Title: A rapid range-wide assessment of bull trout distributions: a crowd-sourced, eDNA-based approach with application to many aquatic species

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Yakama Nation	Bob Rose

**Contributing direct funding
*Contributing by conducting surveys or identifying priority waters

Project summary: The bull trout is an ESA-listed species that relies on cold stream environments across the Northwest and is expected to decline with climate change. Resource managers are charged with maintaining bull trout across their range, but monitoring this species is difficult and many populations have rarely or never been sampled. To reduce this uncertainty (and regulatory gridlock), we propose to coordinate a crowd-sourced field assessment of the distribution of bull trout in the U.S. by using inexpensive, reliable environmental DNA (eDNA) sampling. Samples collected by this multi-partner

effort can be used to evaluate many other species (e.g., a biodiversity assessment) with no additional field costs and can serve as a multi-species baseline for future assessments.

Category: C, Applying science to management

Need: Concerns about the effects of climate change on cold-water fish species in the GNLCC area have prompted considerable research investments during the last five years. The NorWeST stream temperature database and climate scenarios were developed from one such investment and now provide a regionally consistent set of high-resolution predictions developed from data contributed by > 80 resource agencies across jurisdictional boundaries (NorWeST website: http://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html). The NorWeST database and website are accessed ~10,000 times/year and the information is routinely used in a variety of management plan revisions and species climate vulnerability assessments. Linking these temperature maps to biological communities can help our stakeholders identify and maintain large, intact landscapes with naturally functioning aquatic community assemblages, particularly when faced by threats associated with climate change.

Bull trout are a critical component of these assemblages, and a focal species for the GNLCC found in all four ecotypic areas: the Columbia Basin, Rocky Mountains, Sagebrush Steppe, and Cascadia. This species is ESA-listed and occurs at low densities within thousands of streams designated as critical habitat across the region (USFWS 2014). This species is also the most thermally restricted salmonid in the Northwest; over 90% of juvenile bull trout observations are in streams with mean August water temperatures < 11°C (Isaak et al. 2015). Compounding matters is recent evidence that the distribution of bull trout within individuals streams has been contracting in association with climate change (Eby et al. 2014); some historically occupied habitats may no longer support populations. Because of the difficulty and expense of sampling bull trout populations, current information about the species distribution is imprecise and many streams have been sampled infrequently or not at all. That uncertainty comes at a cost; stakeholders may not be able to efficiently target their limited conservation resources, may forego or delay land management critical for other objectives, and may even avoid monitoring populations because of the added burden of obtaining sampling permits.

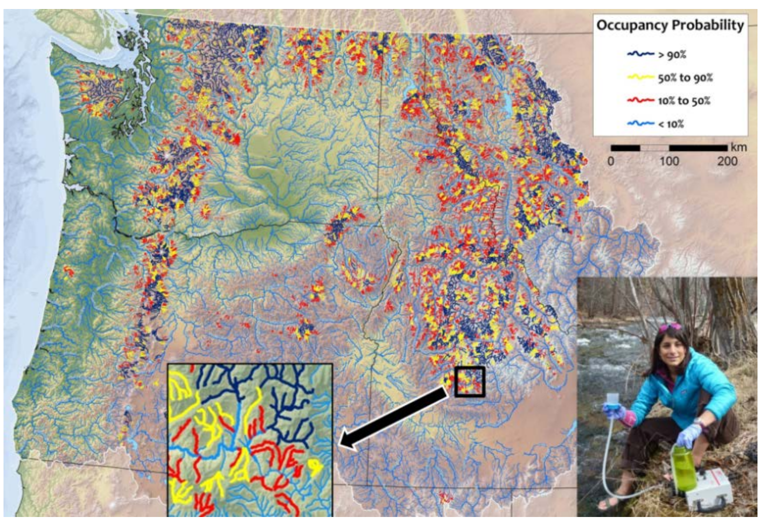

Figure 1. The 5,332 locations that potentially provide spawning and rearing habitat for bull trout in the northwestern U.S. (Isaak et al. 2015). The status of bull trout (present/absent) in 1,000–2,000 of those habitats is unknown because sites have rarely or never been sampled. We propose using inexpensive, highly sensitive eDNA surveys to census these habitats. Photo shows typical eDNA sampling equipment that a single person carries to a site. Field samples would be collected by a suite of partner agencies and Forest Service personnel. Samples would be processed at the National Genomics Center for Wildlife and Fish Conservation.

To reduce this uncertainty, scientists recently developed and published the Climate Shield habitat occupancy model (website: http://www.fs.fed.us/rm/boise/AWAE/projects/ClimateShield.html), which uses NorWeST temperature information with other environmental covariates to accurately predict the probability of bull trout (and cutthroat trout) presence across the Columbia River basin (Figure 1; Isaak et al. 2015). The fish and temperature data used to develop the NorWeST and Climate Shield databases and models were crowd-sourced; the efforts engaged hundreds of biologists working for dozens of agencies and leveraged their raw data to develop databases worth over $10,000,000. Building on those efforts, we propose to conduct a spatially precise, up-to-date, range-wide bull trout status assessment through the use of crowd-sourcing, digital media, and new genomic techniques that are revolutionizing the cost-effectiveness of broad-scale species sampling (http://www.fs.fed.us/research/genomics-center/)

Among the array of genomic techniques now available, environmental DNA (eDNA) sampling has emerged as a powerful method for determining the occurrence of many species reliably and inexpensively (http://www.fs.fed.us/research/genomics-center/edna/). Environmental DNA is DNA shed by organisms and collected by filtering water, and even a single DNA molecule on a filter—and thus a species in a stream—can be detected with high reliability (Wilcox et al. 2013). Our team at the National Genomics Center for Wildlife and Fish Conservation (NGC) has pioneered developments in this field—including the first reliable eDNA assay for salmonid fish species, and the first that distinguishes bull trout from other species of char (Wilcox et al. 2013, 2014). We have also developed a field-proven eDNA sampling protocol that requires only 15 minutes of effort by a single person to collect a sample (Carim et al. 2014). Species detection with eDNA is remarkably sensitive—for brook trout, 100% detection efficiency was achieved despite order-of-magnitude changes in stream discharge (Jane et al. 2014). Subsequent field experiments indicate that detection probability of a single trout in 100 m of stream exceeds 85%, an efficiency several-fold better than one-pass electrofishing (Wilcox et al. in review). Collected samples are easily stored while in the field, can be processed in the lab in under 48 hours, and costs are substantially less than those for electrofishing. Often, an entire 6th-code subwatershed (~20–40 km of stream) can be sampled by a single person in one day. Our protocol (Carim et al. 2014) has been adopted by biologists from partner agencies in most western states, on projects ranging from population inventories to gauging the effectiveness of chemical treatments or electrofishing to remove nonnative species (Table 1). For bull trout, initial studies have been directed at precisely delineating their distribution within select watersheds, as well as confirming their absence from potential habitats and discovering previously unknown populations (Figure 2; McKelvey et al. in review).

Table 1 Organizations participating in eDNA-based surveys for stream fishes with the National Genomics Center for Wildlife and Fish Conservation. An asterisk denotes surveys that involved detecting bull trout.

U.S. Forest Service	U.S. Geological Survey
Region 1*	Arizona Game and Fish Department
Idaho Panhandle National Forest*	California Department of Fish and Wildlife
Lolo National Forest*	Idaho Department of Fish and Game
Helena National Forest*	Montana Fish, Wildlife and Parks*
Region 2	Nevada Department of Wildlife
Grand Mesa, Uncompahgre, and Gunnison National Forests	New Mexico Department of Game and Fish
Region 3	Oregon Department of Fish and Wildlife*
Region 4*	Utah Division of Wildlife Resources
Boise National Forest*	Clark Fork Coalition*
Payette National Forest*	Wild Fish Conservancy*
Salmon-Challis National Forest*	Wildlife Conservation Society
Sawtooth National Forest*	Snoqualmie Tribe*
Region 10	Nez Perce Tribe*
U.S. Fish and Wildlife Service*	Hart Crowser Consultants*

Objectives: In 2015, we will build on previous work by pairing predictions of bull trout habitat occupancy from the Climate Shield model with an optimized eDNA protocol to survey all juvenile bull trout habitats throughout two 4th-code river basins, the Upper Clark Fork River in Montana and the St. Joe River in Idaho. In 2016, we propose to extend this sampling to the entire range of bull trout in the U.S., with expected completion in 2018. Locations to be sampled will include those predicted to be suitable by the Climate Shield model but rarely or never sampled, and those with historical observations of juvenile or spawning bull trout for which there are no recent surveys. The refined estimates of habitat occupancy will be used to build a more robust and precise Climate Shield model for bull trout to more accurately project suitable habitats under climate change. And the comprehensive sampling of eDNA from throughout the Columbia River basin will provide an archive for analyses of the distribution and habitat suitability of any other freshwater species of concern e.g., Pacific lamprey, steelhead/redband trout, westslope cutthroat trout, western pearlshell mussel, or North American river otter.

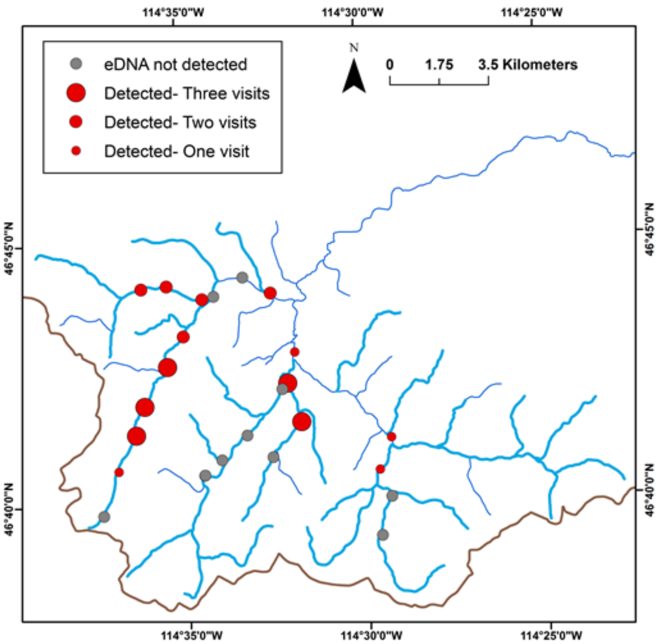

Figure 2. An example of the precision of habitat delineation using eDNA sampling for bull trout in the headwaters of Lolo Creek, Montana (McKelvey et al. in review). This sampling confirmed the presence of bull trout in Granite and North Creeks, confirmed their absence from most of WF Lolo Creek (designated as critical habitat for bull trout), detected seasonal use in portions of Lost Park and Granite Creeks, and revealed a previously unknown population in Lee Creek. Each site was sampled three times at monthly intervals.

Methods: The Climate Shield model makes spatially explicit predictions about habitat occupancy by juvenile bull trout based on habitat characteristics and the presence of brook trout. Candidate habitats will be those with a probability of occupancy > 25% that have little of no recent history of surveys (Appendix 2). Habitats will be sampled at 1.5-km intervals, which produces robust estimates of habitat occupancy in bull trout spawning and rearing habitats (McKelvey et al. in review). A portion of the samples will be sampled on multiple occasions to examine temporal variation in habitat use. The sampling protocol will largely follow Carim et al. (2014), which has been further refined. The majority of fieldwork will be conducted by project partners, and they are providing input on additional waters to be sampled. Our joint 2014 field trials demonstrated the feasibility of crowd-sourcing eDNA sample collection e.g., crews were rapidly and easily trained to collect reliable data, as has been previously done with water temperature. Laboratory analyses will be conducted at the NGC, following Wilcox et al. (2013, in review).

Deliverables:

1. Deliverable relevant to needs identified in Frameworks, Science Plans, and Partner Forums. Our deliverables will be targeted to maintain large intact riverscapes currently threatened by climate change (Goal 1, Page 9 GNLCC Strategic Framework). We will do this by providing the following:

A. A range-wide, high-resolution database of the distribution of bull trout habitats. These data will result in massive increases in efficiency in conservation planning, land management actions, and the direction and allocation of effort to fish monitoring, all deemed essential by project partners. These data will fill a critical gap by providing reliable information on bull trout distributions in areas rarely or never sampled. They will also update managers on those locations historically thought to support bull trout, but about which few data are currently available. Observations of persistence or extirpations from those sites will inform our understanding of the response of these fish to climate change. **Our deliverables will help the GNLCC identify quantifiable measure to assess progress**

in conserving bull trout habitat. Similar to NorWeST and Climate Shield, data will be searchable and downloadable from webpages dedicated to this project and hosted by the Rocky Mountain Research Station's AWAE program. Such data will also form the basis of a large number of peer-reviewed publications with project partners (see **Schedule**) and presentations at local, regional, national, and international conferences.

B. An improved version of the Climate Shield models for predicting occurrence of juvenile bull trout (Isaak et al. 2015). This re-analysis will include the addition of data from thousands of locations sampled using eDNA and hundreds of additional sites based on conventional sampling from Oregon and Washington, and the application of spatial statistical network models (Isaak et al. 2014). The future model will not only be more accurate, particularly in areas that are currently poorly sampled, but much more spatially precise, allowing more fine-grained predictions. This greater precision makes these data more relevant for current project planning, and may prove critical for climate-smart planning by forecasting which habitats are most likely to persist—or be lost—under climate change. Again, results of these analyses will be broadly distributed through the project webpage.

C. An archive of thousands of georeferenced eDNA samples that will represent all species present in streams across the northwestern U.S. These eDNA samples can be stored indefinitely and used to construct distribution maps or occupancy models for any species in the region once an eDNA assay is developed for a species. They can also support the advancement of new genetic or genomic approaches, and we anticipate using them to test multi-species eDNA panels for rapid assessment of aquatic biodiversity and the early detection of invasive species. Their greatest value may not be realized for some time. These samples will constitute a vast and spatially comprehensive benchmark for the distribution of aquatic biota in the early 21st century. For future studies of environmental change and species responses, such archives are likely to be regarded as priceless.

2. Deliverable relevant to on the ground conservation delivery. The actionable science conducted here directly feeds into our stakeholders' resource management plans. A better understanding of the distribution of bull trout is identified in every State Wildlife Action Plan in the study area, the Northern Region's conservation strategy (USFS 2013), and the draft recovery plan under the ESA (USFWS 2014). Because bull trout are listed under the ESA, and bull trout presence places conditions on land and water management, all project partners and stakeholders are unified in needing a better understanding of the distribution of bull trout. Individual partners also have needs specific to their organizations e.g., FERC relicensing of hydropower facilities is contingent on accurate data on bull trout distributions in affected tributaries along the Snake River, and Superfund site remediation in western Montana is being directed by the success of bull trout recovery in affected watersheds. This information need crosses scales from local projects for habitat improvement, culvert removal, or grazing management, to regional conservation efforts partly dedicated to freshwater taxa (the Crown of the Continent initiative), to range-wide conservation assessments of bull trout. As basins are inventoried, individual reports will be sent to the project partners that collected those samples to provide for their specific needs allowing for rapid and effective conservation delivery prior to large-scale dataset analyses. Examples of those reports used to inform and prioritize conservation actions at a local scale are available from the National Genomics Center.

3. Deliverable relevant to effective application on science. Our data outputs and products (outlined above in 1A–C) will extend managers' ability to act strategically by integrating prior GNLCC-funded projects (NorWeST and Climate Shield) with current sampling needs to validate those products while simultaneously providing managers the primary information they have requested (see attached support letters). The interim products will *immediately* provide applicable occupancy data for bull trout and other critical species, while the cumulative analysis will improve or validate existing models. Interim reports

and webinars will allow feedback loops to improve the process. We have already incorporated manager and biologist ideas from summer 2014 into our current sampling protocol and believe this will be an iterative process.

4. Deliverable relevant to partner engagement. Our experience with environmental DNA sampling at the National Genomic Center thus far has shown us that by working with our large list of partners and holding pre-sampling training/listening sessions we can engage additional new partners and show how our eDNA sampling methodology can be adapted for a variety of aquatic biodiversity issues. We will continue these training/listening sessions to share knowledge and prior results. Futhermore, once all the individual level data are assimilated, our validated models can be used outside of the GNLCC jurisdiction.

Appendices:
Appendix 1. References
Appendix 2. Kilometers of potential bull trout streams in the interior Columbia River basin.

Appendix 1. References

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Appendix 2. Kilometers of streams in the interior Columbia River basin with a non-zero probability of being occupied by juvenile bull trout in the absence of brook trout (Isaak et al. 2015). We anticipate applying eDNA sampling to characterize roughly 60% (11,000 km of 18,963 km) of the streams with the greatest uncertainty e.g., those with a 25–75% probability of occupancy, but selected streams from other categories will be surveyed if prioritized by project partners. A full inventory will not be pursued because some hydrologic units are mostly in wilderness and bull trout populations are secure (e.g., SF Flathead River), others have known barriers to bull trout (e.g., the Yaak River), and many have already been extensively sampled with traditional methods. Because brook trout reduce the probability of occupancy by juvenile bull trout, where brook trout are known to be present (e.g., much of Montana and northern Idaho), occupancy probabilities will be recalculated (equations in Isaak et al. 2015) and corrected amounts of potential occupied habitats considered. This may result in the redistribution of effort.

			Probability of occupancy by bull trout (%)				
NorWeST Unit	HUC	NAME	<25	25-50	50-75	75-90	>90
Clearwater	17060301	UPPER SELWAY	244	166	165		129
	17060302	LOWER SELWAY	211	148	84		58
	17060303	LOCHSA	252	292	140	102	
	17060304	MIDDLE FORK CLEARWATER	28				
	17060305	SOUTH FORK CLEARWATER	229	87	60	128	0
	17060306	CLEARWATER	38				
	17060307	UPPER NORTH FORK CLEARWATER	239	337	249	67	203
	17060308	LOWER NORTH FORK CLEARWATER	117	52	18		69
		Total	1358	1082	716	297	458
Mid-Columbia	17060101	HELLS CANYON	9		27	13	
	17060102	IMNAHA	99	45	21	37	82
	17060103	LOWER SNAKE-ASOTIN	41	29	37		
	17060104	UPPER GRANDE RONDE	135	86	33	82	79
	17060105	WALLOWA	15	121	58	65	33
	17060106	LOWER GRANDE RONDE	65	30	40	1	207
	17060107	LOWER SNAKE-TUCANNON	4	19	18	40	42
	17060108	PALOUSE	7				
	17070101	MIDDLE COLUMBIA-LAKE WALLULA	6				
	17070102	WALLA WALLA	54	55		36	117
	17070103	UMATILLA	34			30	
	17070104	WILLOW	0				
	17070105	MIDDLE COLUMBIA-HOOD	169	123	130	105	444
	17070106	KLICKITAT	88	31	85		458
	17070201	UPPER JOHN DAY	142	84	24		138

	17070202	NORTH FORK JOHN DAY	86	61	32		54
	17070203	MIDDLE FORK JOHN DAY	17	31		18	
	17070204	LOWER JOHN DAY	11	11			
	17070301	UPPER DESCHUTES	22	46	60	137	674
	17070302	LITTLE DESCHUTES	11	59	55	52	16
	17070304	UPPER CROOKED	30	8			
	17070305	LOWER CROOKED	12				
	17070306	LOWER DESCHUTES	87	126	148	47	171
	17070307	TROUT	5				
		Total	1151	965	768	663	2515
Mid-Snake	17050102	BRUNEAU	45	59	10		
	17050104	UPPER OWYHEE	4	4			
	17050105	SOUTH FORK OWYHEE	7				
	17050111	NORTH AND MIDDLE FORKS BOISE	106	98	111	109	116
	17050112	BOISE-MORES	58	11	83		
	17050113	SOUTH FORK BOISE	146	242	180	79	198
	17050116	UPPER MALHEUR	37	30	48	62	
	17050120	SOUTH FORK PAYETTE	66	108	99	87	356
	17050121	MIDDLE FORK PAYETTE	89	58	20	59	0
	17050122	PAYETTE	33	24		1	
	17050123	NORTH FORK PAYETTE	136	69	51	92	72
	17050124	WEISER	71	91	26	50	
	17050201	BROWNLEE RESERVOIR	69	35	14	26	
	17050202	BURNT	25				67
	17050203	POWDER	43	109	37	95	
		Total	936	939	680	658	809
Salmon	17060201	UPPER SALMON	273	259	301	444	1052
	17060202	PAHSIMEROI	63	60	22		438
	17060203	MIDDLE SALMON-PANTHER	183	237	236	146	430
	17060204	LEMHI	104	131	186	92	385
	17060205	UPPER MIDDLE FORK SALMON	121	203	213	30	764
	17060206	LOWER MIDDLE FORK SALMON	96	164	122	208	360
	17060207	MIDDLE SALMON-CHAMBERLAIN	305	293	243	148	317
	17060208	SOUTH FORK SALMON	232	390	159	125	229
	17060209	LOWER SALMON	87	50	70		15
	17060210	LITTLE SALMON	73	55	63		43

		Total	1537	1843	1614	1193	4033
Snake-Bear	17040217	LITTLE LOST	67	70	160	65	153
		Total	67	70	160	65	153
SpoKoot	17010101	UPPER KOOTENAI	376	385	184	138	60
	17010102	FISHER	155	107	21	25	1
	17010103	YAAK	133	77	72	0	180
	17010104	LOWER KOOTENAI	43	27	71	88	1
	17010105	MOYIE	31	12	30		
	17010201	UPPER CLARK FORK	336	228	344	157	394
	17010202	FLINT-ROCK	147	306	310	234	570
	17010203	BLACKFOOT	357	323	233	250	1076
	17010204	MIDDLE CLARK FORK	314	210	378	160	177
	17010205	BITTERROOT	463	342	226	321	763
	17010206	NORTH FORK FLATHEAD	75	124	127	0	494
	17010207	MIDDLE FORK FLATHEAD	67	109	65	32	794
	17010208	FLATHEAD LAKE	131	65	38	50	
	17010209	SOUTH FORK FLATHEAD	88	154	264	163	786
	17010210	STILLWATER	94	79	35	121	292
	17010211	SWAN	113	104	143	68	191
	17010212	LOWER FLATHEAD	135	115	72	36	138
	17010213	LOWER CLARK FORK	289	382	213	213	440
	17010214	PEND OREILLE LAKE	107	9	16	31	57
	17010215	PRIEST	131	128	121	80	208
	17010216	PEND OREILLE	146	181	52	165	102
	17010301	UPPER COEUR D'ALENE	277	245	106	67	56
	17010302	SOUTH FORK COEUR D'ALENE	45	27			
	17010303	COEUR D'ALENE LAKE	39			1	
	17010304	ST. JOE	238	253	105	29	152
	17010305	UPPER SPOKANE	9				
		Total	4338	3992	3224	2427	6932
Upper Columbia-Yakima	17020001	FRANKLIN D. ROOSEVELT LAKE	254	170	149	77	39
	17020002	KETTLE	98	184	245	102	120
	17020003	COLVILLE	134	106	114		
	17020004	SANPOIL	257	168	129	181	140
	17020005	CHIEF JOSEPH	17		31		
	17020006	OKANOGAN	71	66		26	117

17020007	SIMILKAMEEN	20	18	25	67	457
17020008	METHOW	103	80	173	135	769
17020009	LAKE CHELAN	16	32	101	18	231
17020010	UPPER COLUMBIA-ENTIAT	107	123	23	51	137
17020011	WENATCHEE	87	47	121	135	258
17030001	UPPER YAKIMA	240	158	118	55	150
17030002	NACHES	107	186	152	94	288
17030003	LOWER YAKIMA	61	81	109	76	
	Total	1572	1419	1492	1019	2707
	Grand total	10957	10309	8654	6322	17607