2013	48.1332	-116.63739
W896BDA	7/23/2014	**48.3779**	**-116.62206**
W896BDB	7/23/2014	**48.3779**	**-116.62206**
W898BDA	8/15/2013	48.4322	-116.66645
W898BDB	8/15/2013	48.4322	-116.66645
W898BDC	8/15/2013	48.4322	-116.66645
W905BDA	8/19/2013	**47.1486**	**-116.48734**
W905BDB	8/19/2013	**47.1486**	**-116.48734**
W905BDC	8/19/2013	**47.1486**	**-116.48734**
W91BDA	8/20/2013	48.7134	-116.87742
W91BDB	8/20/2013	48.7134	-116.87742
W91BDC	8/20/2013	48.7134	-116.87742
W925BDA	7/1/2013	48.0387	-116.57018
W925BDB	7/1/2013	48.0387	-116.57018
W938BDA	8/19/2013	**47.0544**	**-116.40897**
W938BDB	8/19/2013	**47.0544**	**-116.40897**
W942BDA	7/16/2014	**47.2337**	**-116.43912**
W942BDB	7/16/2014	**47.2337**	**-116.43912**
W953BDA	6/8/2013	47.7217	-116.46562
W953BDB	6/8/2013	47.7217	-116.46562
W974BDA	7/15/2014	**47.0606**	**-116.34051**
W974BDB	7/15/2014	**47.0606**	**-116.34051**
W974BDC	7/15/2014	**47.0606**	**-116.34051**
W975BDA	7/16/2014	47.1297	-116.38735
W975BDB	7/16/2014	47.1297	-116.38735
W978BDA	8/18/2013	**47.2462**	**-116.40868**
W978BDB	8/18/2013	**47.2462**	**-116.40868**
W979BDA	6/30/2014	**47.2760**	**-116.40935**
W1006BDA	6/18/2013	48.5004	-116.46627
W1011BDA	6/16/2014	**48.9898**	**-116.44654**

W1032BDA	6/9/2013	47.7887	-116.32120
W1046BDA	8/11/2013	48.4333	-116.38947
W1046BDB	8/11/2013	48.4333	-116.38947
W1048BDA	8/12/2013	48.5074	-116.35610
W1055BDA	7/10/2013	48.8261	-116.41749
W1057V1BDA	5/31/2014	48.9036	-116.38901
W1057V5BDA	8/13/2014	48.9036	-116.38901
W1062BDA	8/15/2013	46.9987	-116.22641
W1062BDB	8/15/2013	46.9987	-116.22641
W1062BDC	8/15/2013	46.9987	-116.22641
W1077BDA	6/11/2013	47.6747	-116.24615
W1080BDA	6/9/2013	47.8074	-116.27443
W1081BDA	6/28/2013	47.8253	-116.26785
W1081BDB	6/28/2013	47.8253	-116.26785
W1081BDC	6/28/2013	47.8253	-116.26785
W1093BDA	6/16/2014	**48.3541**	**-116.29257**
W1093BDB	6/16/2014	**48.3541**	**-116.29257**
W1093BDC	6/16/2014	**48.3541**	**-116.29257**
W1094BDA	8/11/2013	48.4336	-116.30891
W1094BDB	8/11/2013	48.4336	-116.30891
W1094BDC	8/11/2013	48.4336	-116.30891
W1095BDA	6/17/2013	48.4557	-116.30756
W1095BDB	6/17/2013	48.4557	-116.30756
W1095BDC	6/17/2013	48.4557	-116.30756
W1097BDA	6/22/2013	48.5709	-116.32872
W1098BDA	9/3/2013	48.6030	-116.32495
W1098BDB	9/3/2013	48.6030	-116.32495
W1099BDA	8/20/2013	48.6599	-116.34177
W1107BDA	8/9/2013	48.9835	-116.34006
W1111BDA	8/15/2013	47.0315	-116.14223
W1111BDB	8/15/2013	47.0315	-116.14223
W1111BDC	8/15/2013	47.0315	-116.14223
W1113BDA	7/30/2013	47.1316	-116.14650
W1113BDB	7/30/2013	47.1316	-116.14650
W1115BDA	8/19/2013	**47.1991**	**-116.20605**
W1115BDB	8/19/2013	**47.1991**	**-116.20605**
W1132BDA	6/27/2013	47.9740	-116.20650
W1132BDB	6/27/2013	47.9740	-116.20650
W1133BDA	6/27/2013	47.9993	-116.22366
W1133BDB	6/27/2013	47.9993	-116.22366
W1144BDA	8/6/2013	48.5258	-116.21066
W1159BDA	8/16/2013	47.0543	-116.08841
W1159BDB	8/16/2013	47.0543	-116.08841
W1159BDC	8/16/2013	47.0543	-116.08841
W1160BDA	8/18/2013	47.1310	-116.10364
W1160BDB	8/18/2013	47.1310	-116.10364
W1160BDC	8/18/2013	47.1310	-116.10364
W1177BDA	6/30/2013	47.8854	-116.16144

W1188BDA	8/9/2013	48.3791	-116.13732
W1188BDB	8/9/2013	48.3791	-116.13732
W1188BDC	8/9/2013	48.3791	-116.13732
W1188BDD	8/9/2013	48.3791	-116.13732
W1188V3BDA	7/11/2014	48.3791	-116.13732
W1188V3BDB	7/11/2014	48.3791	-116.13732
W1188V3BDC	7/11/2014	48.3791	-116.13732
W1188V4BDA	7/30/2014	48.3791	-116.13732
W1188V4BDB	7/30/2014	48.3791	-116.13732
W1188V4BDC	7/30/2014	48.3791	-116.13732
W1188V5BDA	8/13/2014	48.3791	-116.13732
W1188V5BDB	8/13/2014	48.3791	-116.13732
W1188V5BDC	8/13/2014	48.3791	-116.13732
W1188V6BDA	9/4/2014	48.3791	-116.13732
W1188V6BDB	9/4/2014	48.3791	-116.13732
W1188V6BDC	9/4/2014	48.3791	-116.13732
W1188V7BDA	9/24/2014	48.3791	-116.13732
W1189BDA	8/10/2013	48.4047	-116.19664
W1189BDB	8/10/2013	48.4047	-116.19664
W1189BDC	8/10/2013	48.4047	-116.19664
W1191BDA	7/5/2013	48.5202	-116.17390
W1191BDB	7/5/2013	48.5202	-116.17390
W1192BDA	8/1/2013	48.5494	-116.17631
W1192BDB	8/1/2013	48.5494	-116.17631
W1192QCBDA	8/13/2013	48.5494	-116.17631
W1192QCBDB	8/13/2013	48.5494	-116.17631
W1192QCBDC	8/13/2013	48.5494	-116.17631
W1199BDA	8/9/2013	48.8858	-116.16753
W1206BDA	8/18/2013	47.1548	-116.02982
W1206BDB	8/18/2013	47.1548	-116.02982
W1206BDC	8/18/2013	47.1548	-116.02982
W1210BDA	8/25/2013	47.3428	-116.06513
W1210BDB	8/25/2013	47.3428	-116.06513
W1211BDA	8/25/2013	47.3884	-116.07006
W1211BDB	8/25/2013	47.3884	-116.07006
W1211BDC	8/25/2013	47.3884	-116.07006
W1212BDA	8/17/2013	47.4251	-116.02680
W1212BDB	8/17/2013	47.4251	-116.02680
W1212BDC	8/17/2013	47.4251	-116.02680
W1220BDA	7/1/2013	47.8078	-116.07093
W1220BDB	7/1/2013	47.8078	-116.07093
W1220BDC	7/1/2013	47.8078	-116.07093
W1227BDA	6/26/2013	48.1036	-116.10139
W1227BDB	6/26/2013	48.1036	-116.10139
W1227BDC	6/26/2013	48.1036	-116.10139
W1231BDA	9/5/2013	48.2977	-116.08228
W1231BDB	9/5/2013	48.2977	-116.08228
W1231BDC	9/5/2013	48.2977	-116.08228

W1234BDA	8/10/2013	48.4054	-116.12784
W1234BDB	8/10/2013	48.4054	-116.12784
W1234BDC	8/10/2013	48.4054	-116.12784
W1236BDA	8/25/2013	48.5045	-116.11859
W1236BDB	8/25/2013	48.5045	-116.11859
W1236BDC	8/25/2013	48.5045	-116.11859
W1239BDA	8/12/2013	48.6581	-116.10069
W1239BDB	8/12/2013	48.6581	-116.10069
W1239BDC	8/12/2013	48.6581	-116.10069
W1240BDA	6/15/2014	48.6807	-116.11752
W1245BDA	8/8/2013	48.9199	-116.09908
W1245BDB	8/8/2013	48.9199	-116.09908
W1247BDA	8/8/2013	48.9938	-116.10632
W1247BDB	8/8/2013	48.9938	-116.10632
W1247BDC	8/8/2013	48.9938	-116.10632
W1249BDA	7/15/2013	47.0869	-115.96252
W1249BDB	7/15/2013	47.0869	-115.96252
W1249BDC	7/15/2013	47.0869	-115.96252
W1251BDA	8/19/2013	47.1598	-116.00684
W1251BDB	8/19/2013	47.1598	-116.00684
W1256BDA	8/17/2013	47.4095	-116.01257
W1256BDB	8/17/2013	47.4095	-116.01257
W1256BDC	8/17/2013	47.4095	-116.01257
W1257BDA	8/16/2013	47.4257	-116.00834
W1257BDB	8/16/2013	47.4257	-116.00834
W1257BDC	8/16/2013	47.4257	-116.00834
W1260BDA	7/9/2013	47.5599	-116.02542
W1260BDB	7/9/2013	47.5599	-116.02542
W1260BDC	7/9/2013	47.5599	-116.02542
W1267BDA	7/12/2013	47.8657	-116.00220
W1267BDB	7/12/2013	47.8657	-116.00220
W1287BDA	7/28/2014	48.7784	-116.05181
W1287BDB	7/28/2014	48.7784	-116.05181
W1287BDC	7/28/2014	48.7784	-116.05181
W1294BDA	8/19/2013	47.0733	-115.91806
W1294BDB	8/19/2013	47.0733	-115.91806
W1294BDC	8/19/2013	47.0733	-115.91806
W1295BDA	7/29/2013	47.1120	-115.92621
W1295BDB	7/29/2013	47.1120	-115.92621
W1295BDC	7/29/2013	47.1120	-115.92621
W1301BDA	8/24/2013	47.3921	-115.91964
W1301BDB	8/24/2013	47.3921	-115.91964
W1301BDC	8/24/2013	47.3921	-115.91964
W1346BDA	8/24/2013	47.3324	-115.83236
W1346BDB	8/24/2013	47.3324	-115.83236
W1346BDC	8/24/2013	47.3324	-115.83236
W1347BDA	8/24/2013	47.3996	-115.84660
W1347BDB	8/24/2013	47.3996	-115.84660

W1347BDC	8/24/2013	47.3996	-115.84660
W1348BDA	8/24/2013	47.4349	-115.82944
W1348BDB	8/24/2013	47.4349	-115.82944
W1348BDC	8/24/2013	47.4349	-115.82944
W1387BDA	7/29/2013	47.0216	-115.77673
W1387BDB	7/29/2013	47.0216	-115.77673
W1387BDC	7/29/2013	47.0216	-115.77673
W1396BDA	8/16/2013	47.4299	-115.80214
W1396BDB	8/16/2013	47.4299	-115.80214
W1488BDA	7/14/2013	47.0489	-115.62781
W1488BDB	7/14/2013	47.0489	-115.62781
W1488BDC	7/14/2013	47.0489	-115.62781
W1490BDA	7/29/2014	**47.1136**	**-115.63075**
W1490BDB	7/29/2014	**47.1136**	**-115.63075**
W1490BDC	7/29/2014	**47.1136**	**-115.63075**
W1538BDA	7/27/2013	46.9693	-115.59433
W1590BDA	7/26/2013	47.0793	-115.48946
W1742BDA	7/25/2013	47.1078	-115.30518
W1742BDB	7/25/2013	47.1078	-115.30518
W1742BDC	7/25/2013	47.1078	-115.30518
W1786BDA	7/25/2013	47.0983	-115.28540
W1786BDB	7/25/2013	47.0983	-115.28540
W1786BDC	7/25/2013	47.0983	-115.28540
W1787BDA	7/26/2013	47.1389	-115.28151
W1787BDB	7/26/2013	47.1389	-115.28151
W1817BDA	7/25/2013	47.0916	-115.16090
W1817BDB	7/25/2013	47.0916	-115.16090
W1817BDC	7/25/2013	47.0916	-115.16090
W1924BDA	7/15/2014	**46.9663**	**-116.56323**
W1924BDB	7/15/2014	**46.9663**	**-116.56323**
W1932BDA	7/14/2014	**46.9717**	**-116.48843**
W1932BDB	7/14/2014	**46.9717**	**-116.48843**
W1938BDA	7/14/2014	**47.0097**	**-116.45627**
W1939BDA	7/14/2014	46.9856	-116.40910
W1939BDB	7/14/2014	46.9856	-116.40910
W1939BDC	7/14/2014	46.9856	-116.40910
W1946BDA	7/14/2014	46.9529	-116.34484
W1946BDB	7/14/2014	46.9529	-116.34484
W1956BDA	7/13/2014	**46.9121**	**-116.24325**
W1956BDB	7/13/2014	**46.9121**	**-116.24325**
W1956BDC	7/13/2014	**46.9121**	**-116.24325**
W1961BDA	7/25/2014	**46.9175**	**-116.16200**
W1961BDB	7/25/2014	**46.9175**	**-116.16200**
W1961BDC	7/25/2014	**46.9175**	**-116.16200**
W1966BDA	7/26/2014	46.9612	-116.11333
W1966BDB	7/26/2014	46.9612	-116.11333
W1972BDA	7/25/2014	47.0353	-116.02062
W1972BDB	7/25/2014	47.0353	-116.02062

W1972BDC	7/25/2014	47.0353	-116.02062
W1979BDA	7/24/2014	47.0356	-115.98238
W1979BDB	7/24/2014	47.0356	-115.98238
W1979BDC	7/24/2014	47.0356	-115.98238
W1986BDA	7/24/2014	47.0306	-115.88182
W1986BDB	7/24/2014	47.0306	-115.88182
W1986BDC	7/24/2014	47.0306	-115.88182
W2000BDA	7/26/2014	46.9891	-115.80888
W2000BDB	7/26/2014	46.9891	-115.80888
W2000BDC	7/26/2014	46.9891	-115.80888
W2021BDA	7/28/2014	46.9561	-115.51434
W2021BDB	7/28/2014	46.9561	-115.51434
W2021BDC	7/28/2014	46.9561	-115.51434
W2026BDA	7/28/2014	46.9819	-115.43272
W2026BDB	7/28/2014	46.9819	-115.43272
W2026BDC	7/28/2014	46.9819	-115.43272
W2042BDA	7/25/2014	46.9681	-115.27082
W2042BDB	7/25/2014	46.9681	-115.27082
W2042BDC	7/25/2014	46.9681	-115.27082

***Bolded** locations fuzzed within 500 m because survey was conducted on private property.

AppendixIIIc: Private Land Request and Return Postcard

We requested permission to access privatley owned wetlands with a written letter. We asked landowners who were willing to allow access to fill out and return a postcard providing written permission.

Return Card

Please fill out this card and drop in the mail

1. I grant permission for 1-2 Idaho Fish and Game employees to conduct a survey for frogs and salamanders on my property Yes____No____ (If No for #1, please the drop card in mail)
2. Is there a wetland or "wet area" on your property? Yes___No___
3. If Yes to #2, approximately how big is your wetland (ie. acres, distance around, or length) and what is it made up of (ie. Cattail swamp, pond, spring/seep, wet field, etc.). Size:_____
   Type of wetland:_____
4. Have you ever heard or seen any amphibians there before and if you know, what are they?
   a. Amphibians present? Yes:___No:___ What kind: _____
5. Are you interested in receiving a species list based on our findings of your wetland? Yes __No __

Printed Name:_____
Signature:_____
Date:_____

Thank you for taking the time to fill this out and send it back to us!

IDAHO DEPARTMENT OF FISH AND GAME
PANHANDLE REGION
2885 West Kathleen Avenue
Coeur d'Alene, Idaho 83815

C.L. "Butch" Otter / Governor
Virgil Moore / Director

Dear Landowner,

During the spring and summer of 2014 Idaho Fish and Game will be conducting surveys for frogs and salamanders across the Idaho Panhandle. Our goal is to determine how widespread and abundant each species of frog and salamander is in the region.

The information collected will be used in the next revision of Idaho's State Wildlife Action Plan (Plan), which will be published in 2015. The goal of the Plan is to prevent federal listings of species and maintain state authority of wildlife by determining which species of wildlife may be at risk of being listed under the Endangered Species Act. Once we decide which species may be at risk, we develop actions which will help conserve these species and avoid ESA listing.

Our efforts can only be successful if landowners are willing to allow us to conduct surveys, thus we are seeking your permission to conduct a survey for frogs and salamanders on the property indicated on the enclosed maps. Any pond, marsh, or wet area that may or may not have these animals is a potential survey site. We will do whatever we can to accommodate any concerns you may have about where, how, and when we would conduct the survey. We would greatly appreciate your return of the enclosed, postage paid card with the information filled out at your earliest convenience.

If you grant permission to conduct a survey:

- We will contact you within the next several weeks with more information
- We will contact you before we conduct the survey
- We would conduct the survey in the spring/summer of 2014
- If you wish, we will be happy to provide you the results of our survey of your wetland

If you have any specific questions about this project we would appreciate a call or email, anytime.
We truly appreciate whatever help you can provide us in accomplishing this project.

Sincerely,

Shannon Ehlers
Wildlife Research Biologist
(208)659-9229
shannon.ehlers@idfg.idaho.gov

AppendixIIId: Environmental DNA vs. Dipnetting Field Assessment

eDNA Tools for Monitoring Amphibians

12-JV-11221633-112

Final Report

Caren S. Goldberg, University of Idaho/Washington State University

31 March 2014

Introduction

The goal of this pilot project was to determine detection probabilities and most efficient sampling design for using environmental DNA (eDNA) methods to monitor amphibian populations in north Idaho.

Field sampling methods

Four lakes were sampled by Michael Lucid and Idaho Fish and Game staff in June and July of 2012. At each lake, three 250 mL water samples were collected and filtered through 0.45 µm pore size cellulose nitrate filters in disposable filter funnels (Whatman) at approximately every 50 m around the perimeter (Figure 1). Field negatives (distilled water) were filtered at the beginning of each sampling session and after every 5 sampling sites. Locations of amphibians detected visually while collecting water samples were recorded. Water sample collection was followed with a dip-net survey where locations of all detected amphibians were recorded.

eDNA assay development

After sampling, collaborators on this project (Michael Lucid, Sam Cushman, and Caren Goldberg) decided that analysis should focus on the species identified through field surveys: long-toed salamanders (*Ambystoma macrodactylum*), western toads (*Anaxyrus boreas*), Columbia spotted frogs (*Rana luteiventris*), and American bullfrogs (*Lithobates catesbeianus*). I developed a species-specific qPCR assay for each species using previously developed genetic data (Austin et al. 2004, Funk et al. 2008, Goebel et al. 2009, Lee-Yaw and Irwin 2012) with Primer Express software (Life Technologies) and PrimerBLAST (NCBI), except for Columbia spotted frogs, where the test is only for the northern clade. I validated each assay using tissue samples from all north Idaho amphibians (except Coeur d'Alene salamanders, which I do not have tissue samples for). This consisted of 10 of each of the target species and 5 each of the following: Pacific treefrog (*Pseudacris sierrae*), Rocky Mountain tailed frog (*Ascaphus montanus*), and Idaho giant salamander (*Dicamptodon aterrimus*). Reactions were run using Quantitect Multiplex PCR Mix (Qiagen, Inc.) with recommended multiplexing concentrations (1X QuantiTect Multiplex PCR mix, 0.2 µM of each primer, and 0.2 µM of each probe) on an Applied Biosystems 7500 Fast Real-Time PCR System. Reactions were 15 µl in volume and each included 3 µl of sample. Cycling began with 15 min at 95°C followed by 50 cycles of 94°C for 60 s and 62°C for 60 s and went for 50 cycles. All of the samples from the target species tested positive and all of the samples from non-target species tested negative.

Sample analysis

DNA was extracted from filters with the Qiashredder/DNeasy method described in Goldberg et al. (2011). All filter sample extractions and qPCR reaction preparations were conducted in a lab dedicated to low-quantity

DNA samples. Researchers are required to shower and change clothes before entering this room after being in a high-quality DNA or post-PCR laboratory, and no amphibian tissues have been handled in this room. A negative extraction control was included with each set of extractions and an additional negative qPCR control was run with each plate of samples. We used a multi-tube approach for analysis, where multiple reactions were conducted for each sample, to increase the probability of detecting each species (Taberlet et al. 1999). We analyzed each sample in triplicate in 1 – 3 reactions (i.e. in 3-9 wells) and included an internal positive control (IC; Qiagen) in each well. A positive sample was defined as any sample that showed exponential amplification in all three wells from one run or in one or more wells from two separate reactions (samples were rerun whenever triplicate wells within a reaction yielded inconsistent results). Quantitative standards consisted of diluted skin tissue derived DNA quantified on a Nanodrop spectrophotometer and diluted 10^{-3} through 10^{-6}, run in duplicate.

We compared results of eDNA sampling with field sampling to evaluate detection probabilities of target species with eDNA sampling.

Results

All negative controls tested negative. Detection probabilities of all species were high with one exception: at Dennick Lake, many samples failed to detect any species, indicating an issue with sample preservation or eDNA degradation due to water chemistry at that site.

Long-toed salamanders were detected with eDNA at every segment where they were detected with field methods, with an overall detection probability per sample of 0.92 given that it was in the segment. The per sample probability of detection given that long-toed salamanders were in the lake was 0.64 for Playa, 1 for Copper, 0.96 for Long Mountain, and 0.08 for Dennick. At Dennick, the one observation of a long-toed salamander was made halfway between two eDNA sampling points; long-toed salamander eDNA was detected at one of these sampling points but not the other.

Western toads were also detected with eDNA at every segment where they were detected with field methods, with an overall detection probability per sample of 0.66 given that it was in the segment. The per sample probability of detection given that western toads were in the lake was 0.21 for Playa and Copper and 0.04 for Long Mountain (no evidence of western toads was found at Dennick Lake). At Playa Lake, western toad eDNA was detected 21 m in one direction from an egg mass but not 28 m in the other direction. Additionally, new larvae were detected at a site where no western toad eDNA was detected, indicating either misidentification or low detection probability. At Copper Lake, western toad eDNA was detected 61 m from the nearest observed toad, and at Long Mountain Lake western toad eDNA was detected at only one location.

Columbia spotted frogs were detected with eDNA at all sites at Copper Lake and most sites at Playa Lake where they were detected with field methods. No evidence of Columbia spotted frog presence was detected at Long Mountain Lake, and many sites at Dennick Lake where the species was detected by field crews were not detected using eDNA. At Copper and Playa Lakes, Columbia spotted frogs had an overall detection probability per sample of 0.91 given that it was in the segment. For Dennick, this estimate was 0.37. The per sample probability of detection given that Columbia spotted frogs were in the lake was 0.66 for Playa, 1.0 for Copper, and 0.21 for Dennick. At Playa Lake, there was one sampling site where an adult spotted frog was seen at the sampling site but not detected in the eDNA sample (although western toads were detected in the eDNA sample at this site), but at another sampling site, spotted frog eDNA was detected with the nearest field observation at 85 m.

Bullfrog eDNA was not detected during this study. Bullfrogs were only seen at Dennick Lake, where eDNA samples had very low overall detection. However, Columbia spotted frogs were detected in the area of the lake where bullfrog observations where noted. There is some possibility that field identification was incorrect, although the unexplained low detection of eDNA at this site may also have reduced detection of this species.

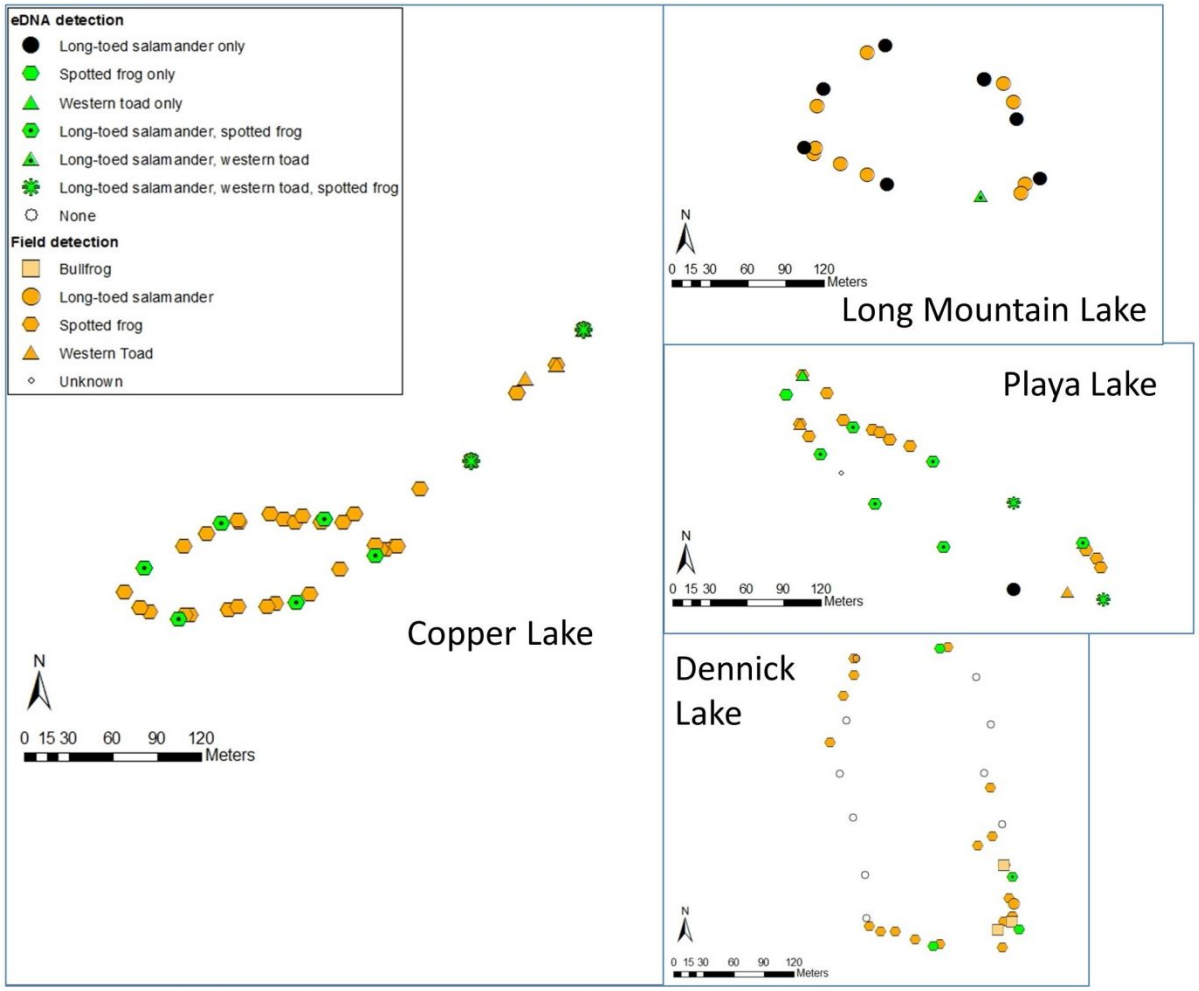

Figure 1. Field observations and environmental DNA sampling results from 4 lakes in northern Idaho. Environmental DNA samples were taken in triplicate from each location.

Conclusions

Environmental DNA showed very high detection probabilities for long-toed salamanders and Columbia spotted frogs and slightly lower detection probabilities for western toads given that they were within 50 m of the sampling site. For >95% probability of detection of these species, two samples need to be taken in a segment for long-toed salamanders and Columbia spotted frogs and three for western toads. At the scale of the lake, we found that some sites <25 m away from an observation (and some right at an observation, if field identification was accurate) did not detect the species using eDNA. Overall lake detection probabilities indicate that for average lakes, 3 samples in a lake (evenly spaced) is enough to detect long-toed salamanders and Columbia spotted frogs with >95% certainty. However, for western toads, this number may range from

13 for breeding populations to 74 for individual adults passing through (one adult western toad was found 200 m from Long Mountain Lake).

Environmental DNA results found species in areas of the lakes where they were not detected with visual sampling, and also detected species that were missed in surveys of the whole lake with visual and dipnet sampling (long-toed salamanders at Copper and Playa Lakes, western toads at Long Mountain Lake). This indicates a complementary role for eDNA in detecting and monitoring amphibian species in northern Idaho, even those that appear to have high detection probabilities. To conduct this complementary sampling, water samples would be taken before dipnet surveys begin and placed in the shade during field surveying. If all target species are detected during the survey, the water is discarded. However, if not all target species are detected, the water can be filtered and the filter preserved for later analysis.

Finally, the low detection probabilities at Dennick Lake demonstrate that there are additional factors to be considered in the collection and preservation of eDNA samples. This site should be further investigated and resampled to try and determine why eDNA sampling showed such low detection at that site compared with the other sites from this study and similar studies with eDNA throughout the area and globally.

Literature cited

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AppendixIIIe: Photographs of Purported *Rana sylvatica* and Confirmed *Rana pipiens* Museum Specimens

Taxonomy was confirmed for all historic observations of *Rana pipiens* for which specimens were available. Taxonomy was not confirmed for all historic observations of *Rana sylvatica* for which specimens were available. All purported *R. sylvatica* specimens were determined to be *R. luteiventris*.

Columbia spotted frogs (Rana luteiventris) samples which had been misidentified as wood frogs (Rana sylvatica) when collected:

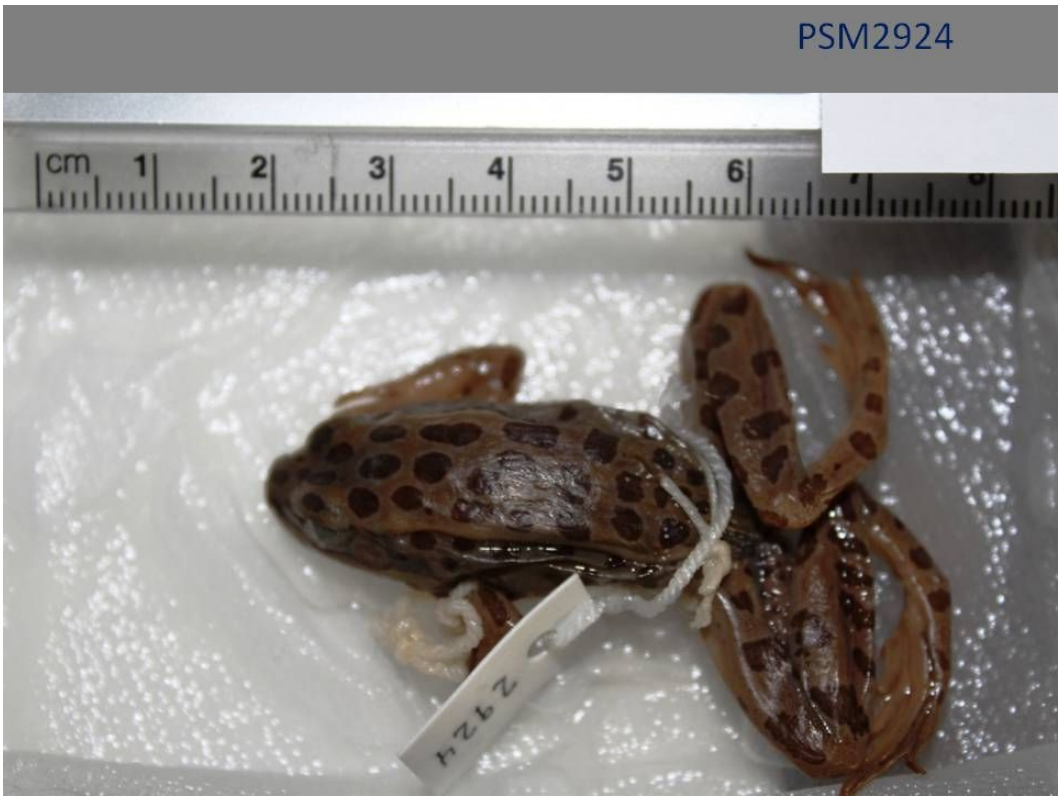

LACM76528
76528
76523
76529


LACM76529
76529


LACM76532


LACM76533
76533

Confirmed northern leopard frog (Rana pipiens) Museum Specimens:



PSM2927

PSM2931


PSM2932
cm
1
2
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PSM10767
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