Northwest **Power** and **Conservation** Council

April 4, 2017

MEMORANDUM

TO: **Fish and Wildlife Committee members**

FROM: **Nancy Leonard**

SUBJECT: **Putting aquatic species on the map: the eDNAtlas and eDNArchive for aquatic taxa in western North America**

BACKGROUND:

Presenter: Michael Young, Research Fisheries Biologist (USFS)
Dan Isaak, Research Fisheries Biologist (USFS)

Summary: We describe eDNA sampling and share first-year field results from the range-wide eDNA-based inventory of bull trout in the Northwestern U.S., featuring crowd-sourced sampling of ~3,000 sites. Project results constitute the first phase of the Aquatic eDNAtlas, an open-access database depicting eDNA sampling results throughout Western North America and the eDNArchive, and eDNA-based biodiversity catalog.

Relevance: Bull trout is one of the focal (important) resident fish species for the Program (Appendix N). Bull trout are addressed under the *Resident Fish Mitigation* and the *Mainstem Hydrosystem Flow and Passage Operations* Program Strategies (see general measures section). The 2014 Program has an interim bull trout population objective to maintain a stable and increasing population trend. Bull Trout is one of the species targeted as part of the Program's *refine program goals and objectives task*.

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Background: Effective conservation and management of societally important cold-water and other native fishes during an era of rapid environmental change, nonnative species invasions, and urbanization will require unprecedented levels of interagency coordination and high-quality information to guide decision-making. Strategic investment strategies and prioritization will be required because conservation needs always exceed available resources. Fundamental to any prioritization scheme is precise information about species distributions across broad areas to show current status, trends, and risks. One focal species for such efforts is the bull trout, an ESA-listed species that occurs at low densities within thousands of streams designated as critical habitat across the Northwest. Because gauging the status of bull trout at broad scales is precluded by the difficulty and expense of traditional sampling, estimates of its present distribution are imprecise and changes in occupancy status uncertain. 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.

To reduce this uncertainty, the Boise Spatial Streams Group developed and published the Climate Shield habitat occupancy model, which accurately predicts the probability of bull trout (and cutthroat trout) presence across the Columbia River basin (Figure 1) and makes spatially explicit projections (1-km resolution) about climate refugia for species under a suite of climate and invasive species scenarios. For this and related projects e.g., the NorWeST stream temperature model and database, we engaged hundreds of biologists working for dozens of agencies and leveraged their raw data to develop databases worth over $10,000,000, attesting to the effectiveness of crowd-sourcing environmental data collection. But the Climate Shield project also re-emphasized the need for a coordinated, broad-scale effort to precisely categorize habitat occupancy by bull trout across its historical range in the U.S., because many of these potential climate refugia have rarely or never been sampled.

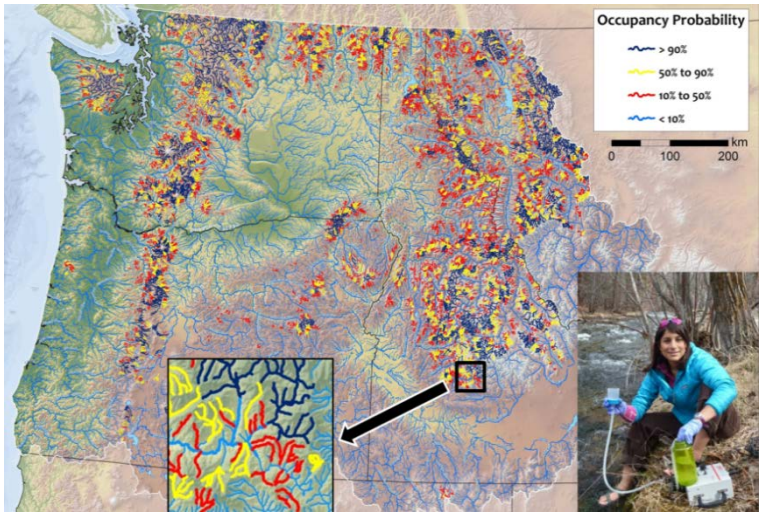


Figure 1. The 5,332 locations that potentially provide spawning and rearing habitat for bull trout in the northwestern U.S. (Isaak et al. 2015). The status of bull trout (present/absent) in 1,000–2,000 of those habitats is unknown because sites have rarely or never been sampled. We are using cost-efficient, highly sensitive eDNA surveys to census these habitats. Photo shows typical eDNA sampling equipment that a single person carries to a site.

A revolutionary advance in detecting aquatic species—environmental DNA (eDNA) sampling—provides a way forward. Environmental DNA is DNA shed by organisms and collected by filtering water, and scientists at the National Genomics Center for Wildlife and Fish Conservation (NGC) have pioneered developments in this field, which include the first reliable eDNA assay for salmonid fish species, the first that distinguishes bull trout from other species of char, the first to demonstrate the efficiency of detection of salmonids in streams, and the first to apply eDNA sampling at broader

scales to describe salmonid species occupancy. Following an NGC protocol that was field-tested by hundreds of resource agency partners, a one-person crew can collect an eDNA sample in under 15 minutes. And because even a single DNA molecule on a filter can be detected with high reliability, species detection with eDNA sampling is remarkably sensitive. Heightened interest in using eDNA methods has driven collaborations between the NGC and biologists from partner agencies throughout western North America on projects including population inventories, seasonal patterns of species movement, invasive species detection, and effectiveness monitoring of chemical treatments or electrofishing to remove nonnative species.

Foremost among those efforts is the range-wide, eDNA-based inventory of local populations of bull trout across its U.S. range. This relies on crowd-sourced eDNA sampling of potential natal habitats identified by the Climate Shield model or designated as critical habitat for spawning and rearing by the U.S. Fish and Wildlife Service. Biologists from dozens of agencies have contributed time and matching funds to collect ~3,000 samples to date, a total expected to exceed 10,000 at the project's conclusion in 2018. The results are expected to be invaluable to researchers trying to understand patterns of habitat occupancy by bull trout. To be most valuable to all stakeholders, however, these data need to be easily shared within a consistent database structure that permits user-driven data summaries and analyses that are essential for decision making. To that end, we are developing the aquatic **eDNAtlas**, an online, open-access database of eDNA sampling results. Building on the foundation of the NorWeST and Climate Shield webpages, an interactive ArcGIS Online-based website is being developed for the eDNAtlas that will provide downloadable data in formats desired by users. An electronic pipeline for delivering consistent results from the NGC to the Boise Spatial Streams Group has been built and tested. Bull trout are the flagship species for this effort and those data are available now, but we envision extending this to the 30+ species that are tested at the NGC (Figure 2) on the ~10,000 samples to by analyzed by late summer 2017, with new data to be added semiannually. In addition, the samples themselves constitute a near-permanent catalog of biodiversity—an **eDNArchive**—because each sample can be stored indefinitely and analyzed for the presence of many species at any later time. Collectively, these data tools will enable users to make efficient, strategic assessments of species status, trend, and distribution, detect and track nonnative species invasions, and evaluate habitat restoration success and fish passage.

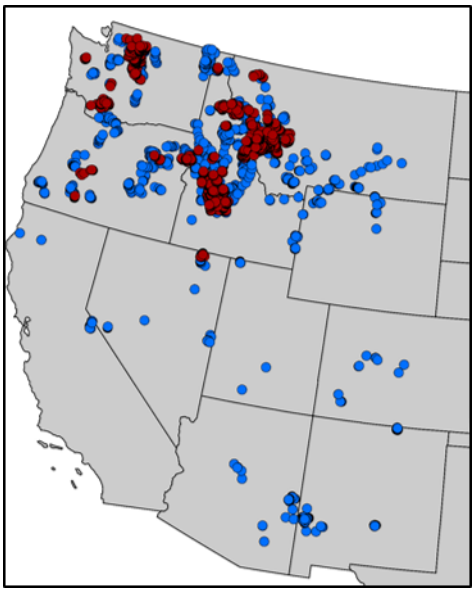


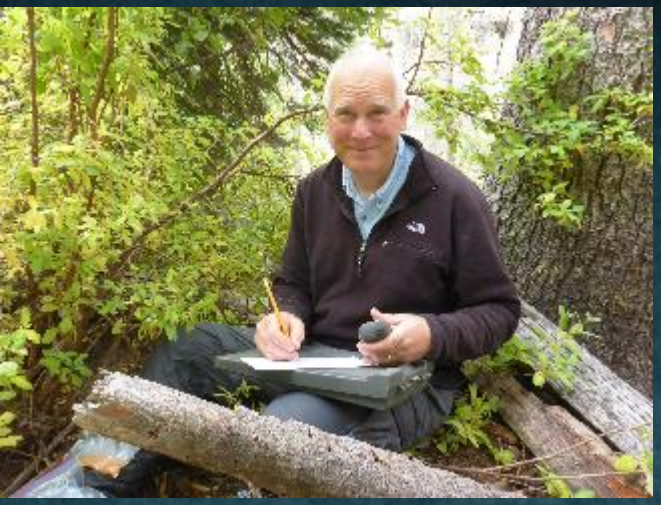
Figure 2. Locations already sampled as part of eDNA-based projects involving the NGC (excluding AK and Canada) as of March 2016. Color denotes those samples related to the range-wide bull trout project (red; *n* = 2,988) and those representing sampling for all other species (blue; *n* = 3,495).

More Info:

Range-wide Bull Trout eDNA project:
https://www.fs.fed.us/rm/boise/AWAE/projects/BullTrout_eDNA.html
Cold-Water Climate Shield project:
https://www.fs.fed.us/rm/boise/AWAE/projects/ClimateShield.html
National Genomics Center for Wildlife & Fish Conservation:
https://www.fs.fed.us/research/genomics-center/edna/
National Stream Internet project:
https://www.fs.fed.us/rm/boise/AWAE/projects/NationalStreamInternet.html
NorWeST Stream Temperature project:
https://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html

Putting aquatic species on the map: The eDNAtlas and Archive for aquatic taxa in western North America

Michael Young, Dan Isaak, Kevin McKelvey, Michael Schwartz
U.S. Forest Service, Rocky Mountain Research Station



NGC members

Kevin McKelvey
Michael Schwartz
Kellie Carim
Taylor Wilcox
Tommy Franklin*
Caleb Dysthe
Samuel Greaves

BSSG members

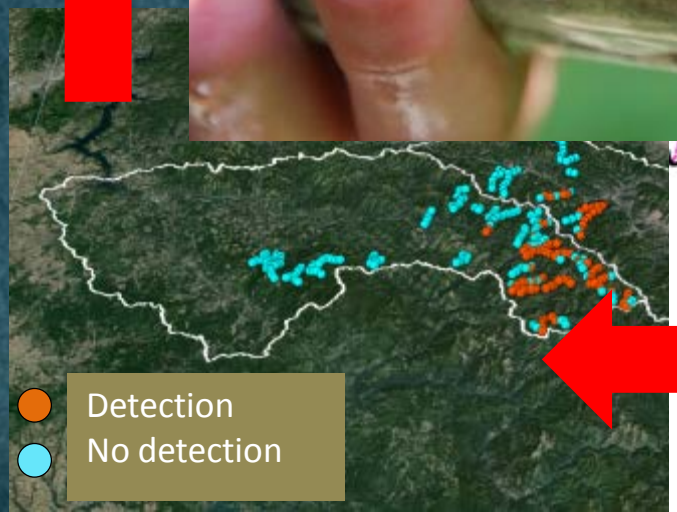
Dan Isaak
Dave Nagel
Dona Horan
Sherry Wollrab
Sharon Parkes
Matt Groce

National Genomics Center
FOR WILDLIFE AND FISH CONSERVATION

Boise Spatial Streams Group

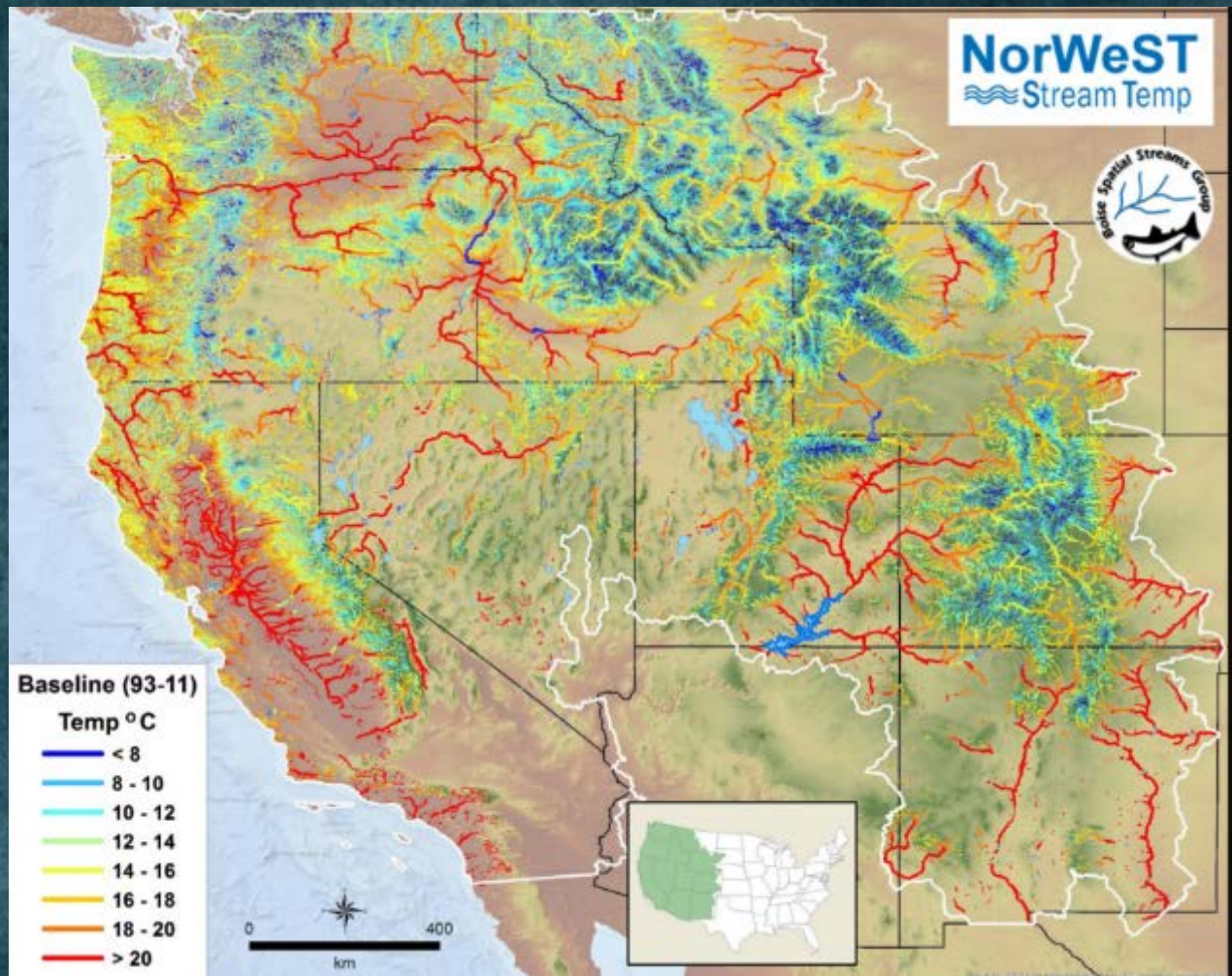
Project evolution

- Origin: concern about a focal species
  - Juvenile bull trout
- Understanding its distribution
  - Climate Shield model
  - Uncertainty
- eDNA sampling
  - What is it
  - Why use it
- Bull trout + eDNA
  - Where to look
  - Early results
- All species + eDNA
  - eDNAtlas
  - eDNArchive



Why choose juvenile bull trout?

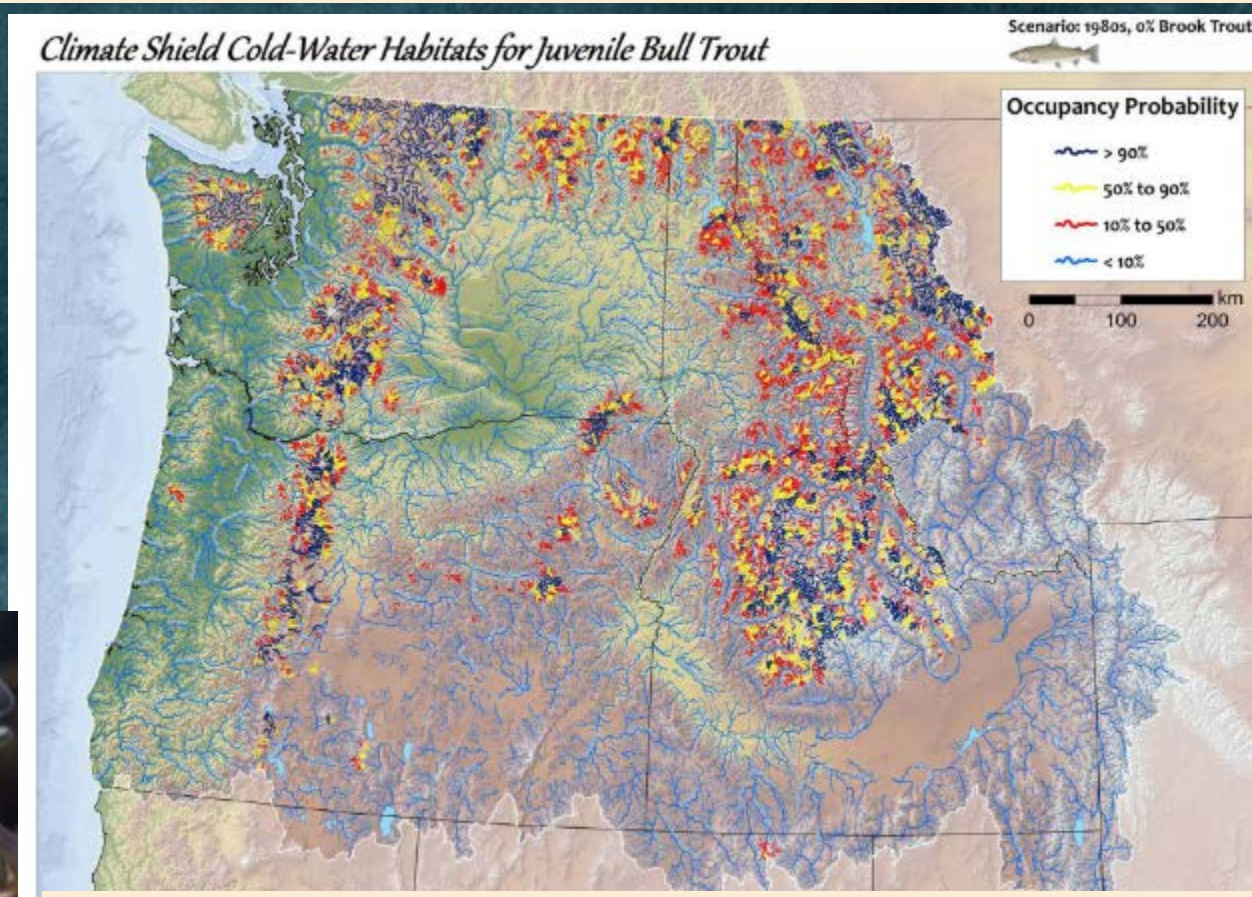
- ESA-listed as threatened
- Presence dictates land & water management & planning
- Widespread in PNW
- Often rare
- Difficult to detect
- Juveniles constrained by water temperature, vulnerable to nonnative spp.
- = candidate for occupancy modeling to identify suitable habitat

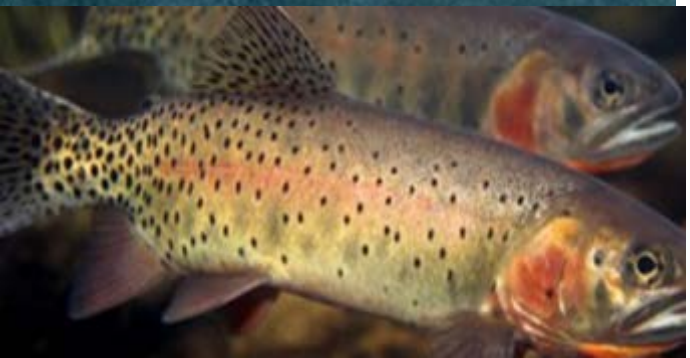


Identifying climate refugia for native trout – the Climate Shield

- Climate to cold-water habitat
- Predictions
  - Accurate & sufficient
  - Address invasive species
  - Empirical
  - Precise & range-wide
- Projections
  - Address climate change
- Many unsampled potential habitats
  - **Validation?**

https://www.fs.fed.us/rm/boise/AWAE/projects/ClimateShield.html
or Google “cold-water climate shield”



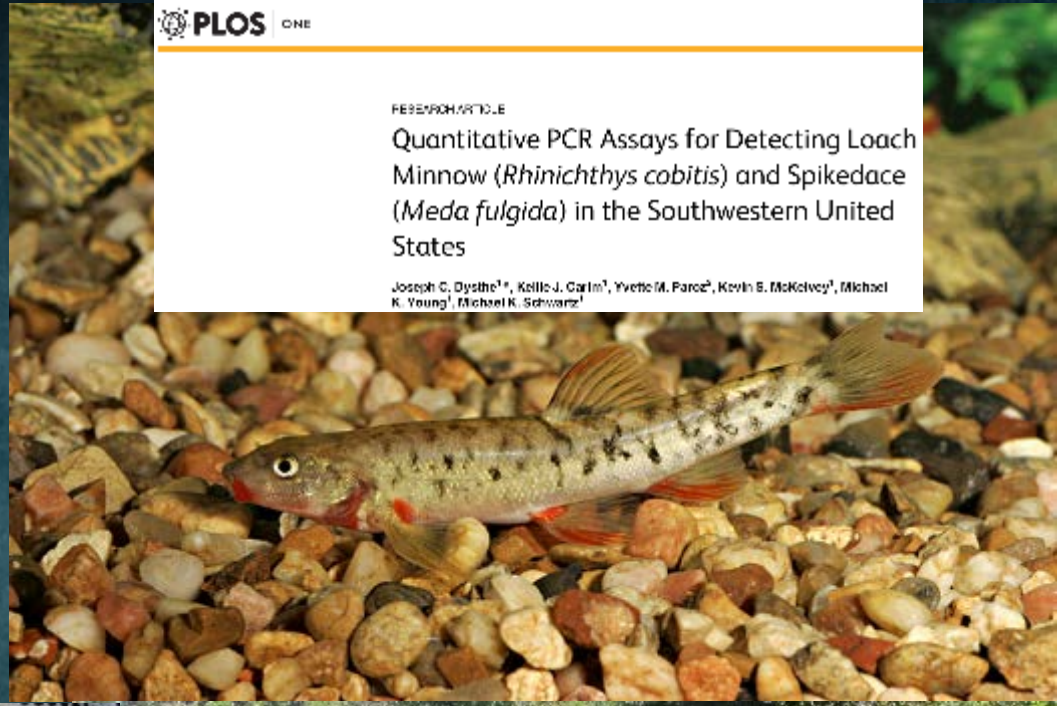


~3700 potentially occupied cold-water habitats: which ones are?

Conventional sampling issues

- Harmful
- Expensive & time-consuming
- Ineffective
  - Rare native species
  - Invasion fronts & removal survivors

- Is there an alternative?



What is eDNA sampling?

- Collection of DNA from the environment
- The indirect detection of species presence
  - Bird dog
  - Elk tracking

Siberian permafrost cores contain DNA from prehistoric plants and mega-fauna in the absence of preserved fossils

American Bullfrog

- Fish & Wildlife
  - Mammoths in permafrost
  - Neanderthal in soil
- Aquatic application
  - American bullfrogs in France in 2008

Why use eDNA sampling: efficiency

- Fast
- Portable
- Stable
- Cost: pennies on the dollar, minutes on the hour

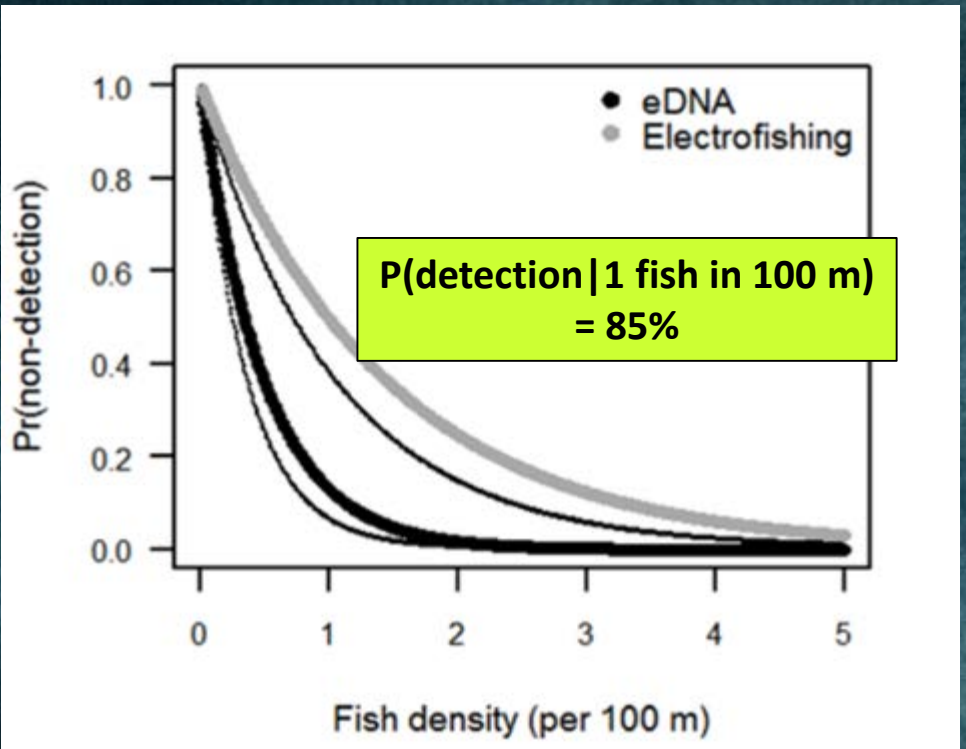
- <u>Rapid, broad-scale surveys are feasible</u>

Why use eDNA sampling: accuracy

- Reliably* species-specific
- Sensitivity: high & quantified
  - Release rate: ~500 copies/sec
  - Detection threshold: 1 copy
- Very good at detecting rare species

- Occupancy estimates are robust

DNA Source	DNA Concentration Copies / ul	N	Proportion Successful
Brook Trout	315.5	40	1
	62.5	40	1
	12.5	40	1
	2.5	40	1
	0.5	40	0.825



OPEN ACCESS Freely available online PLOS ONE

Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity

Taylor M. Wilcox[1]*, Kevin S. McKelvey[1], Michael K. Young[1], Stephen F. Jane[2], Winsor H. Lowe[3], Andrew R. Whiteley[2], Michael K. Schwartz[1]

ELSEVIER Contents lists available at ScienceDirect Biological Conservation journal homepage: www.elsevier.com/locate/biocon

Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*

Taylor M. Wilcox[a,b,*], Kevin S. McKelvey[a], Michael K. Young[a], Adam J. Sepulveda[c], Bradley B. Shepard[d,1], Stephen F. Jane[e], Andrew R. Whiteley[e], Winsor H. Lowe[b], Michael K. Schwartz[a]

Why use eDNA sampling: revolutionary

- Apply a consistent approach
- Craft a sampling design
- Engage the stakeholder community

- Defensible, precise, broad-scale occupancy estimates for priority species in real time for reasonable cost

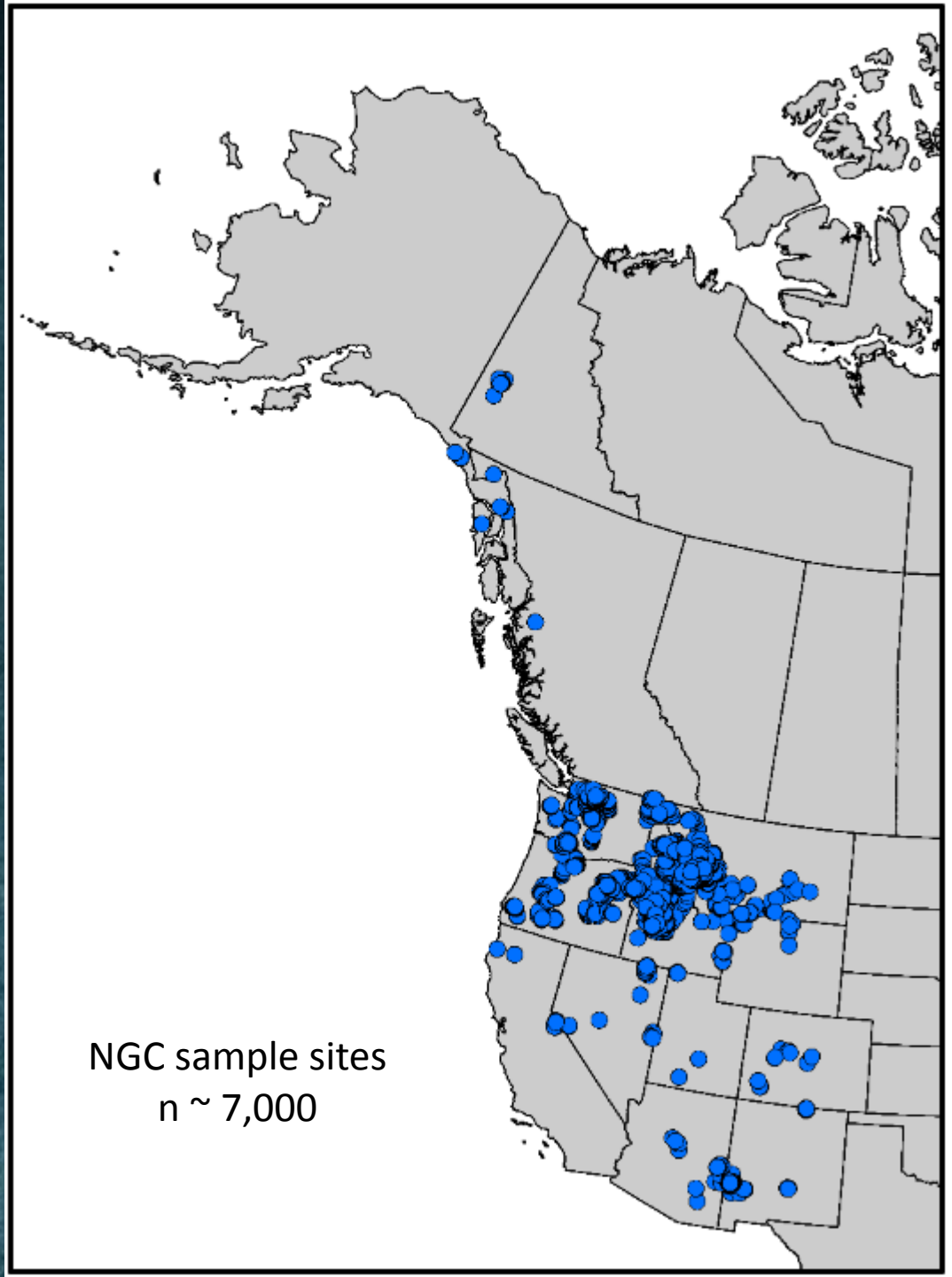

USDA
United States Department of Agriculture

A Protocol for Collecting Environmental DNA Samples From Streams

Kellie J. Carim, Kevin S. McKelvey, Michael K. Young, Taylor M. Wilcox, and Michael K. Schwartz

eDNA: many species

- Trout: rainbow, westslope cutthroat, Yellowstone cutthroat, brown
- Charr: bull, brook, Dolly Varden, lake, Arctic
- Salmon: Chinook, chum, coho, pink, sockeye
- Arctic grayling
- Any salmonid
- Pacific & brook lamprey
- Game fish/invaders: northern pike, sauger, walleye, smallmouth bass
- Non-game fish: sculpin (several), northern leatherside chub, loach minnow, spikedace
- Amphibians: Rocky Mountain tailed frog, western toad
- Mussels: western pearlshell, California floater
- Invertebrates: opossum shrimp, Snake River Physa
- North American river otter
- Harlequin duck
- Your species here...



Applications:
Detecting invasive species

- Have non-native species arrived?
- Have they been eradicated?
- Does the non-native species barrier work?

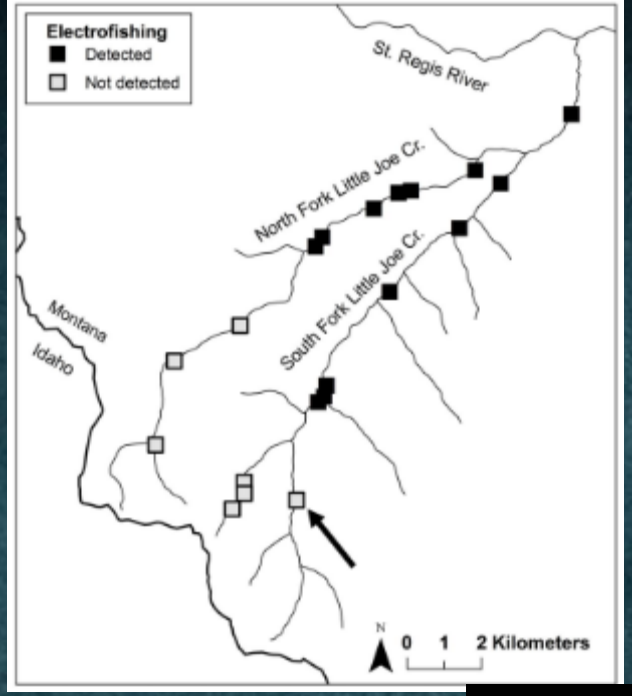
- Where to sample?

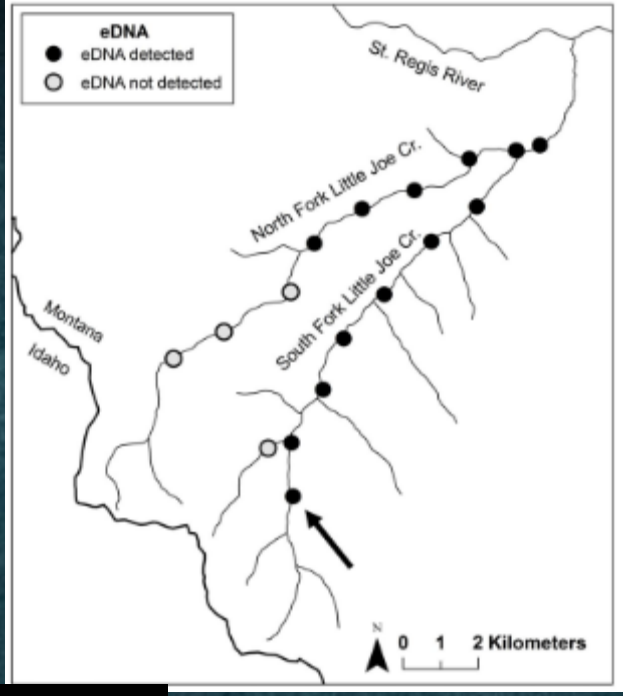
Utah DWR 2014:
chemical treatment
to remove brook trout

Applications: detecting bull trout

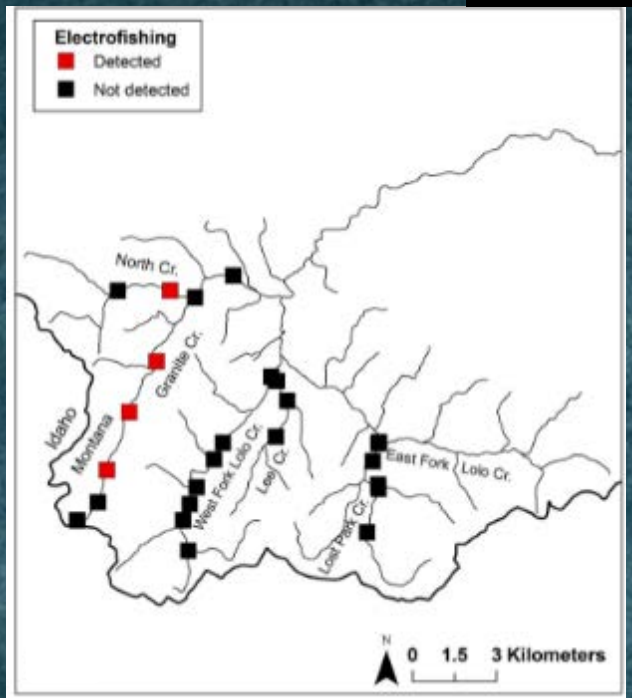
- ESA listed as threatened
- Dictates land & water management & planning
- Widespread - rare
- Difficult to detect
- Juveniles constrained by environment/community
- = ideal candidate for eDNA sampling

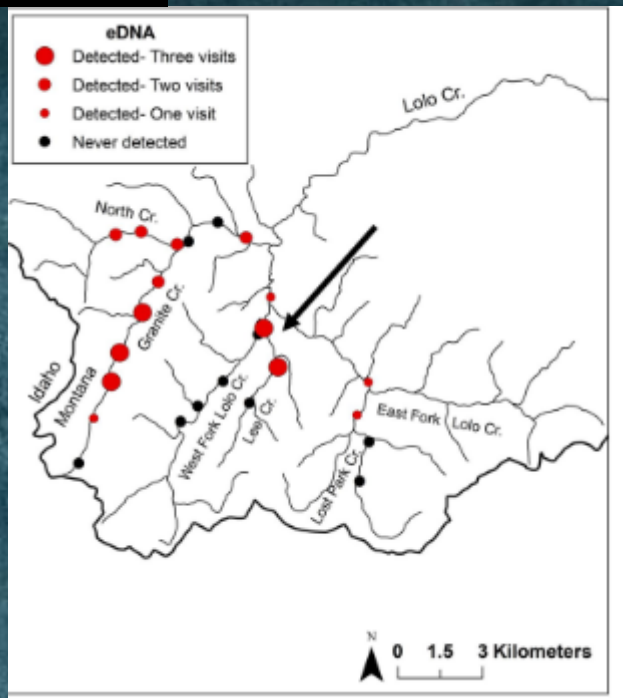
- Test: Montana 2014
- Confirmed known habitats
- Discovered new ones





McKelvey et al. 2016





The range-wide, eDNA-based inventory of bull trout: Coordinators

Michael Young, Dan Isaak, Kevin McKelvey, Michael Schwartz, Tommy Franklin, Kellie Carim, Taylor Wilcox, Wade Fredenberg, Matt Groce, Dave Nagel, Dona Horan, Sherry Wollrab

Collaborators

Bureau of Land Management
Bureau of Reclamation
Chehalis Tribe
Clark Fork Coalition
Coeur d'Alene Tribes
Great Northern LCC
Idaho Conservation League
Idaho Department of Environmental Quality
Idaho Department of Fish and Game
Idaho Power Company
Kalispel Tribes
Lewis River Bull Trout Working Group
Montana Department of Natural Resources Conservation
Montana Fish, Wildlife & Parks
Mount Rainier National Park
National Fish and Wildlife Foundation
The Nature Conservancy
Nez Perce Tribes
North Cascades National Park
Oregon Department of Fish and Wildlife
Trout Unlimited
University of Washington
U.S. Fish and Wildlife Service
National Forests:
Beaverhead-Deer Lodge, Boise, Colville, Deschutes, Flathead, Gifford Pinchot, Helena, Idaho Panhandle, Lolo, Mount Baker-Snoqualmie, Nez Perce-Clearwater, Payette, Salmon-Challis, Sawtooth, Umatilla, Wallowa-Whitman, Wenatchee
Regions 1, 4, and 6
Washington Department of Fish and Wildlife
Whitefish Institute
Wild Fish Conservancy
Yakama Nation

Sponsors

Great Northern LANDSCAPE CONSERVATION COOPERATIVE

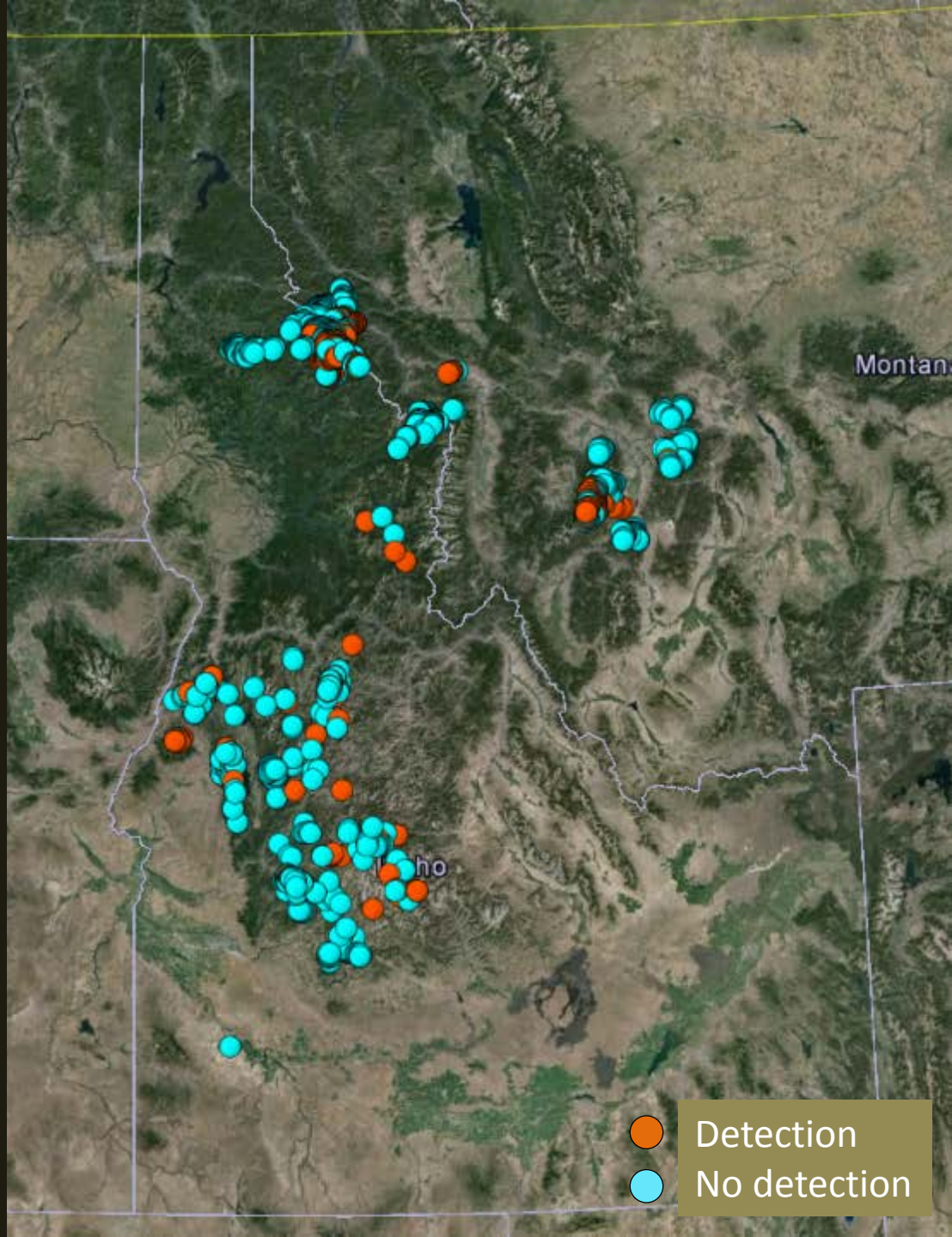
USDA U.S. FOREST SERVICE Region 1

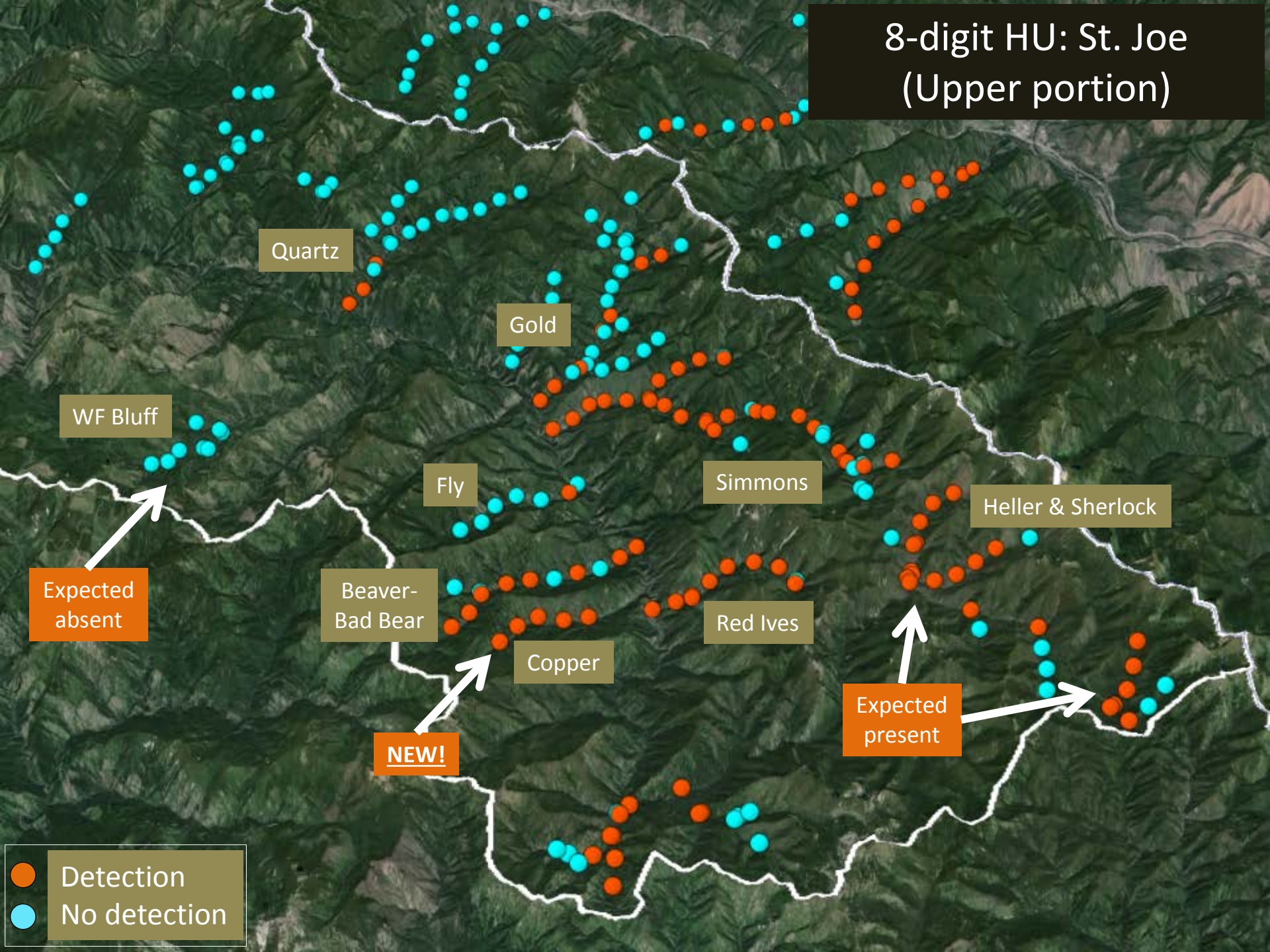
Institutional Support

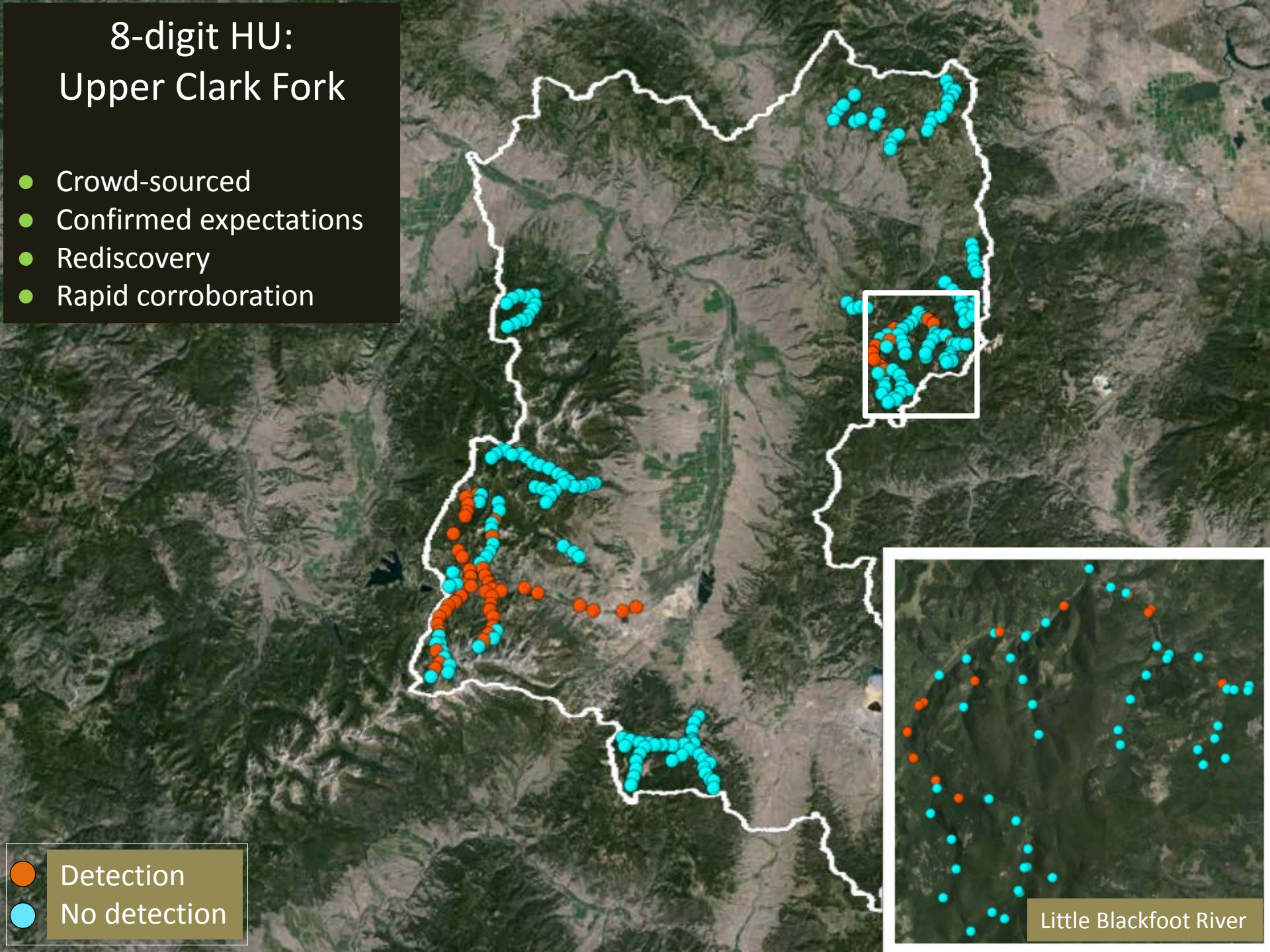
USDA Forest Service National Genomics Center for Wildlife and Fish Conservation

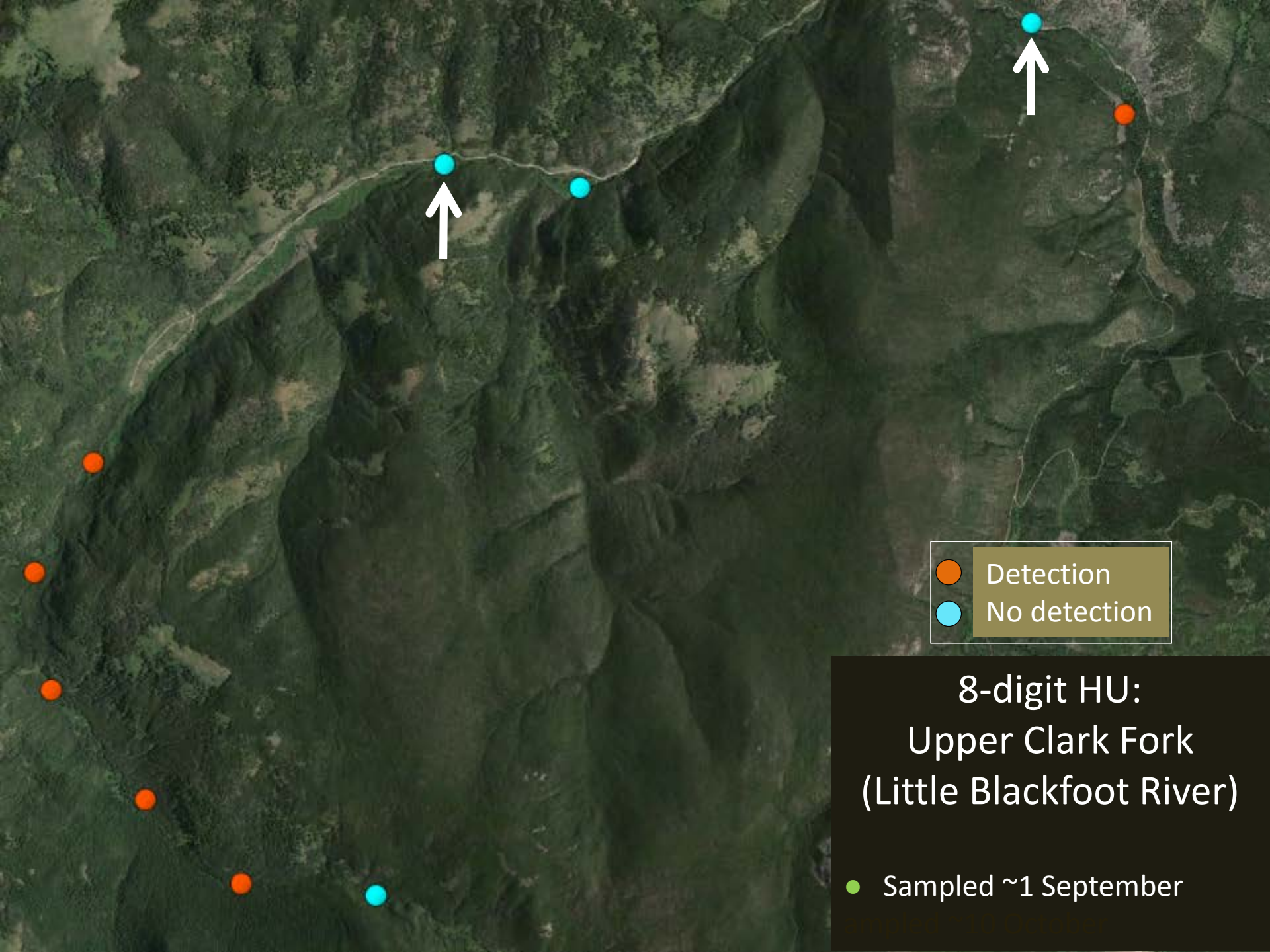
Project framework

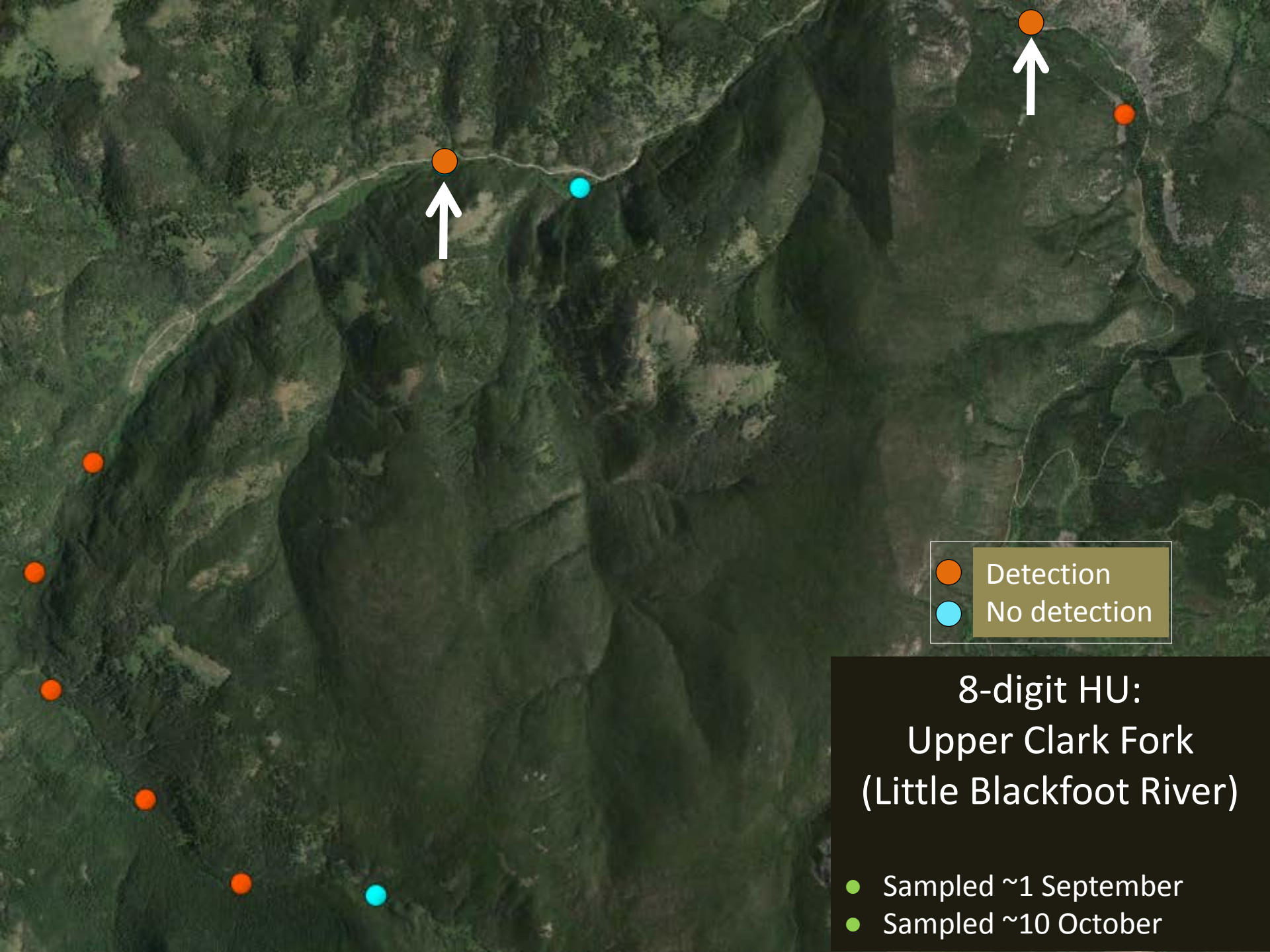
- Target: natal bull trout habitats
  - Cold-water habitats that are part of the Climate Shield
  - USFWS-designated critical habitat for bull trout spawning & rearing
- Grain & Scope
  - Sites at 1-km intervals
  - All 8-digit U.S. HUs
- Timing
  - 2015: 500+ samples
  - 2016: 3,000+ samples
  - 2018: the rest of the range
- Goals
  - Better ability to forecast bull trout futures
  - Consistent, reliable, range-wide map of bull trout
  - **<u>Support the stakeholders</u>**




8-digit HU: St. Joe
(Upper portion)
Quartz
Gold
WF Bluff
Fly
Simmons
Heller & Sherlock
Expected absent
Beaver-Bad Bear
Red Ives
Copper
NEW!
Expected present
Detection
No detection

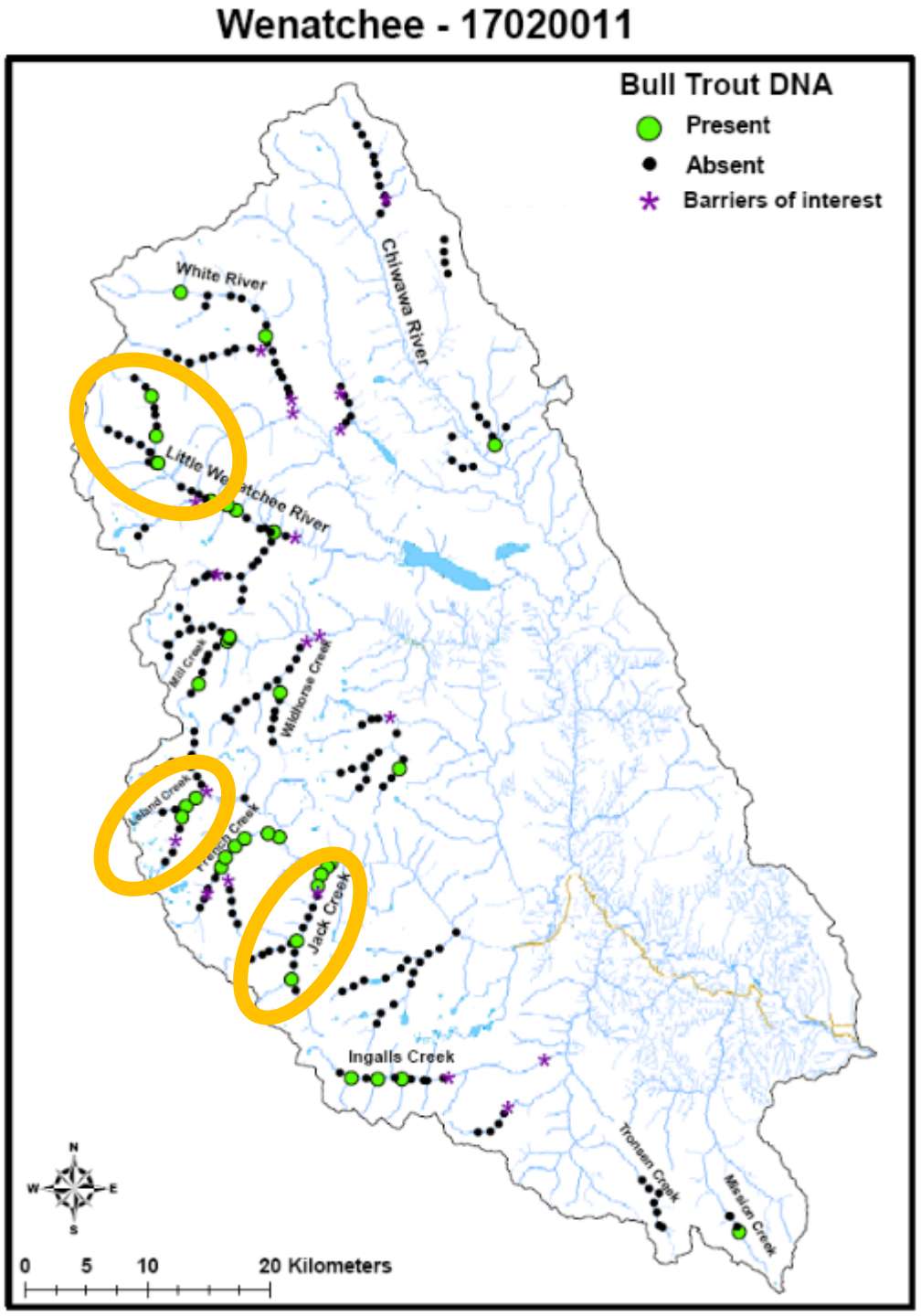

8-digit HU:
Upper Clark Fork
Crowd-sourced
Confirmed expectations
Rediscovery
Rapid corroboration
Detection
No detection
Little Blackfoot River


Detection
No detection
8-digit HU:
Upper Clark Fork
(Little Blackfoot River)
Sampled ~1 September


Detection
No detection
8-digit HU:
Upper Clark Fork
(Little Blackfoot River)
Sampled ~1 September
Sampled ~10 October

8-digit HU: Wenatchee

- WNTI-supported
- USFWS/WDFW/WFC sampling
- New populations above barriers



The Rangewide Bull Trout eDNA Project: want to help?

- Visit our website: www.fs.fed.us/rm/boise/AWAE/projects/BullTrout_eDNA.html

or Google "rangewide bull trout eDNA project"

- Contact us to get your "library card"
- Follow the simple instructions

A Protocol for Collecting Environmental DNA Samples From Streams

Kellie J. Carim, Kevin S. McKelvey, Michael K. Young, Taylor M. Wilcox, and Michael K. Schwartz

Website: Get bull trout hunting directions

Participating in the Bull Trout eDNA Survey

rather, if you can sample one or more cold-water habitats in their entirety, then we welcome your participation.

To make that possible, we will provide you with all you need to conduct eDNA field sampling for juvenile bull trout. That includes:

1) **A protocol that explains how to collect eDNA samples.**

2) **Additional guidelines specific to the bull trout eDNA survey project.**

3) **A map and spreadsheet of eDNA points to guide your sampling.**

4) The loan of a pump set with a battery & charger. We operate a "tool library" i.e., you can reserve a pump set for use during a particular time. The number of pump sets is limited and demand is high, so it's important to reserve one. It's also critical to return it when you are done to permit others to start their sampling. If you want to buy your own pump set—which gives you more flexibility with respect to when you sample—we can give you the specifications.

5) Field kits for the collection and storage of eDNA samples. To ensure consistency in sampling and guarantee sterility of the supplies, we prefer to provide the field kits to you.

Once sampling is complete, return the pump set, field kits, and collected samples. In a few weeks, we'll share with you whether and where bull trout were present. And at the end of each year, we'll post an interactive map of the results of sampling across the range of bull trout on our **results page**.

Bull trout eDNA Sample Sites

…ur eDNA surveys for
…here throughout their
…) were identified using the
…712 streams (**Isaak et al.**
…are also shown on the
…ed, so please review the
field sampling instructions (**"Participating in the Bull Trout eDNA Survey, Important caveats"**). To get started, print or plot the map of showing the 8-digit HUC to be sampled and identify which potential bull trout streams are of interest (and can be sampled in their entirety). Files may be periodically updated, so confirm that you have the most current versions before starting field work. Next, download the Excel file with the eDNA sample site coordinates (note that a GPS unit will be required for accurate field navigation).

Bull Trout eDNA Sample Sites

Scenario: 1980s, 0% Brook Trout

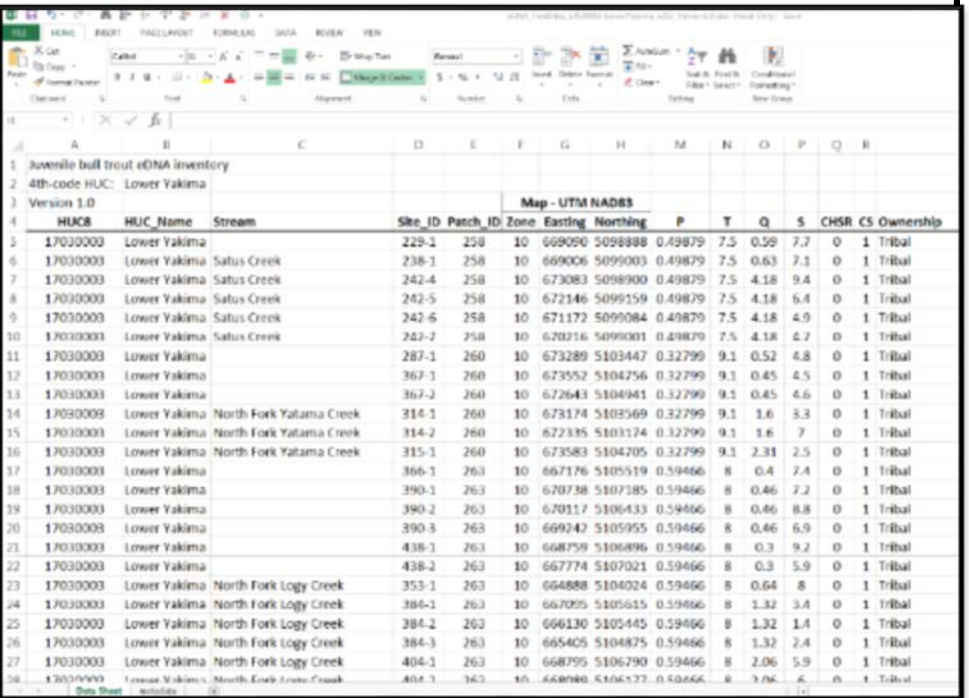
NHD Unit: 17080002 (Lewis)

Legend

- **eDNA Sample Site (N=355)**
  Note: The 355 sites on this map occur on streams having <10% slope and a probability of bull trout occurrence >25%.
- **Intermittent Flowlines**
- **Slope > 10%**
- **Probability of Occupancy (%)**
  - 10 - 25
  - 25 - 50
  - 50 - 75
  - 75 - 90
  - 90 - 100
- **Bull Trout Critical Habitat**
- Waterfalls

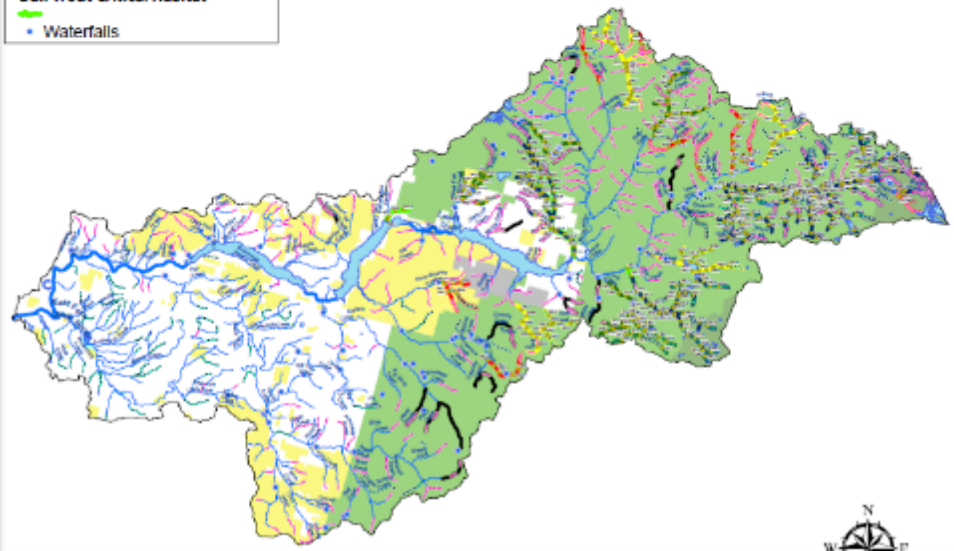
Land Ownership

No Data	NPS
BLM	Other Federal
BOR	Tribal
USFWS	State/City
USFS - Nonwilderness	TNC
USFS - Wilderness	Private
COE	Other/Unknown



1 Juvenile bull trout eDNA inventory
2 4th-code HUC: Lower Yakima
3 Version 1.0

					Map - UTM NAD83								
HUC8	HUC_Name	Stream	Site_ID	Patch_ID	Zone	Easting	Northing	P	T	Q	S	CHSR	CS	Ownership
17030003	Lower Yakima		229-1	258	10	669090	5098888	0.49879	7.5	0.59	7.7	0	1	Tribal
17030003	Lower Yakima	Satus Creek	238-1	258	10	669006	5099003	0.49879	7.5	0.63	7.1	0	1	Tribal
17030003	Lower Yakima	Satus Creek	242-4	258	10	673083	5098900	0.49879	7.5	4.18	9.4	0	1	Tribal
17030003	Lower Yakima	Satus Creek	242-5	258	10	672146	5099159	0.49879	7.5	4.18	6.4	0	1	Tribal
17030003	Lower Yakima	Satus Creek	242-6	258	10	671172	5099084	0.49879	7.5	4.18	4.9	0	1	Tribal
17030003	Lower Yakima	Satus Creek	242-7	258	10	670216	5099001	0.49879	7.5	4.18	4.7	0	1	Tribal
17030003	Lower Yakima		287-1	260	10	673289	5103447	0.32799	9.1	0.52	4.8	0	1	Tribal
17030003	Lower Yakima		367-1	260	10	673552	5104756	0.32799	9.1	0.45	4.5	0	1	Tribal
17030003	Lower Yakima		367-2	260	10	672643	5104941	0.32799	9.1	0.45	4.6	0	1	Tribal
17030003	Lower Yakima	North Fork Yakama Creek	314-1	260	10	673174	5103569	0.32799	9.1	1.6	3.3	0	1	Tribal
17030003	Lower Yakima	North Fork Yakama Creek	314-2	260	10	672335	5103174	0.32799	9.1	1.6	7	0	1	Tribal
17030003	Lower Yakima	North Fork Yakama Creek	315-1	260	10	673583	5104705	0.32799	9.1	2.31	2.5	0	1	Tribal
17030003	Lower Yakima		366-1	263	10	667176	5105519	0.59466	8	0.4	7.4	0	1	Tribal
17030003	Lower Yakima		390-1	263	10	670738	5107185	0.59466	8	0.46	7.2	0	1	Tribal
17030003	Lower Yakima		390-2	263	10	670117	5106433	0.59466	8	0.46	8.8	0	1	Tribal
17030003	Lower Yakima		390-3	263	10	669242	5105955	0.59466	8	0.46	6.9	0	1	Tribal
17030003	Lower Yakima		438-1	263	10	668759	5106896	0.59466	8	0.3	9.2	0	1	Tribal
17030003	Lower Yakima		438-2	263	10	667774	5107021	0.59466	8	0.3	5.9	0	1	Tribal
17030003	Lower Yakima	North Fork Logy Creek	353-1	263	10	664888	5104024	0.59466	8	0.64	8	0	1	Tribal
17030003	Lower Yakima	North Fork Logy Creek	386-1	263	10	667095	5105615	0.59466	8	1.32	3.4	0	1	Tribal
17030003	Lower Yakima	North Fork Logy Creek	384-2	263	10	666130	5105445	0.59466	8	1.32	1.4	0	1	Tribal
17030003	Lower Yakima	North Fork Logy Creek	384-3	263	10	665405	5104875	0.59466	8	1.32	2.4	0	1	Tribal
17030003	Lower Yakima	North Fork Logy Creek	404-1	263	10	668795	5106790	0.59466	8	2.06	5.9	0	1	Tribal



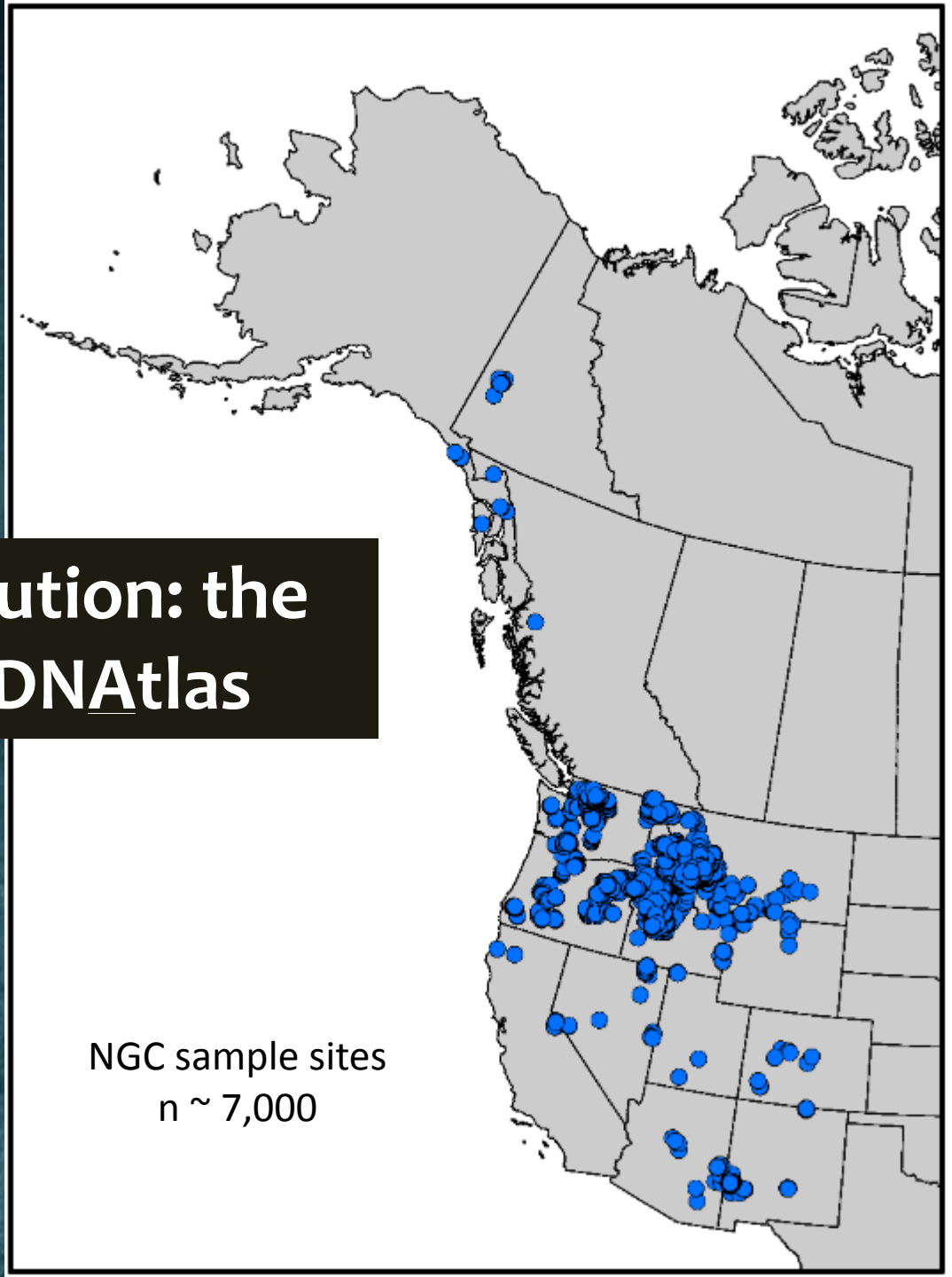

Go sample, mail everything back, and then...
chaos or efficiency?

eDNA results:

chaos or efficiency

- Ease can equal redundancy
- Data often regarded as proprietary
- Lack of consistent data delivery

Solution: the eDNAtlas



NGC sample sites
n ~ 7,000

Steps in eDNAtlas Database Development

Data collected with standard protocol

A Protocol for Collecting Environmental DNA Samples From Streams

Kellie J. Carim, Kevin S. McKelvey, Michael K. Young, Taylor M. Wilcox, and Michael K. Schwartz

General Technical Report
RMRS-GTR-355

QA/QC procedures (laboratory & data)

	A	B	C
1			
2	**Stream:**	Elk Creek	
3	**Georeference:**	610234 E, 4402546 W	
4			
5	**Date**	**Time**	**Temp (°C)**
6	7/15/2005	21:23	15.59
7	7/15/2005	21:53	15.11
8	7/15/2005	22:23	14.64
9	7/15/2005	22:53	14.32
10	7/15/2005	23:23	13.86
11	7/15/2005	23:53	13.55
12	7/16/2005	0:23	13.24

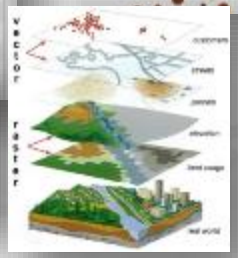
Pipeline for data entry (relational & geospatial)

DATABASE

NHDPlus

ESRI ArcGIS

Metadata documentation & website delivery in user-friendly formats



PDF Adobe

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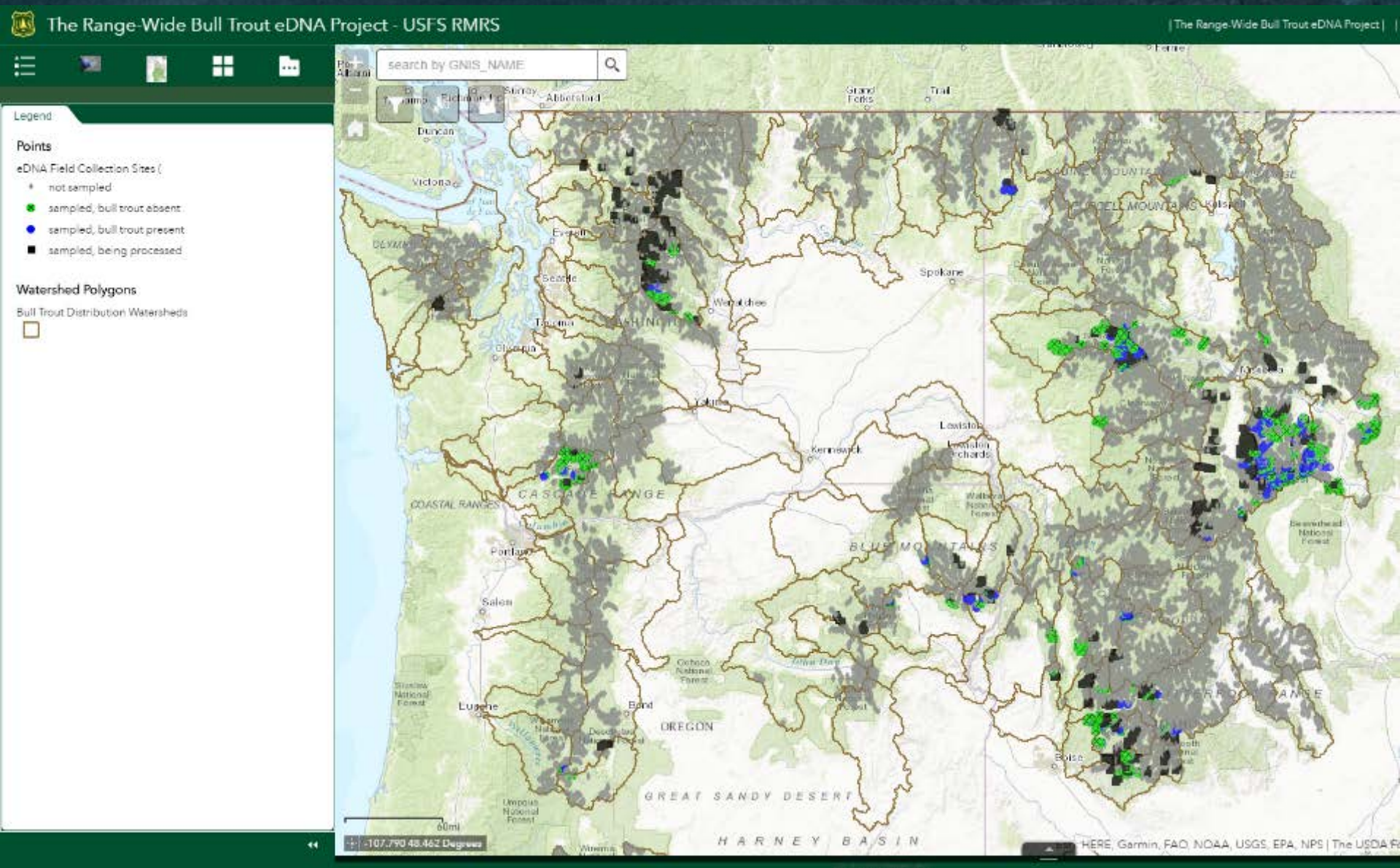
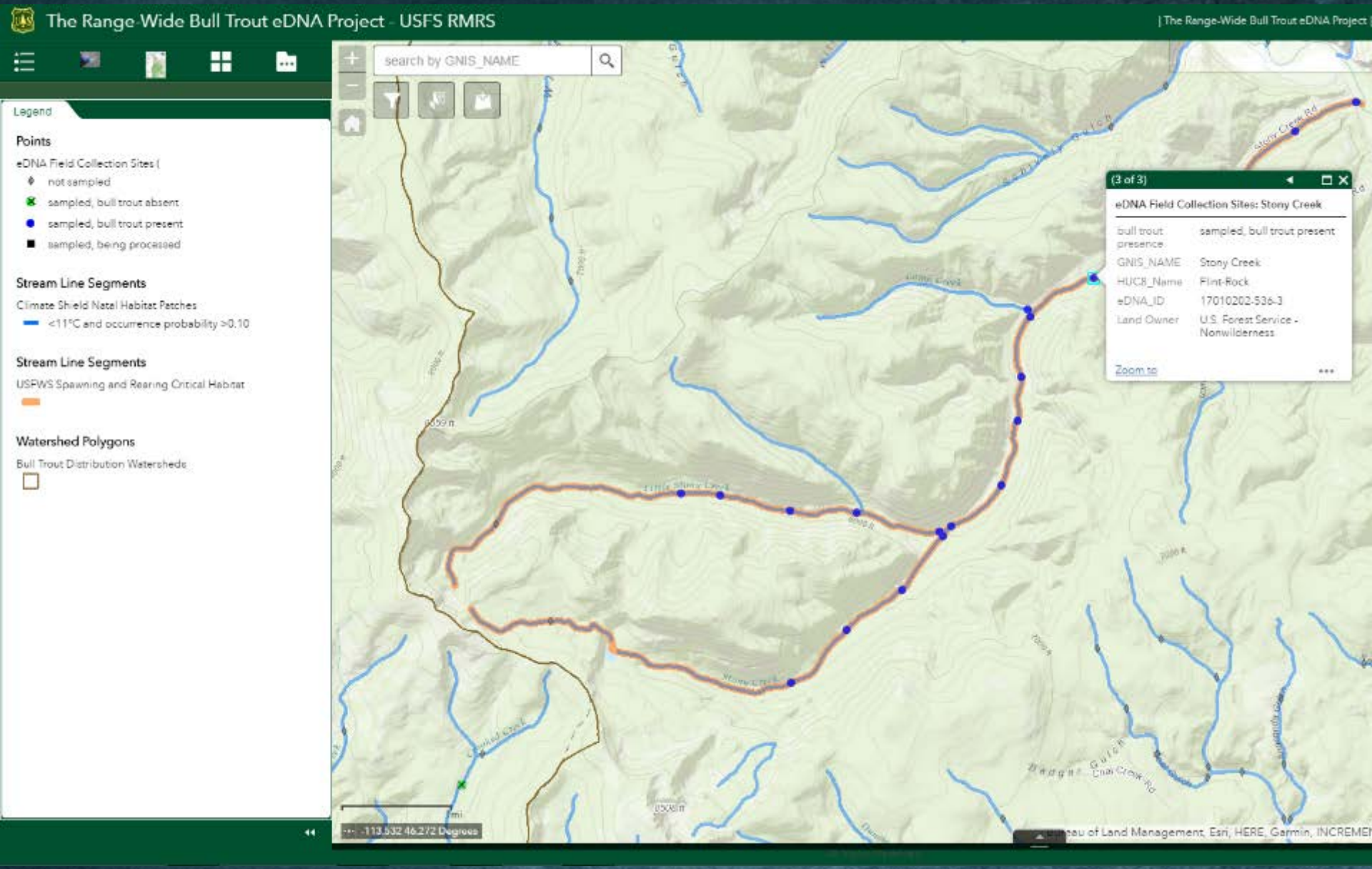
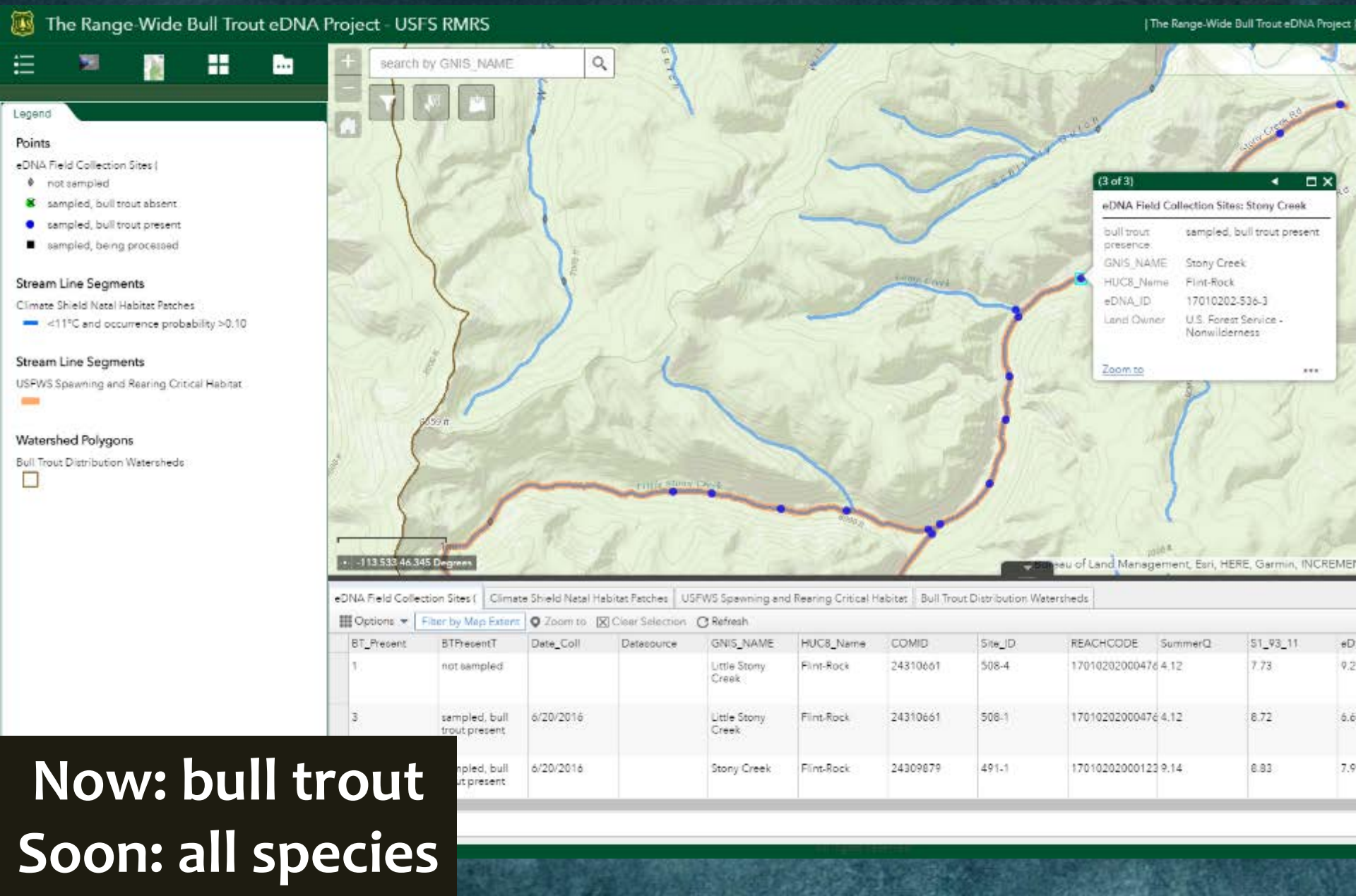
eDNAtlas: open-access data portal



eDNAtlas: open-access data portal



eDNAtlas: open-access data portal

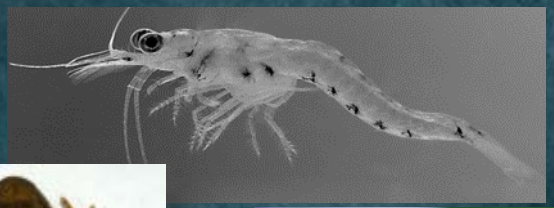


Now: bull trout
Soon: all species

eDNArchive

- 1 eDNA sample = many species
- Permanent archives of biodiversity
- ~10% of samples run for other spp.










eDNAtlas & Archive Advantages

1) Efficiencies of scale: each contributor is part of a massive biological sensing network

2) As the database grows, its value compounds

3) Fieldwork savings: analyze archived samples

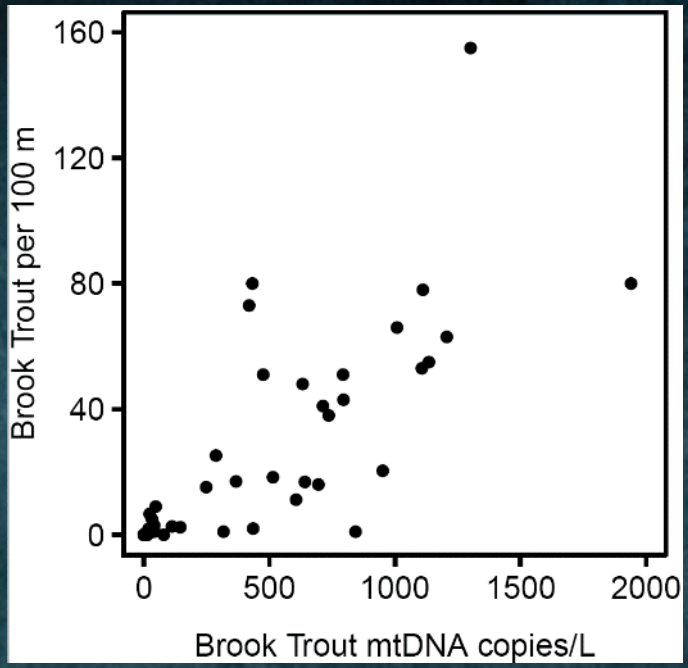
4) Database evolves with input from managers

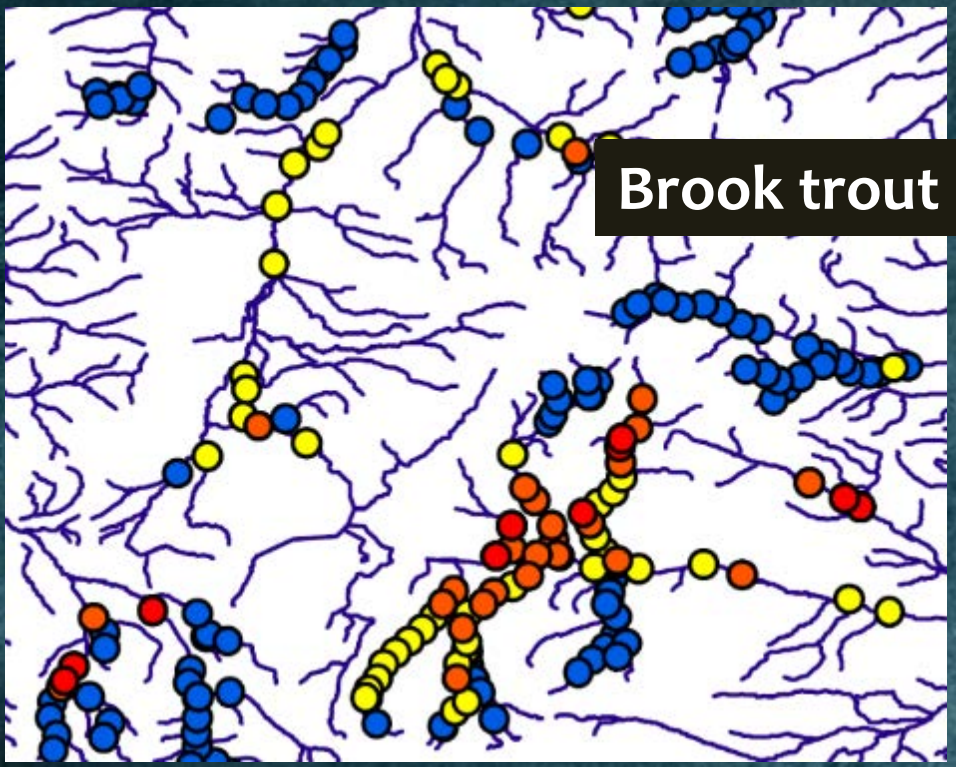
5) Consistency & open access fosters communication within & among agencies

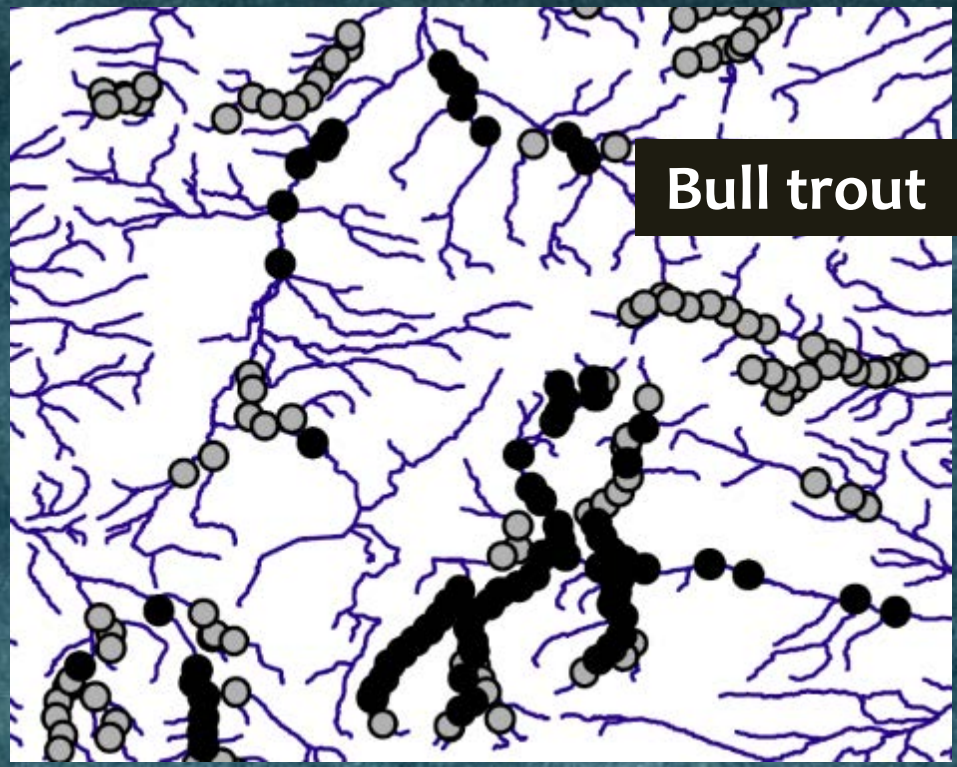
6) No reinventing of technical wheels (i.e., website/database design, geospatial stuff, sampling protocols, etc.)

Other options

- eDNA assay development
  - $7.5K/taxon
  - 2–3 months*
- Sample analysis
  - $85, 1st species
  - $35, all other species
  - 56-hour turnaround
  - All gear provided*
- Beyond presence
  - Abundance
  - Co-occupancy
  - Multi-species assessments
- Questions?








The End