

The Next Generation of eDNA Research: Applications for Fish Conservation

Kellie J. Carim, Ph.D.

National Genomics Center for Wildlife and Fish Conservation



Lamprey Conservation Team and Technical Workgroup

Information Exchange

December 6th, 2017



What is Environmental DNA?

DNA released from an organism into the surrounding environment





Species detection using environmental DNA from water samples

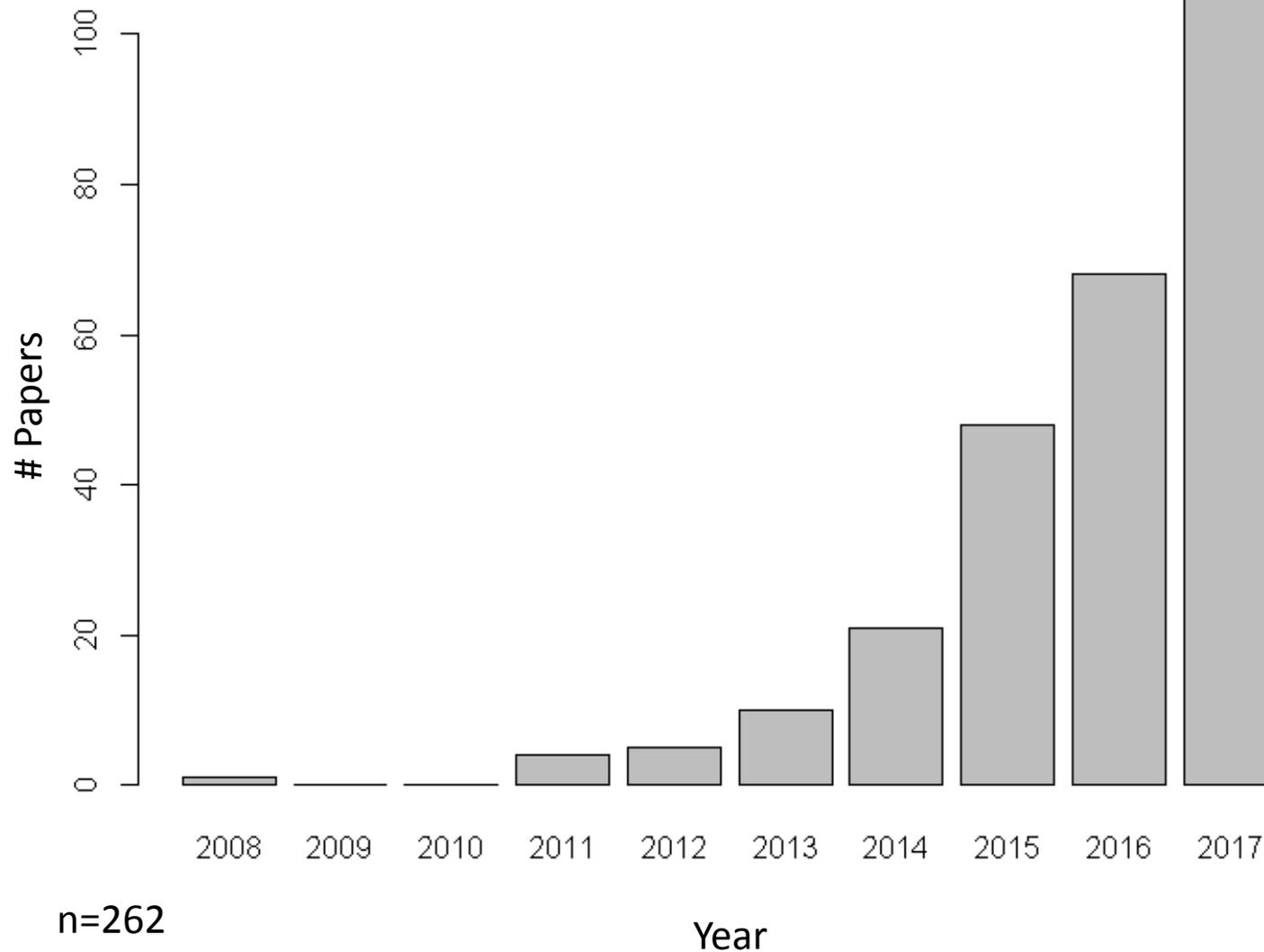
Gentile Francesco Ficetola, Claude Miaud, François Pompanon and Pierre Taberlet

Biol. Lett. 2008 **4**, 423-425
doi: 10.1098/rsbl.2008.0118

“We showed that this technique is able to discriminate between species absence and presence, even at low densities.”
– Ficetola et al. 2008

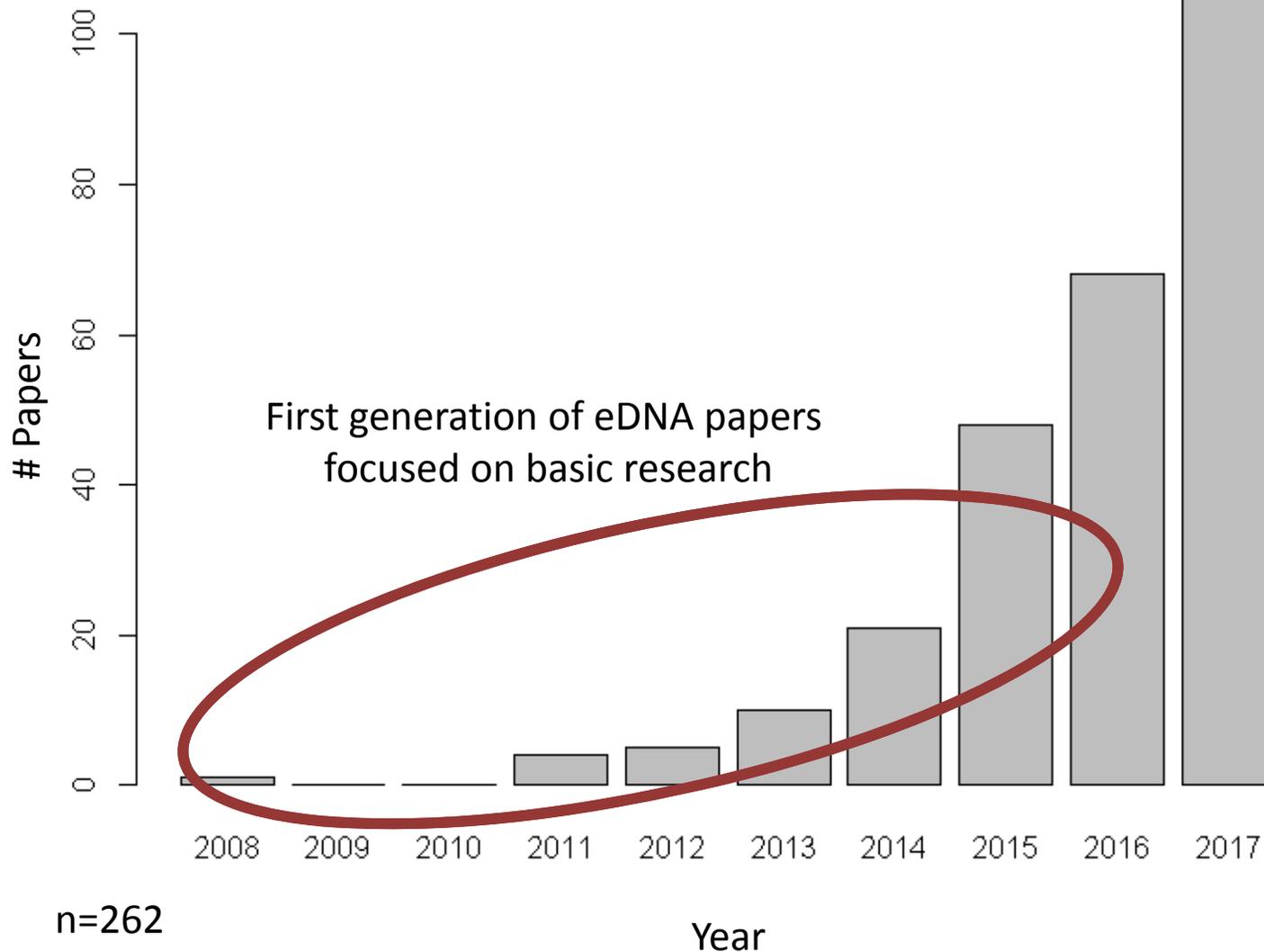
eDNA Publications by Year

<https://taylorwilcox.weebly.com/environmental-dna.html>



eDNA Publications by Year

<https://taylorwilcox.weebly.com/environmental-dna.html>



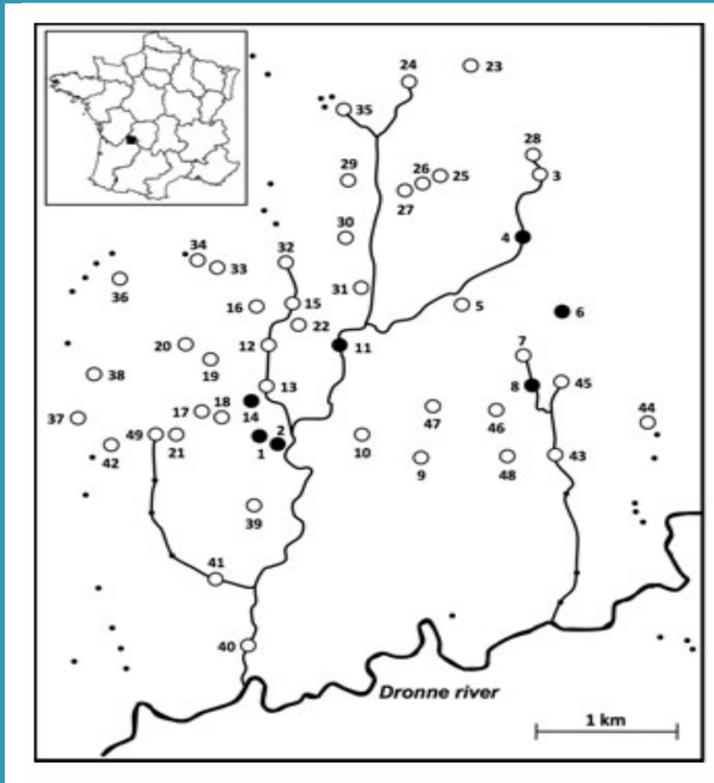
Environmental DNA: Basic Research

- How does eDNA compare to traditional sampling methods?

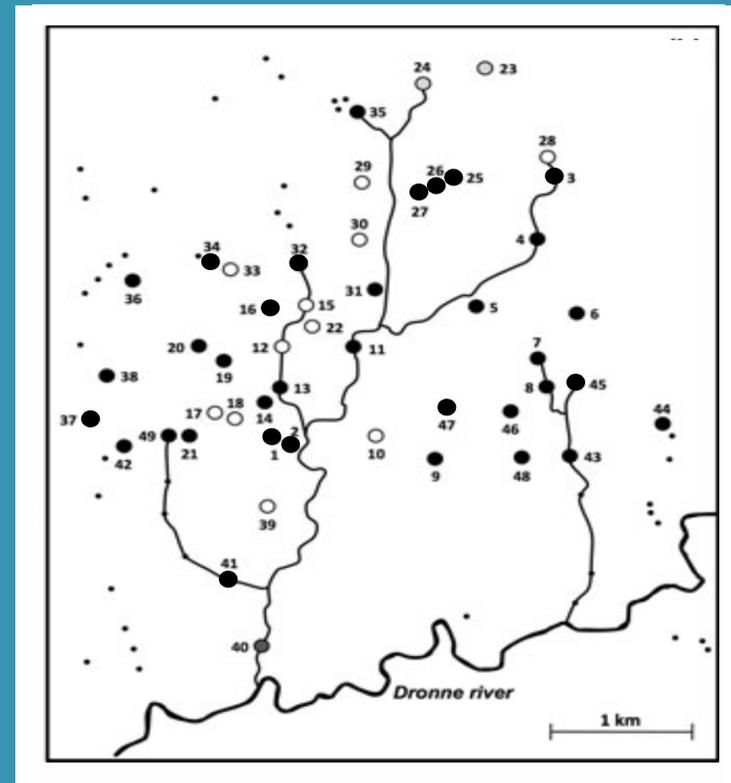


Photo: Jeremy Monroe

eDNA vs Traditional Sampling Methods



Traditional Methods:
Frogs detected at 7 sites



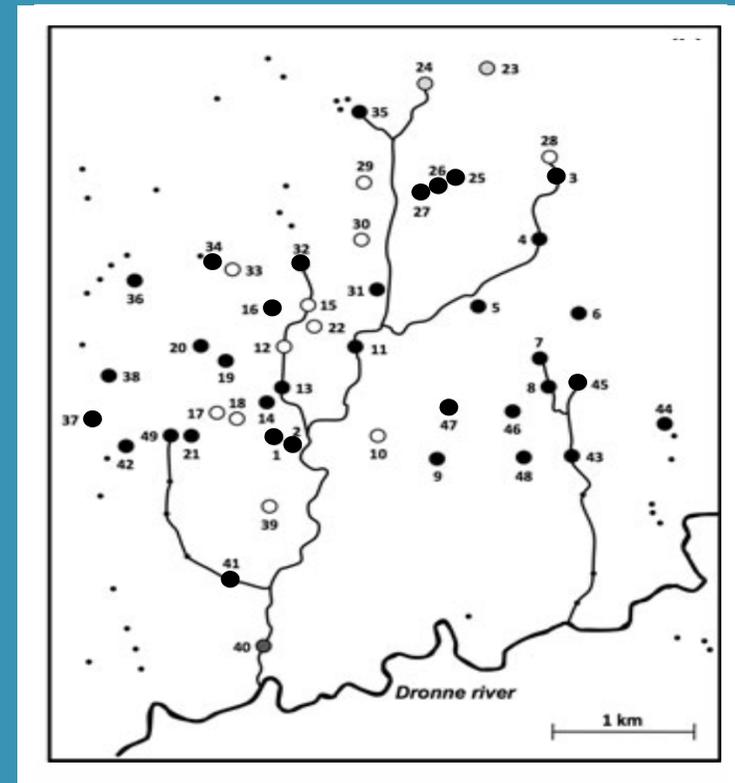
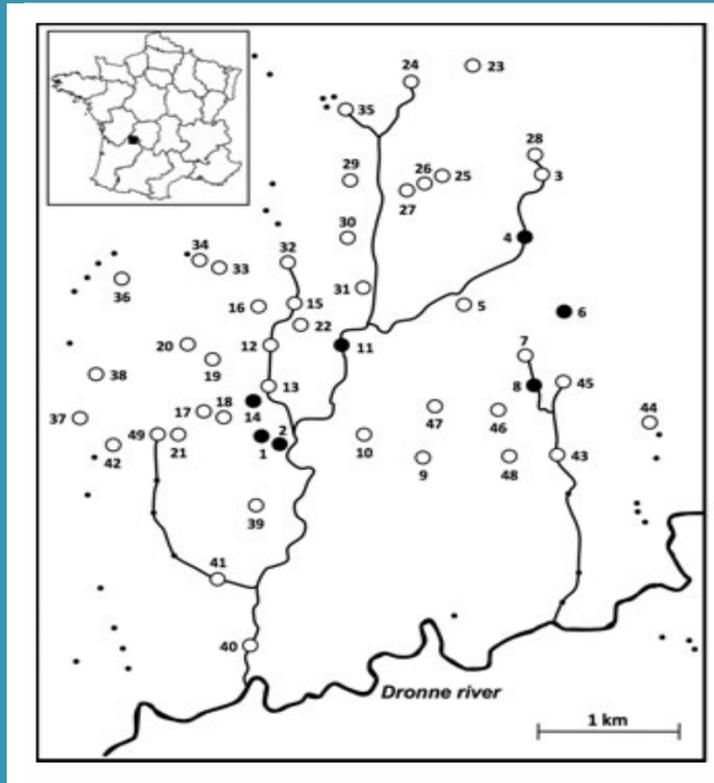
eDNA Methods:
Frogs detected at 38 sites



- Not Detected
- Detected
- Ponds not yet surveyed

Dejean et al. (2012)

eDNA vs Traditional Sampling Methods



eDNA based methods are more sensitive, accurate, and time and cost efficient than traditional sampling methods.

Environmental DNA: Basic Research

- How does eDNA compare to traditional sampling methods?
- What are best methods for sample collection?



Photo: Jeremy Monroe

What are the best methods for sample collection?



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



Rocky Mountain
Research Station

General Technical Report
RMRS-GTR-355

August 2016



Prepared in cooperation with Washington State University

Environmental DNA Sampling Protocol—Filtering Water to Capture DNA from Aquatic Organisms

Chapter 13 of
Section A, Biological Science
Book 2, Collection of Environmental Data

Techniques and Methods 2–A13

U.S. Department of the Interior
U.S. Geological Survey



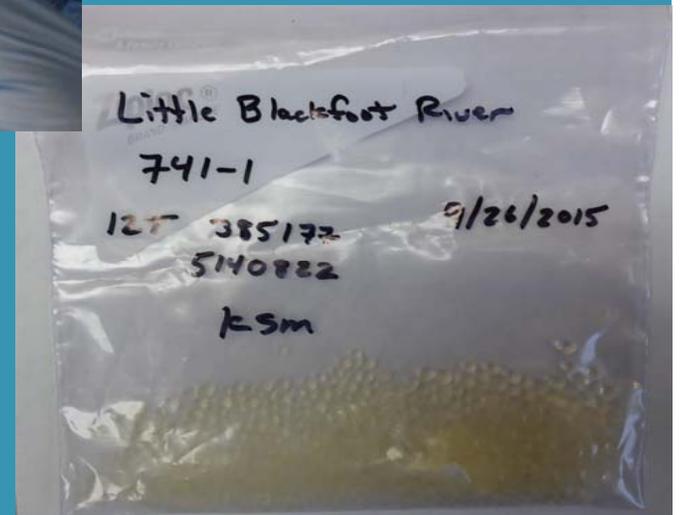
Carim et al. 2016; Laramie et al. 2015

Maximizing probability of detection, minimizing contamination risk

A single copy of DNA can cause contamination and false positive results...



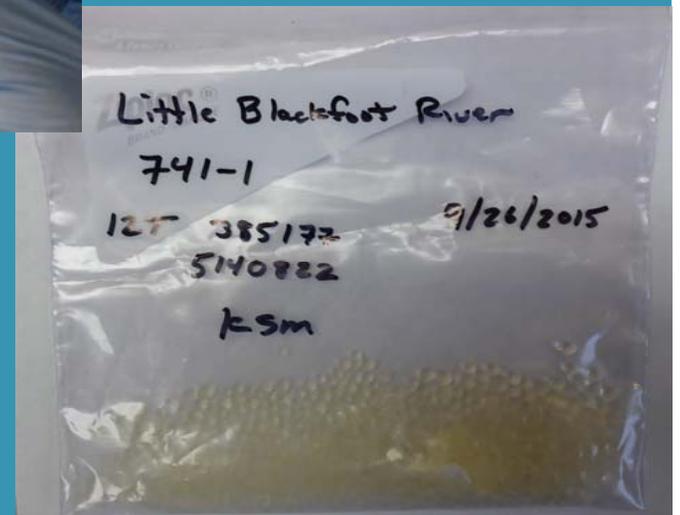
... but if properly collected, a single copy of DNA may be all you need to confirm presence.



Maximizing probability of detection, minimizing contamination risk



- Take precautions to avoid contamination
- Filter and store properly to preserve DNA



Environmental DNA: Basic Research

- How does eDNA compare to traditional sampling methods?
- What are best methods for sample collection?
- How is eDNA detected in a sample?

Species specific eDNA markers identify target species DNA in sea of non-targets

Bull Trout

Salvelinus confluentus

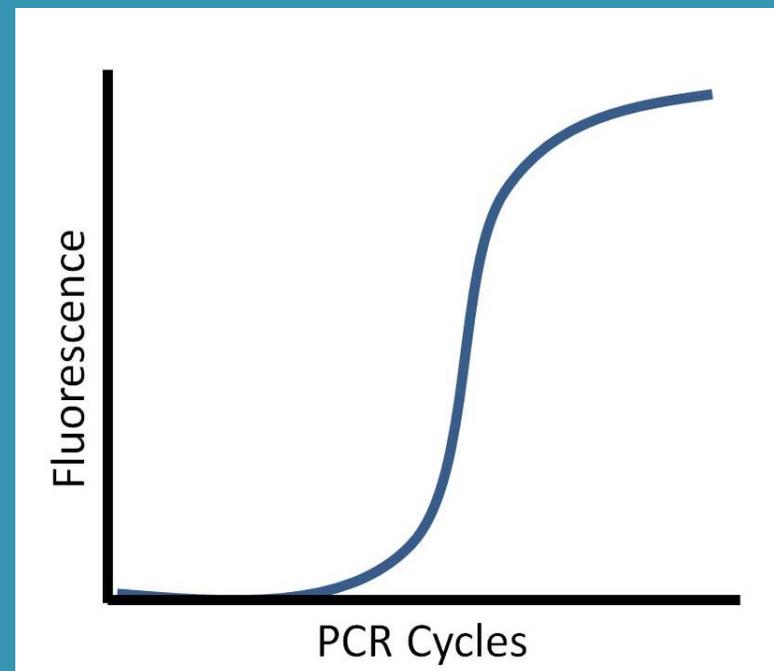


ATTCCTGC



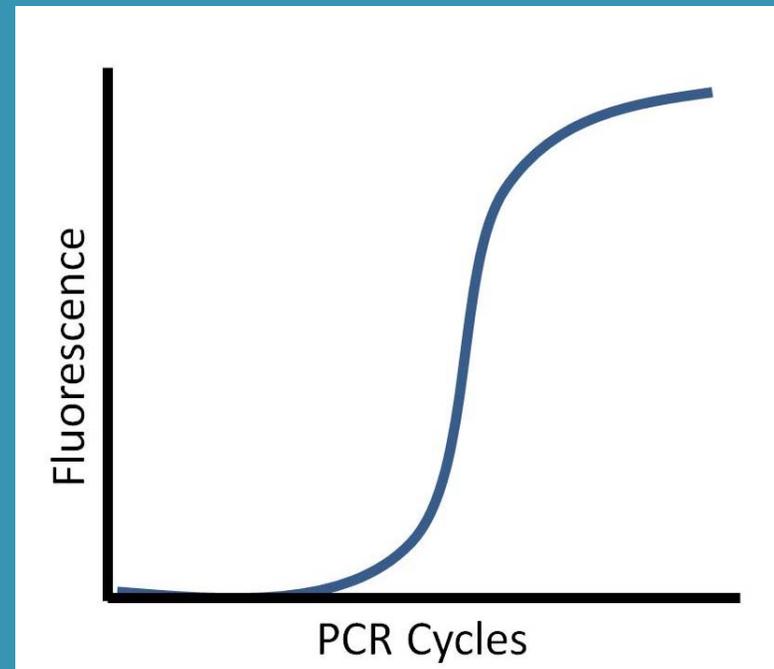
Quantitative PCR Analysis

Highly sensitive process that allows for DNA detection and quantification



Quantitative PCR Analysis

Currently the most reliable and commonly used method for eDNA analysis



Detecting DNA of a Target Species

Bull Trout

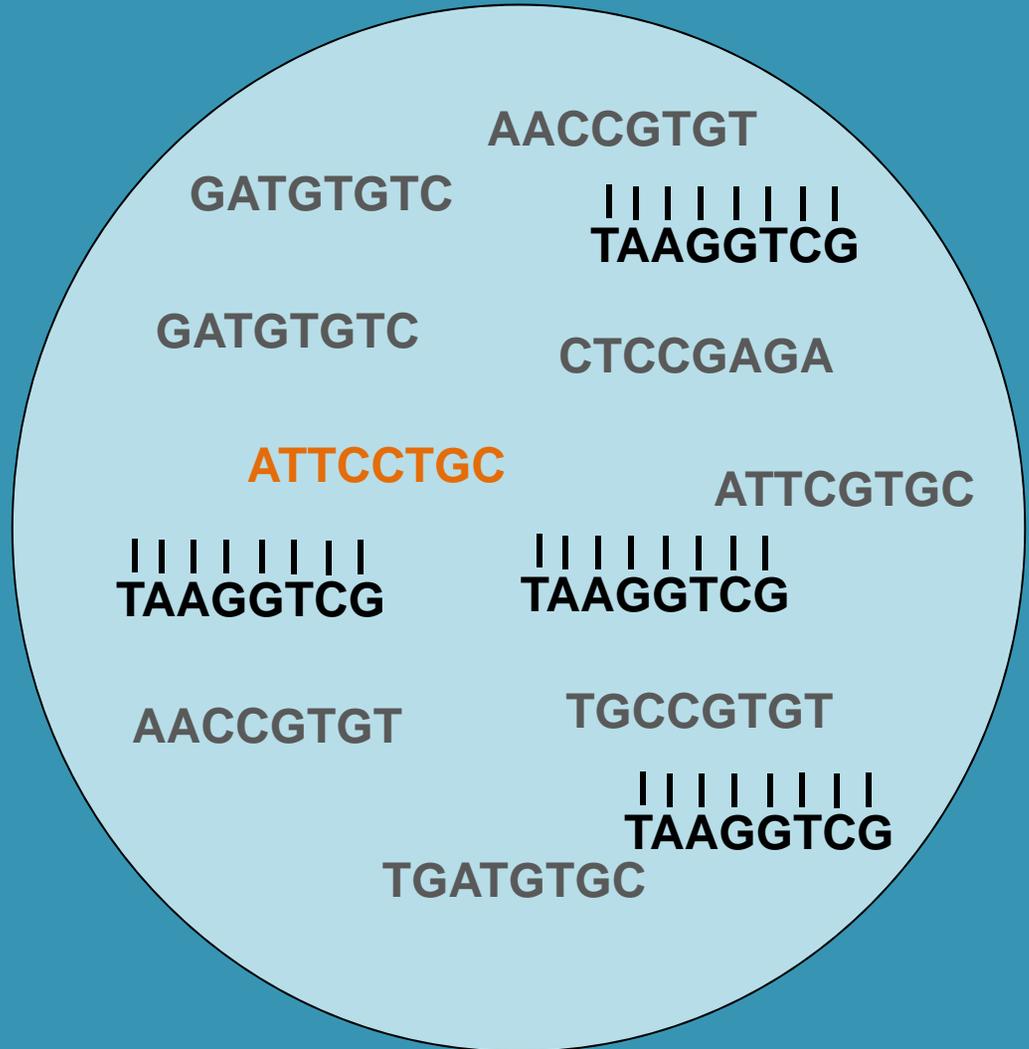
Salvelinus confluentus



ATTCCTGC
| | | | | | | |
TAAGGTCG



eDNA marker is an exact
compliment to target DNA



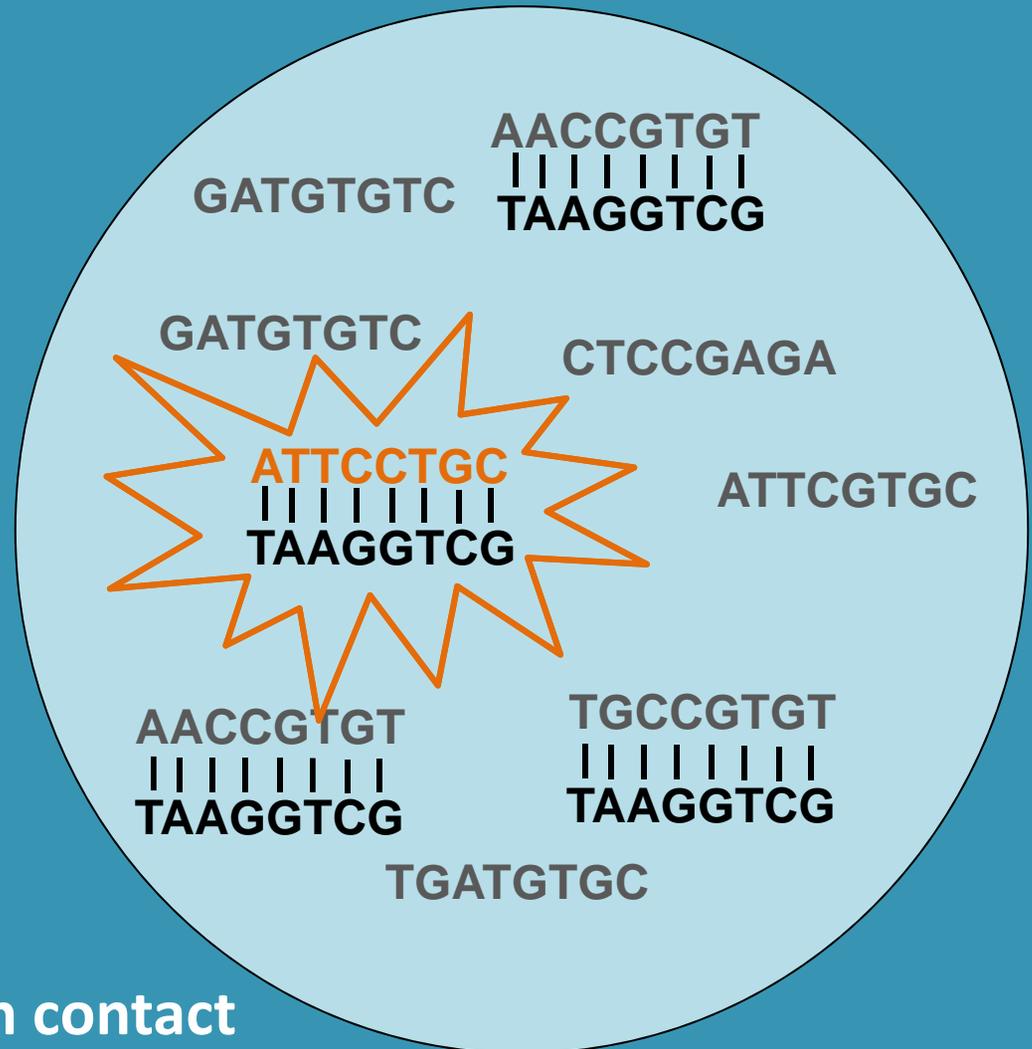
Detecting DNA of a Target Species

Bull Trout

Salvelinus confluentus

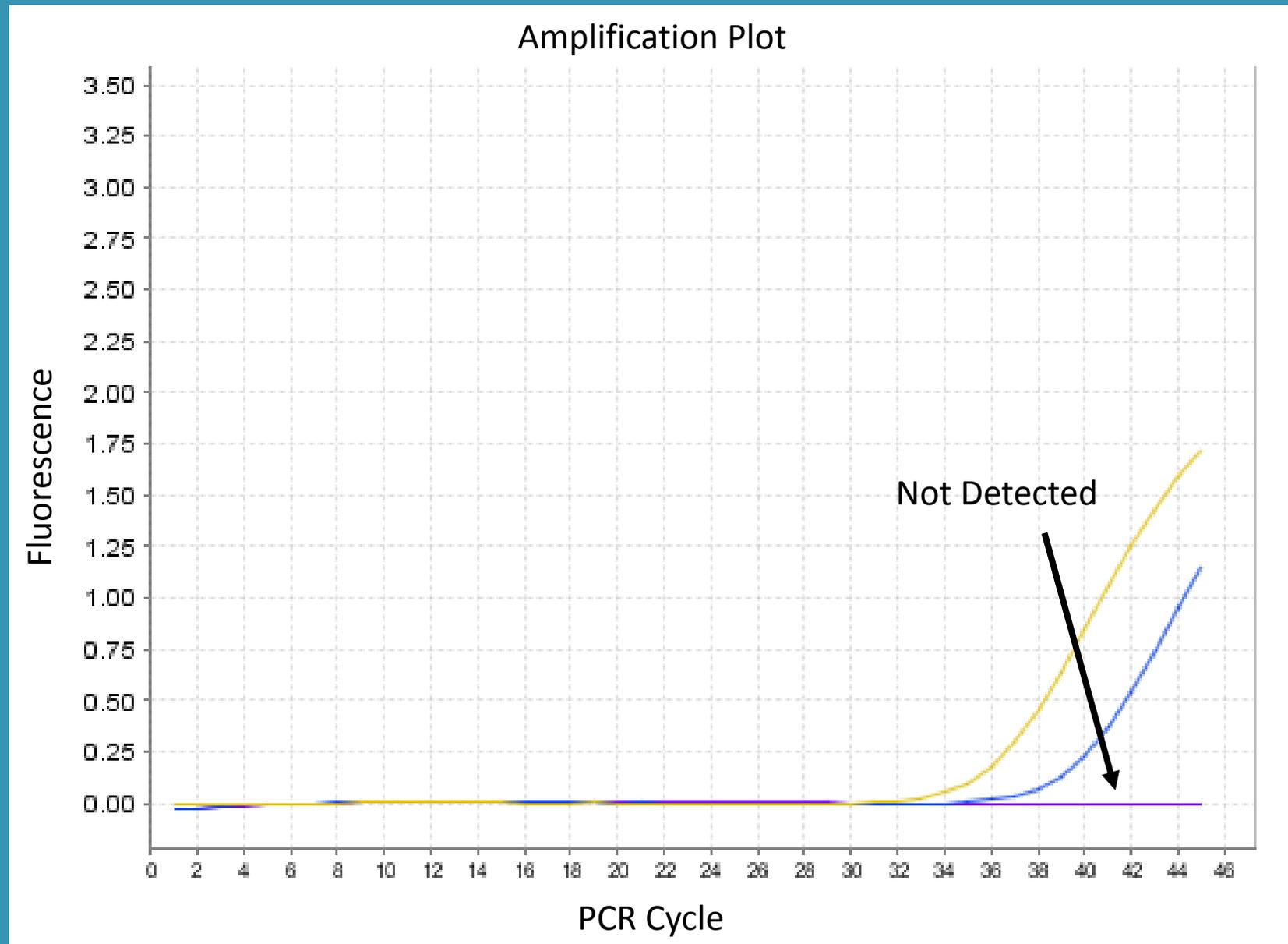


ATTCCTGC
| | | | | | | |
TAAGGTCG

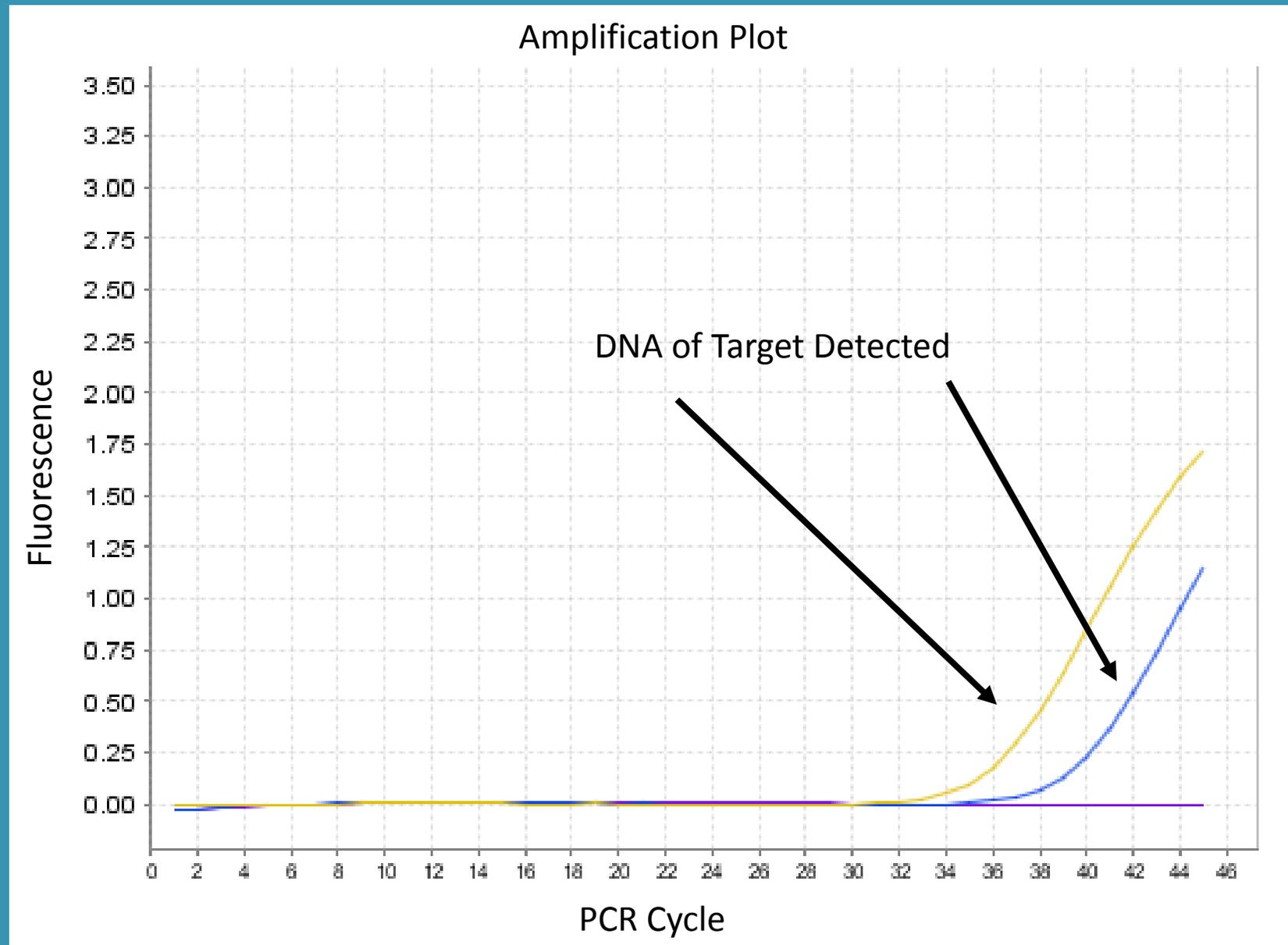


When the eDNA marker is in contact with target DNA, light is emitted

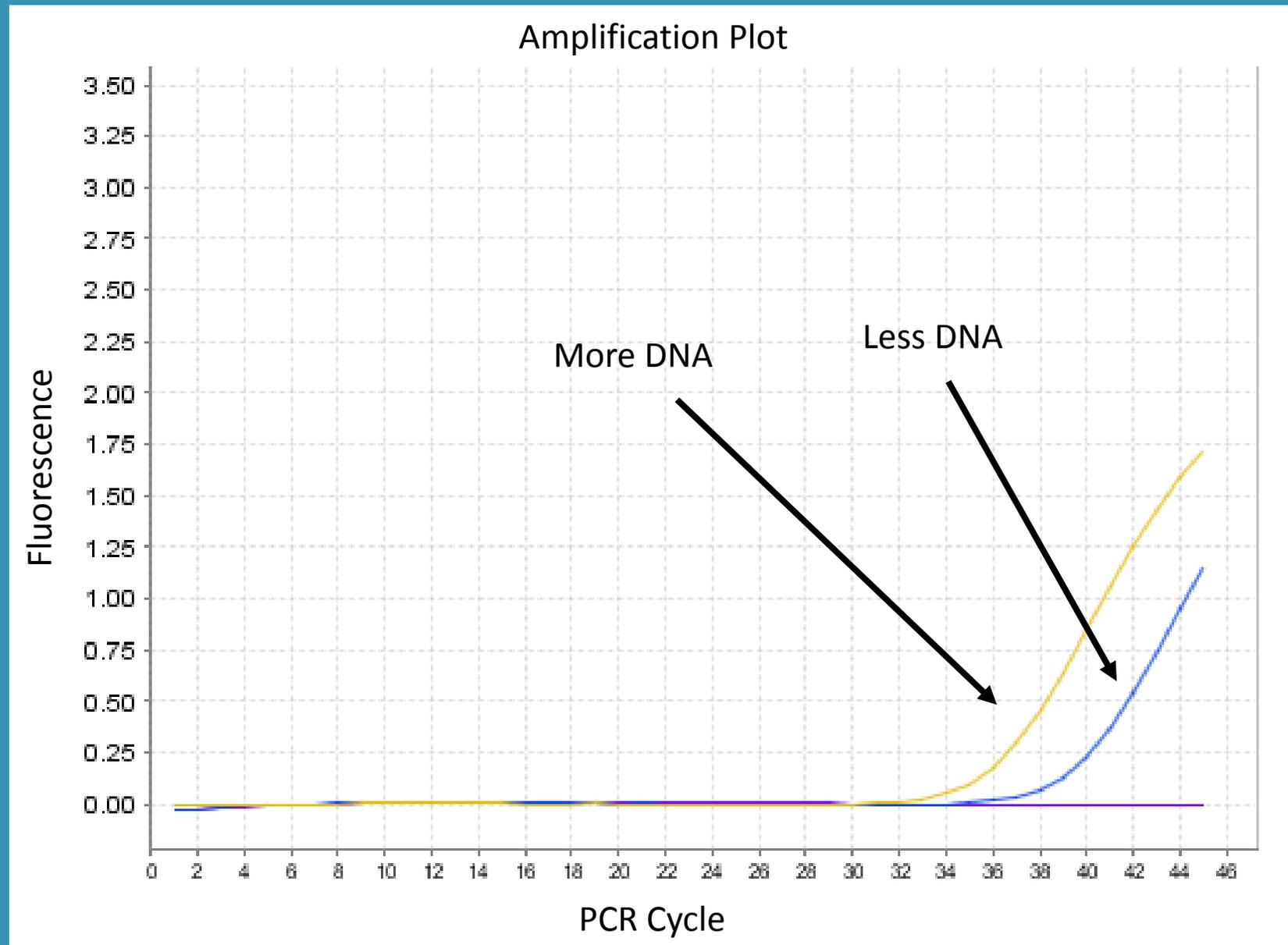
Quantitative PCR Analysis- Results



Quantitative PCR Analysis- Results



Quantitative PCR Analysis- Results



Environmental DNA: Basic Research

- **How does eDNA compare to traditional sampling methods?**
- **What are best methods for sample collection?**
- **How is eDNA detected in a sample?**
- **How are eDNA markers developed?**

Considerations in eDNA Marker Design

Sensitivity- detecting **ALL** of your target species

Specificity - detecting **ONLY** the species of interest

National Genomics Center

eDNA Marker Design Checklist

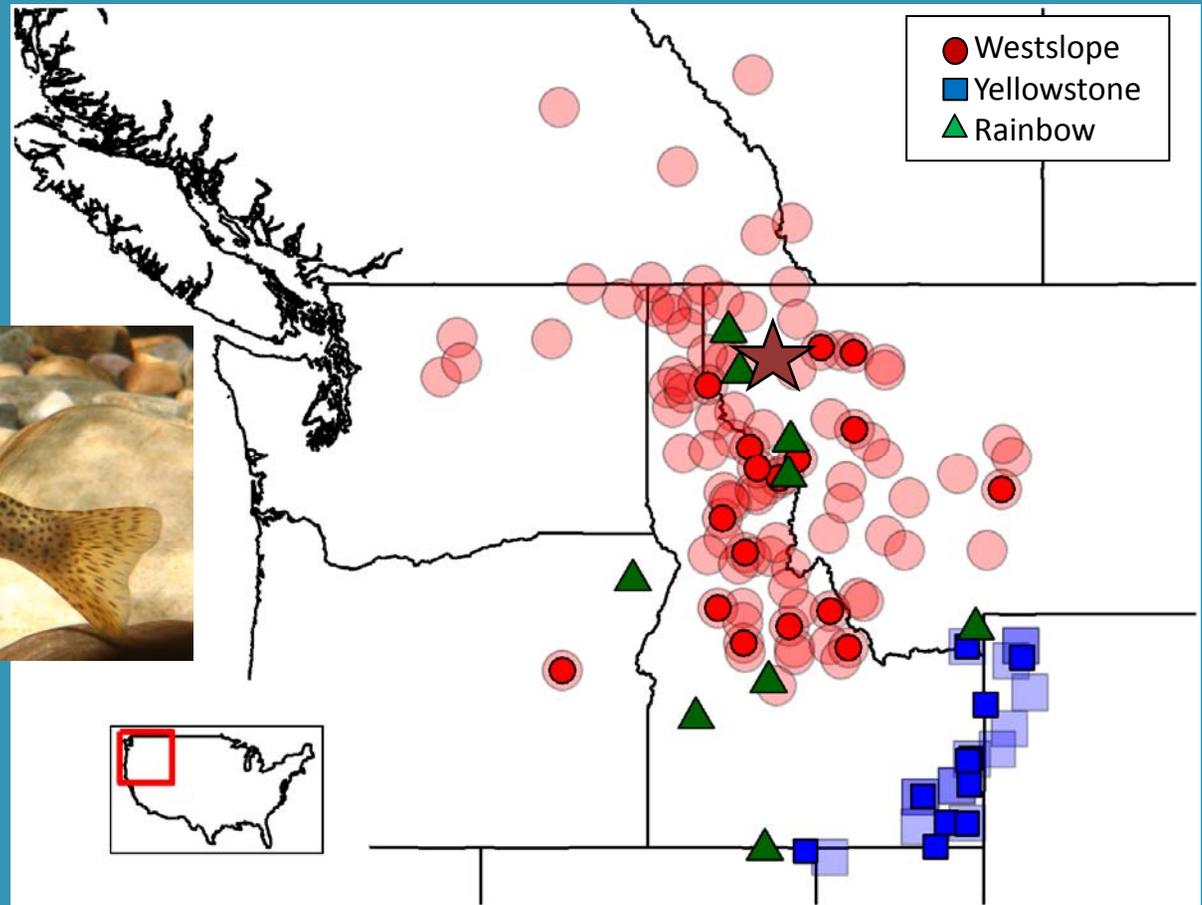
1. Compile genetic data across geographic range
2. Screen mitochondrial DNA for a region unique to target species
3. Design marker to maximize genetic differences with DNA of non-targets
4. BLAST to estimate specificity
5. Test against DNA of target and non-target species
6. Optimize assay concentrations and evaluate efficiency
7. Test against environmental DNA samples with expected presence/absence

Wilcox et al. 2013, 2015; Dysthe et al. In review



National Genomics Center
— FOR WILDLIFE AND FISH CONSERVATION —

Sensitivity of eDNA markers- detecting ALL of your target species



The marker successfully detected westslope cutthroat trout except for one fish in one population

Specificity of eDNA markers - detecting ONLY the species of interest



RESEARCH ARTICLE

A Noninvasive Tool to Assess the Distribution of Pacific Lamprey (*Entosphenus tridentatus*) in the Columbia River Basin

Kellie J. Carim^{*✉}, J. Caleb Dysthe[✉], Michael K. Young, Kevin S. McKelvey, Michael K. Schwartz

- Will also detect Pit-Klamath lamprey (*E. lethophagus*)
- Not useful as a species specific marker where multiple *Entosphenus spp.* occur

Environmental DNA: Basic Research

- How does eDNA compare to traditional sampling methods?
- What are best methods for sample collection?
- How is eDNA detected in a sample?
- How are eDNA markers developed?
- How do proximity, stream flow, water temperature, animal density, etc. affect detection probabilities?

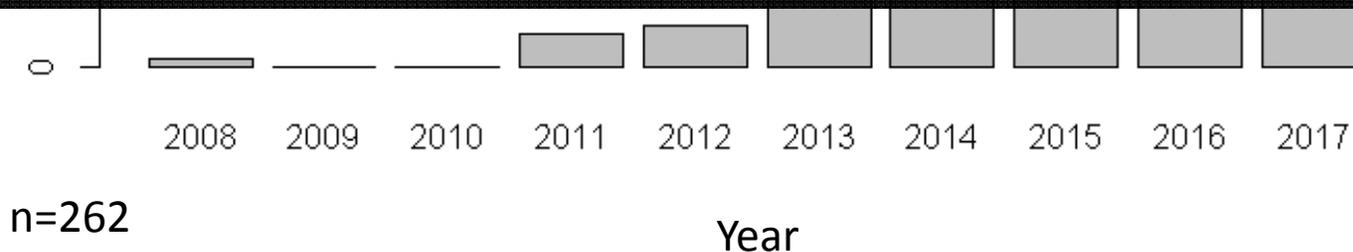
eDNA Publications by Year

<https://taylorwilcox.weebly.com/environmental-dna.html>

A powerful presence/absence tool

A somewhat useful tool for abundance

**A poor tool for age/size structure,
genetic diversity, hybridization**



Environmental DNA: Basic Research

- **How does eDNA compare to traditional sampling methods?**
- **What are best methods for sample collection?**
- **How is eDNA detected in a sample?**
- **How are eDNA markers developed?**
- **How do proximity, stream flow, water temperature, animal density, etc. affect detection probabilities?**
- **How can we adjust protocols to maximize probability of detection?**

How can we adjust protocols to maximize probability of detection?

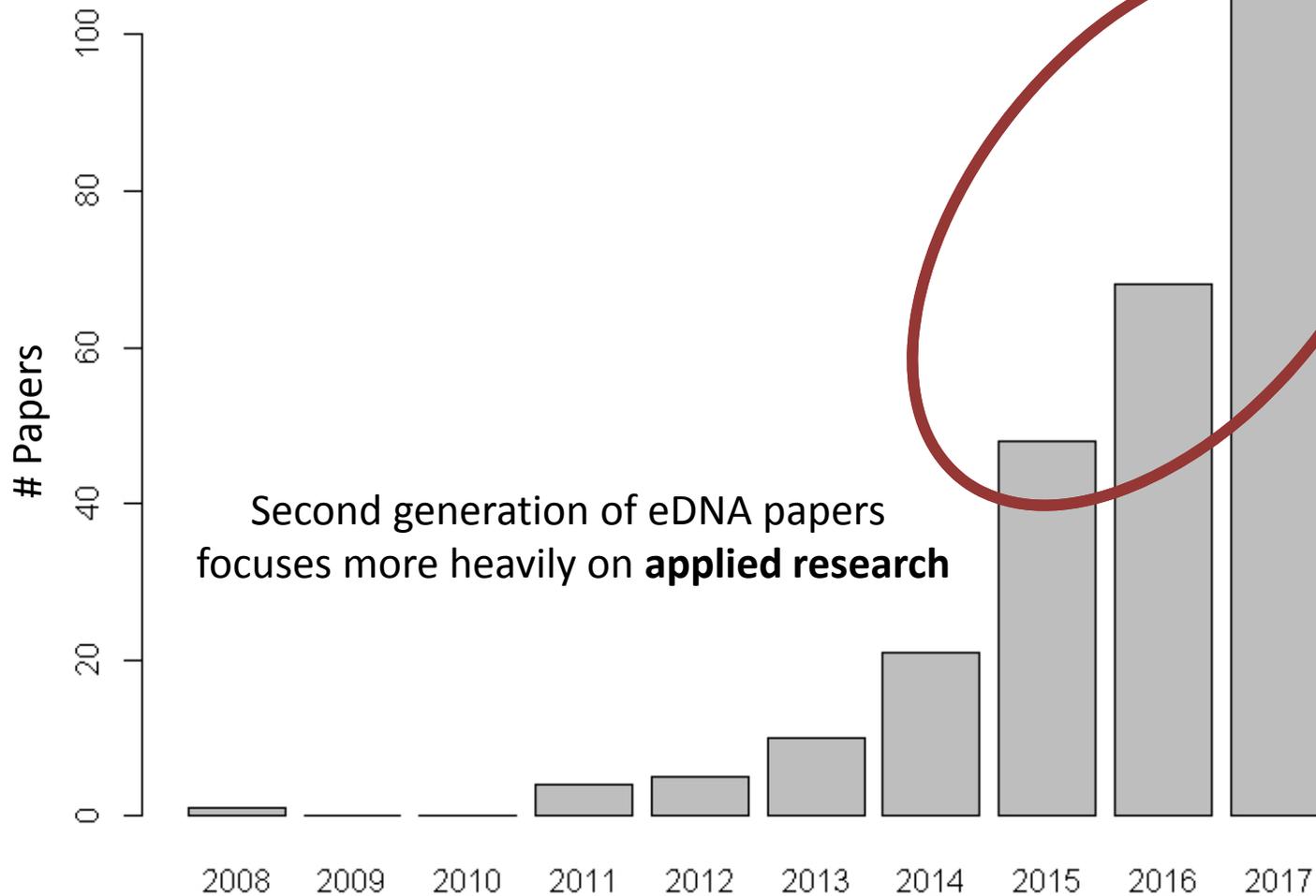


**eDNA of Pacific Lamprey Ammocoetes in Sediment:
Controlled Laboratory Testing to Refine and Validate
Field Sampling Methods**

Marty Liedtke, USGS Western Fisheries Science Center

eDNA Publications by Year

<https://taylorwilcox.weebly.com/environmental-dna.html>



n=262

Year

Environmental DNA Applications for Fisheries Conservation and Management

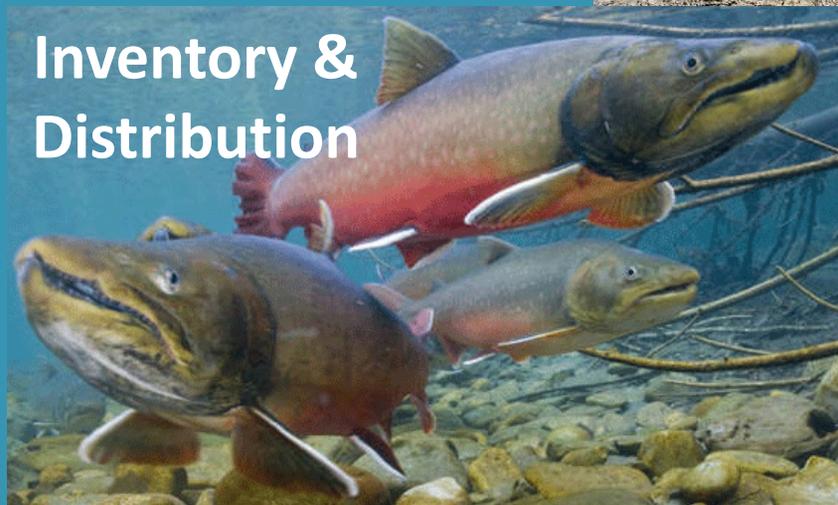
Reintroduction Efforts



Eradication Efforts



Inventory & Distribution





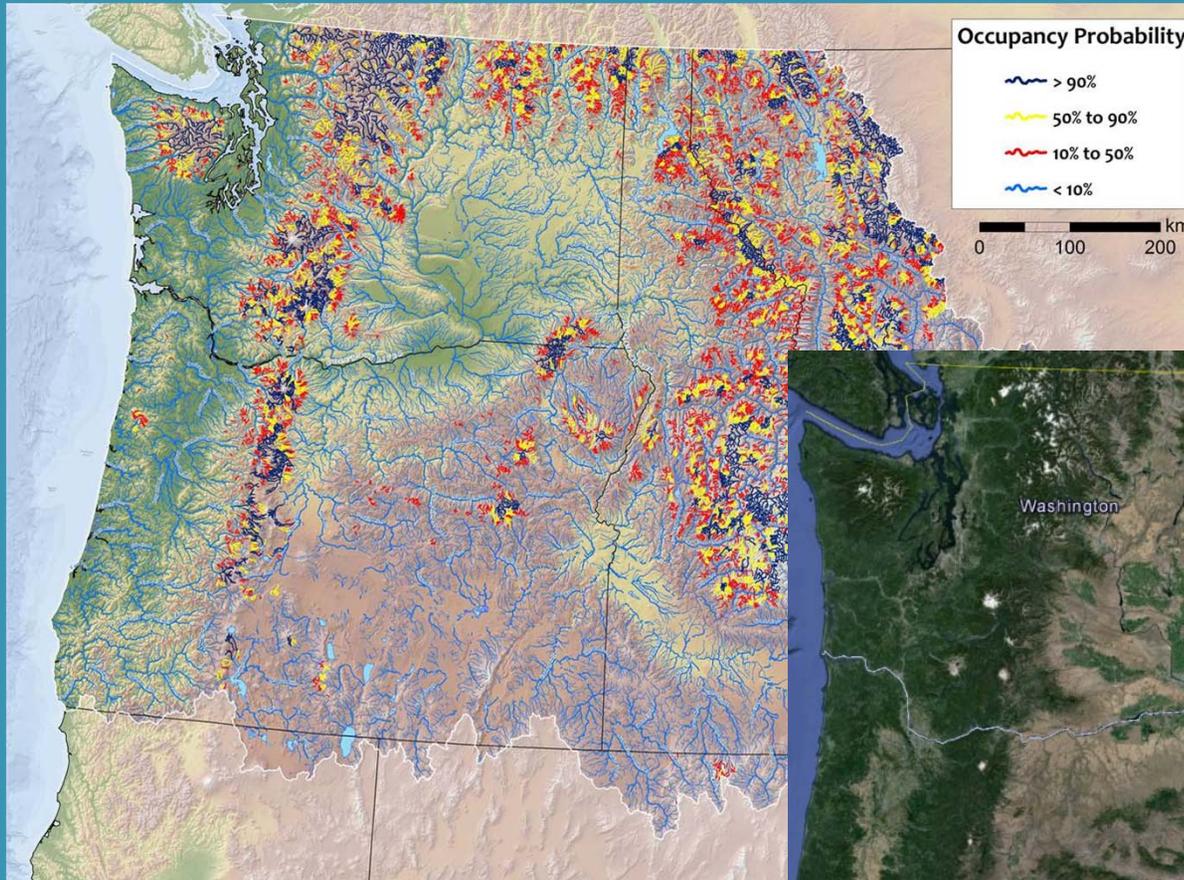
Jeremy Monroe, Freshwaters Illustrated

Three Lamprey eDNA Field Studies: What We Learned by Applying eDNA Methodology

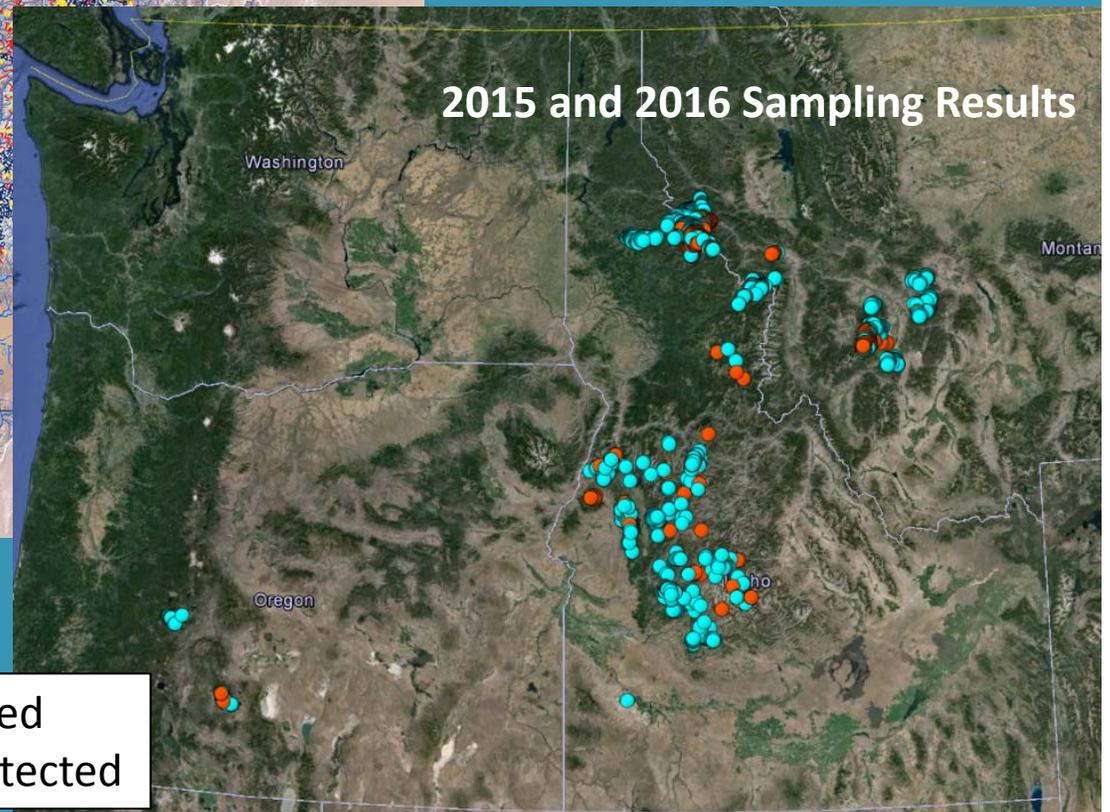
Carl Ostberg, USGS Western Fisheries Science Center

eDNA and Bull Trout Inventory

Collaborative effort with > 30 state, federal and tribal partners



Identify suitable habitat



National Genomics Center
FOR WILDLIFE AND FISH CONSERVATION

Lamprey Research at the National Genomics Center



National Genomics Center
— FOR WILDLIFE AND FISH CONSERVATION —

Development and application of
Lampetra and *Entosphenus* markers

- Presence
- Distribution
- Monitoring
- Recolonization

U.S. Fish and Wildlife Service

Using eDNA Sampling to Detect Pacific
Lamprey in a Large River:
2016 Wenatchee River Pilot Study



Ann B. Grote
U.S. Fish and Wildlife Service
Mid-Columbia Fish and Wildlife Conservation Office
Leavenworth, WA 98826

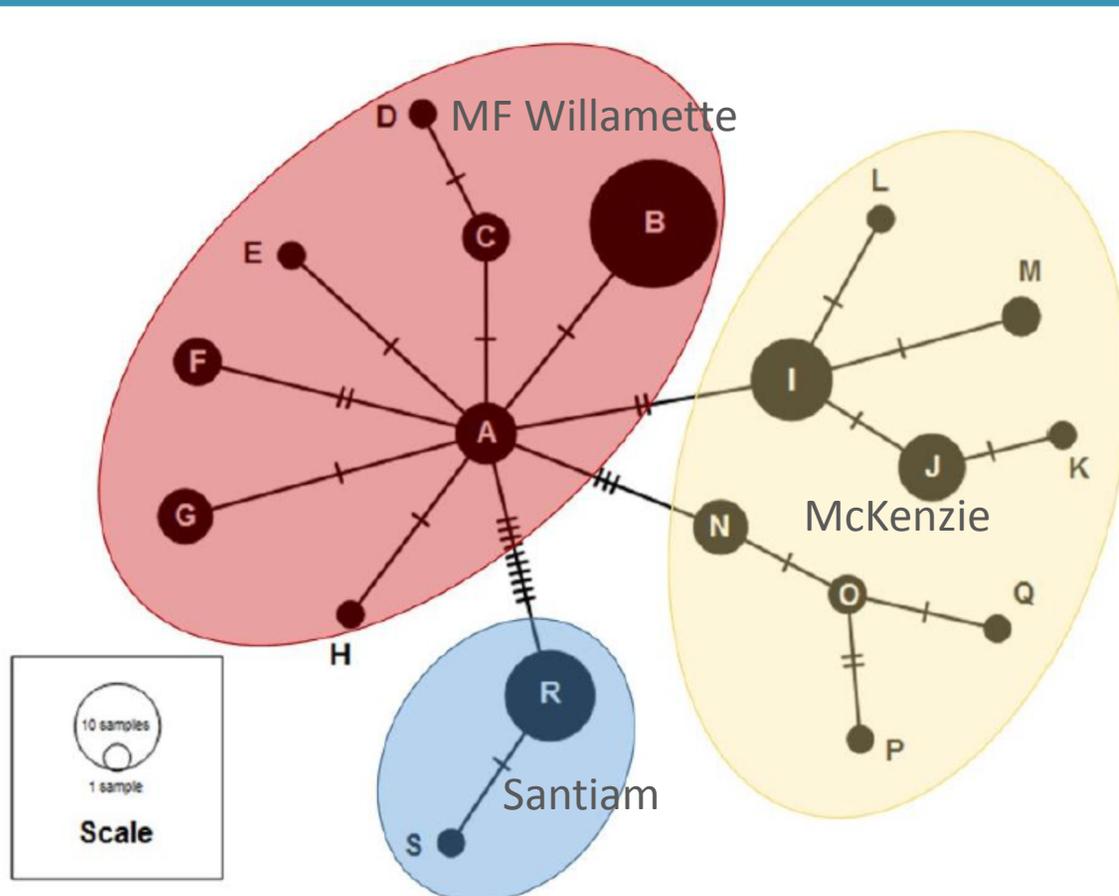
Kellie J. Carim
U.S.D.A. Forest Service
National Genomics Center for Wildlife and Fish Conservation
Missoula, MT 59801

Lamprey Research at the National Genomics Center



National Genomics Center
— FOR WILDLIFE AND FISH CONSERVATION —

Phylogenetic analysis to understand species relatedness, and landscape level diversity



Lampetra Haplotype Diversity in the Willamette River Basin

Lamprey Research at the National Genomics Center



Thank You to Our Colleagues and Collaborators

Nez Perce - Clearwater National Forest

Payette National Forest

Sawtooth National Forest

Willamette National Forest

U.S. Fish and Wildlife Service

Colville Tribe

CRITFC

Nez Perce Tribe

Shoshone Bannock Tribes

Umpqua Tribe

Yakama Nation

University of Manitoba

University of Montana

Questions?



National Genomics Center
— FOR WILDLIFE AND FISH CONSERVATION —

kelliejcarim@fs.fed.us

406-542-3252

www.fs.fed.us/research/genomics-center

eDNA for Monitoring Species Reintroduction Efforts



National Genomics Center
— FOR WILDLIFE AND FISH CONSERVATION —

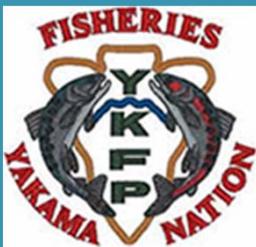


Photo: Jeremy Monroe

Reintroduction of Pacific Lamprey in the Wenatchee River

Reintroduction of Pacific Lamprey in the Wenatchee River



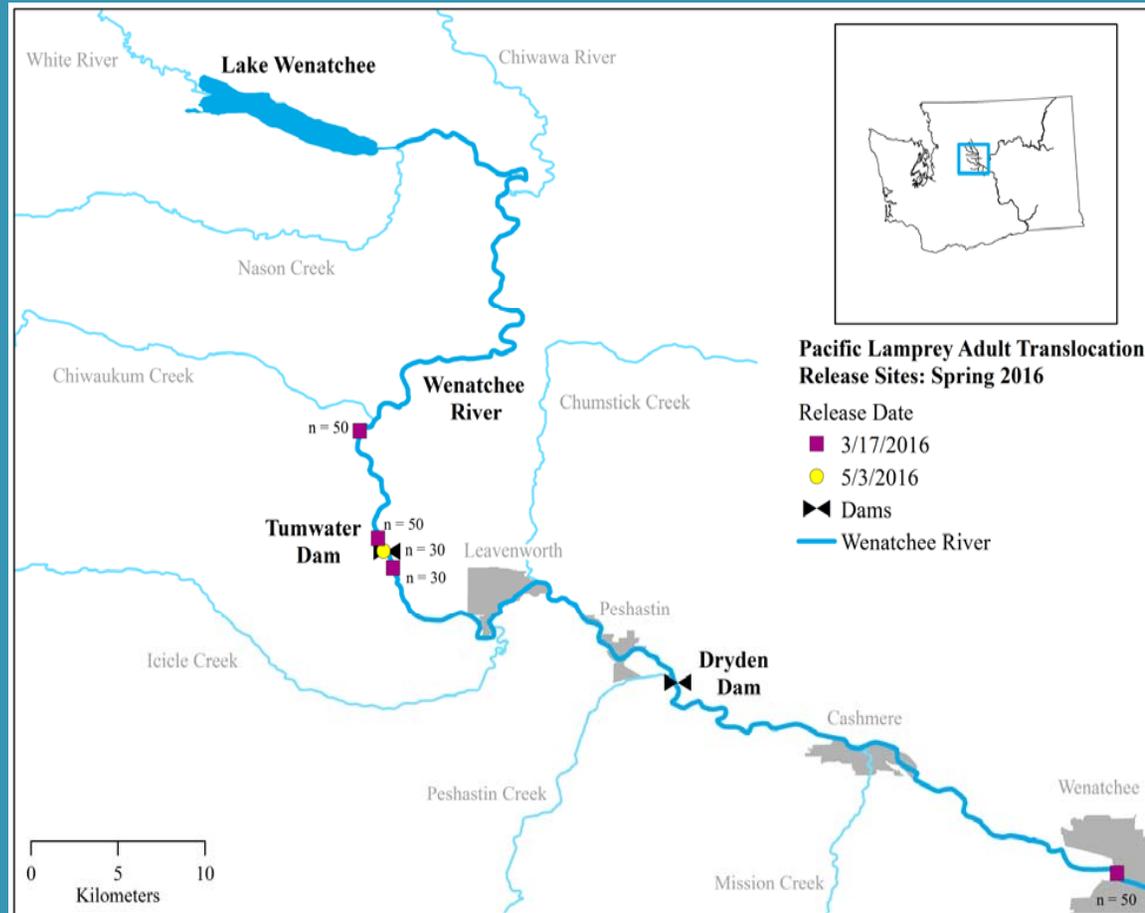
2009 electrofishing surveys by US Fish and Wildlife Service to determine distribution

No Pacific lamprey observed above Tumwater Dam

In 2016 Yakima Nation Fisheries Program began translocations

What can eDNA sampling tell us about fish movement post-translocation in a large river system?

Lamprey Reintroduction by the Numbers

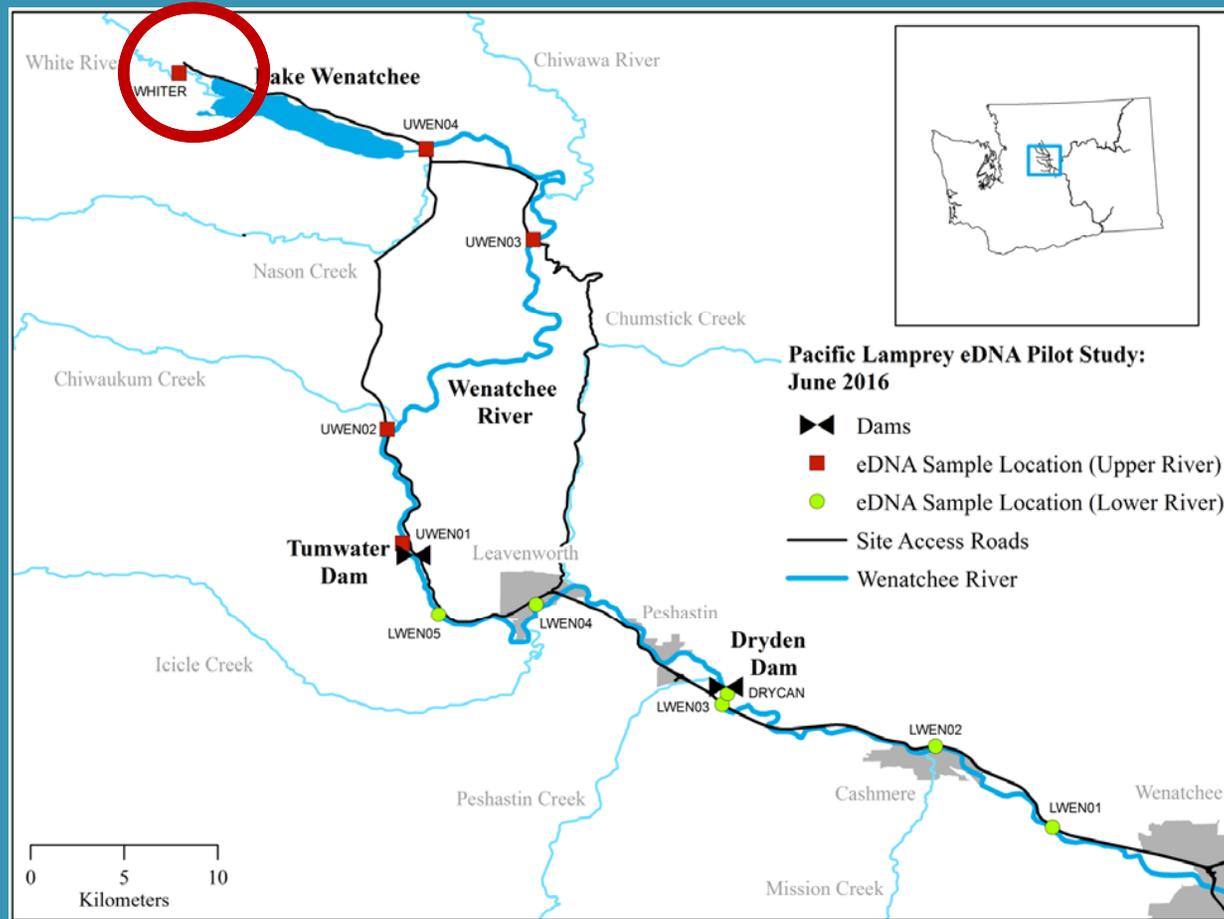


March 17th, 2016: 180 PIT tagged fish in “lower” and “upper” River

May 3rd, 2016 : 30 additional PIT tagged fish released in “upper” River

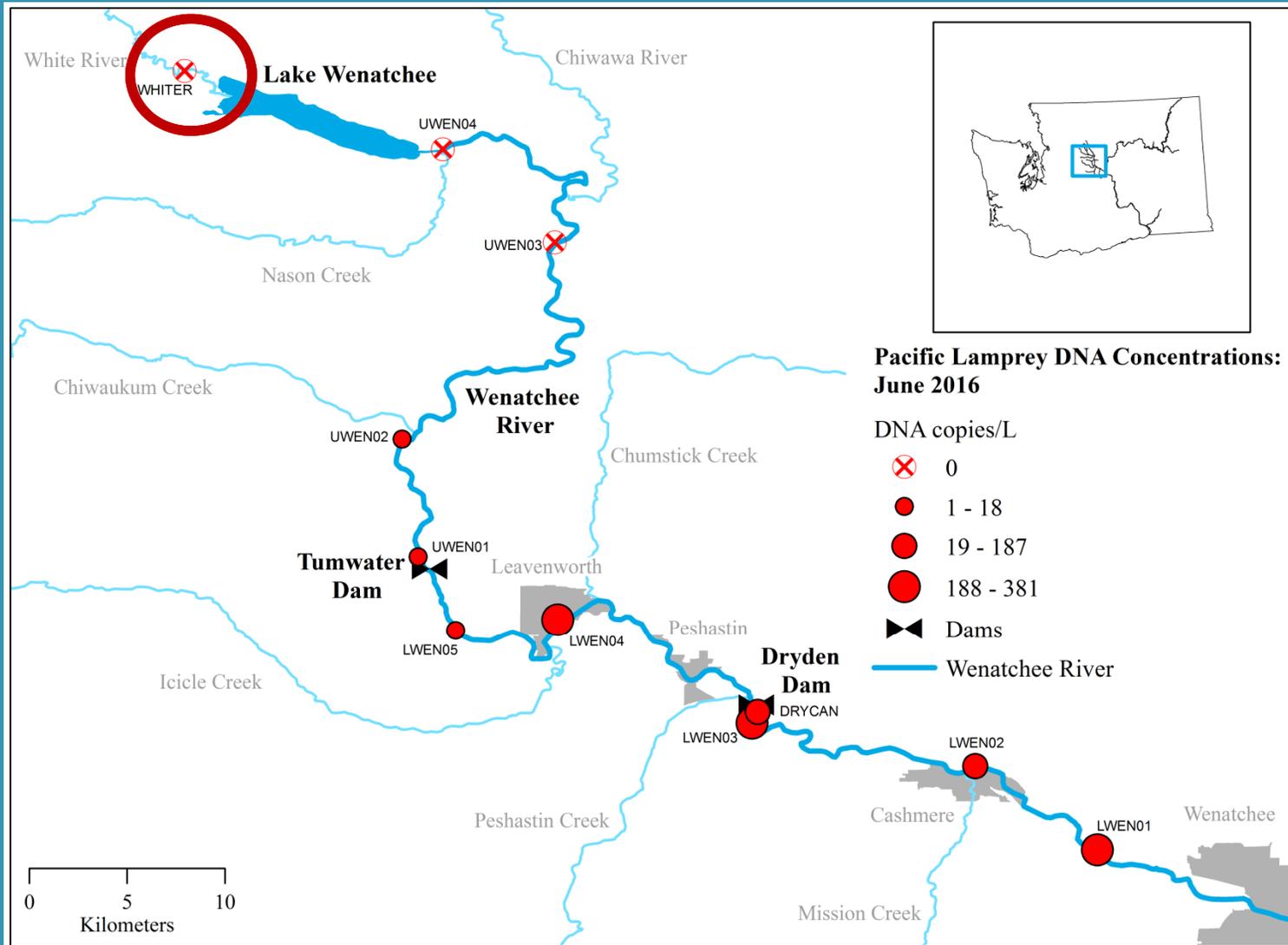
June 7th 2016 : Single PIT tagged adult detected at White River

eDNA Sampling Post-Reintroduction



Samples collected June 13th – 21st, 2016 (pre-spawning)
Locations based on ease of access (road crossings)
Analyzed for presence and quantity of Pacific lamprey DNA

Results: eDNA Lamprey Detections



Conclusions and Sampling Recommendations

Sampling was effective in a large river system

Provided a non-invasive method to monitor translocated fish

Sampling at sentinel sites through time could indicate timing of migration

Sampling at tighter spatial intervals identify upper extent of occupied habitat

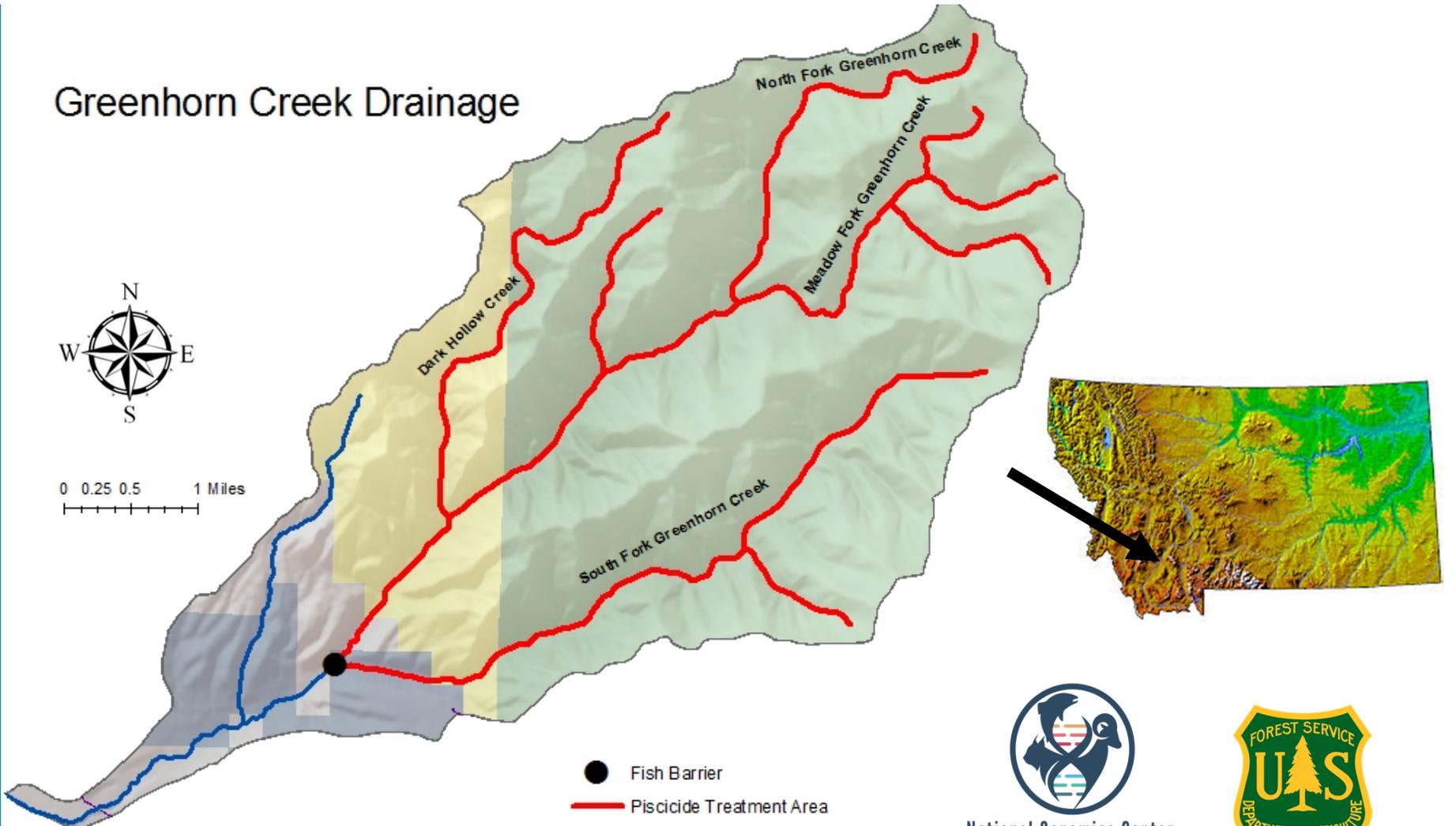


eDNA for Evaluating Eradication Efforts



A case study in Greenhorn Creek, MT

Greenhorn Creek Drainage



- Fish Barrier
- Piscicide Treatment Area
- ~ Stream
- Roads
- Drainage

- Public Land Ownership**
- OWNER**
- Montana State Lands
 - US Bureau of Land Management
 - US Forest Service

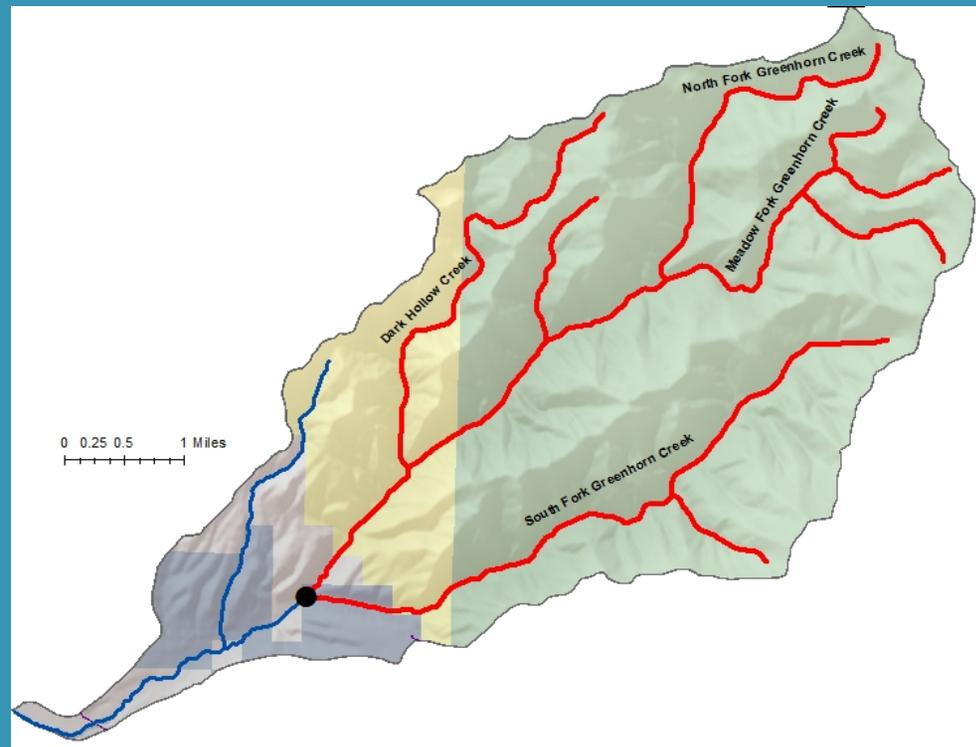


Background on Upper Greenhorn Basin

Treated with rotenone in 2013 and 2014

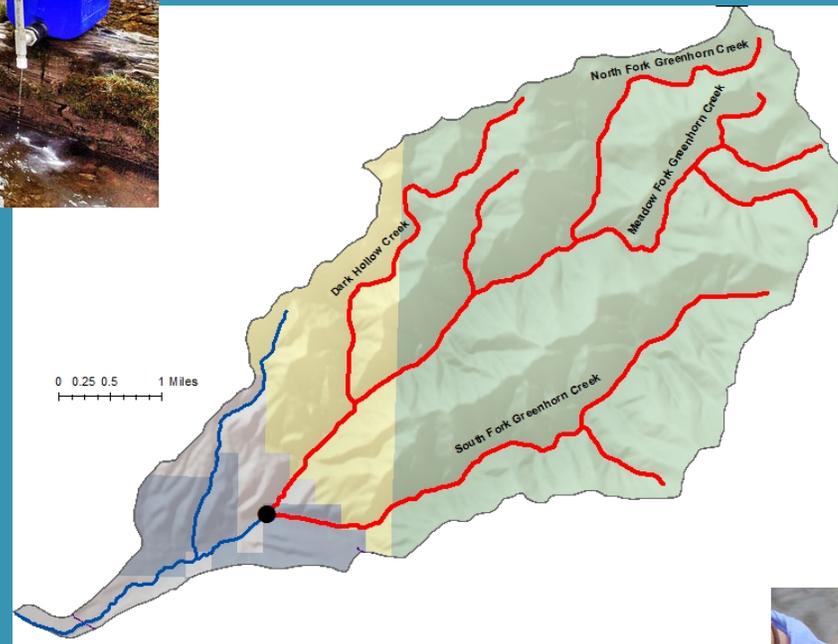
Targets: brook trout and rainbow- cutthroat hybrids

Pure cutthroat present in Dark Hollow
(Upper 1.4 miles not treated)



Intensive electrofishing planned for July & August 2015

**Piscicide treatments are expensive;
time and labor intensive**



**Can eDNA sampling save time and money
through more effective evaluations?**



Methods for Evaluating Piscicide Treatment in the Greenhorn Basin, MT



Sampled entire treated area July 12th- 15th 2015

Collected eDNA samples at 250m intervals, 122 samples total

Analyzed all for brook trout, westslope (excluded Dark Hollow)

Continuously electrofished entire basin following eDNA sampling

Results: Fish Detections Post-Treatment

Electrofishing recovered two fish (one of each target)

Westslope Cutthroat Trout

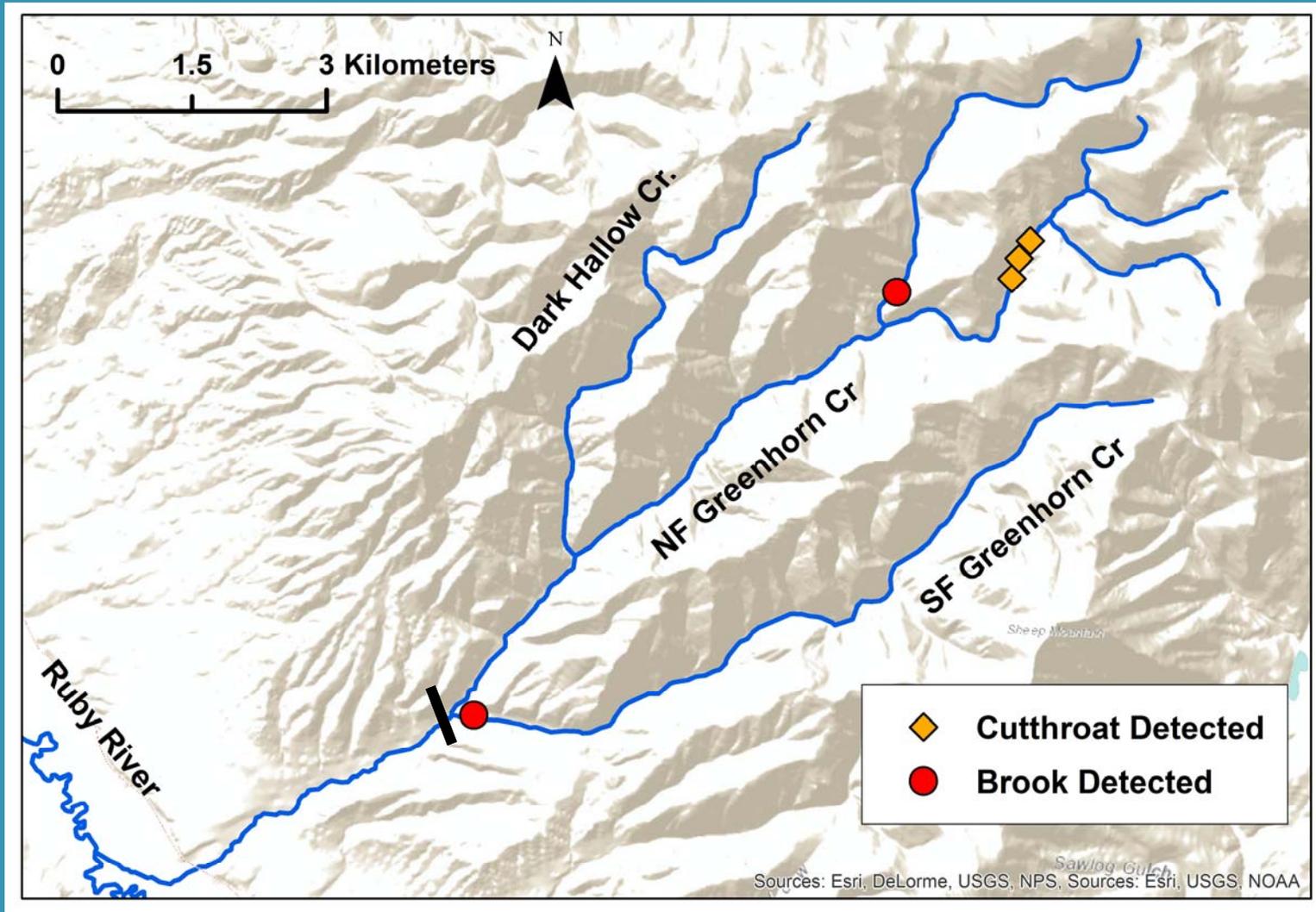


Brook Trout

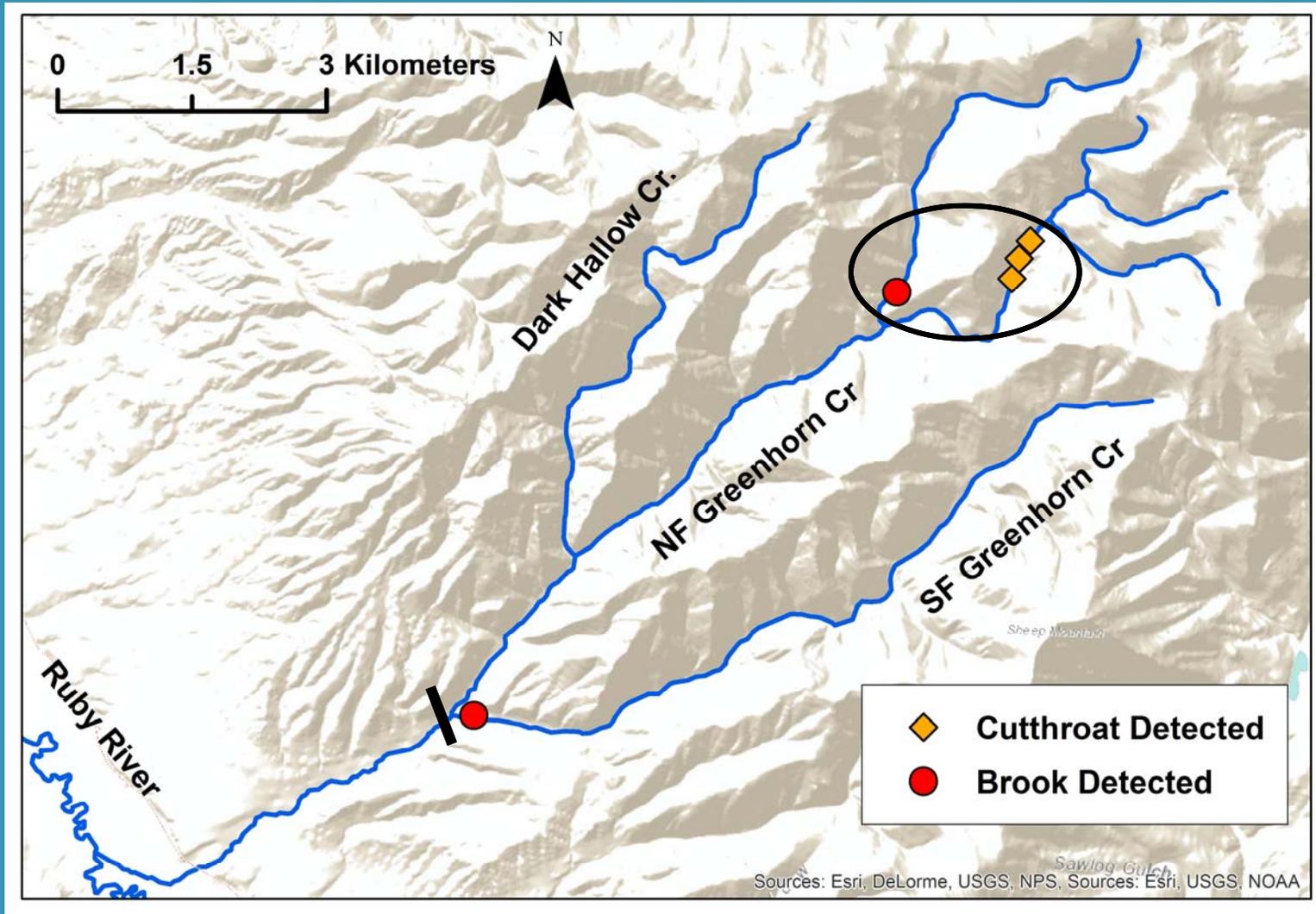


eDNA samples were run blind; detected both species
in multiple locations

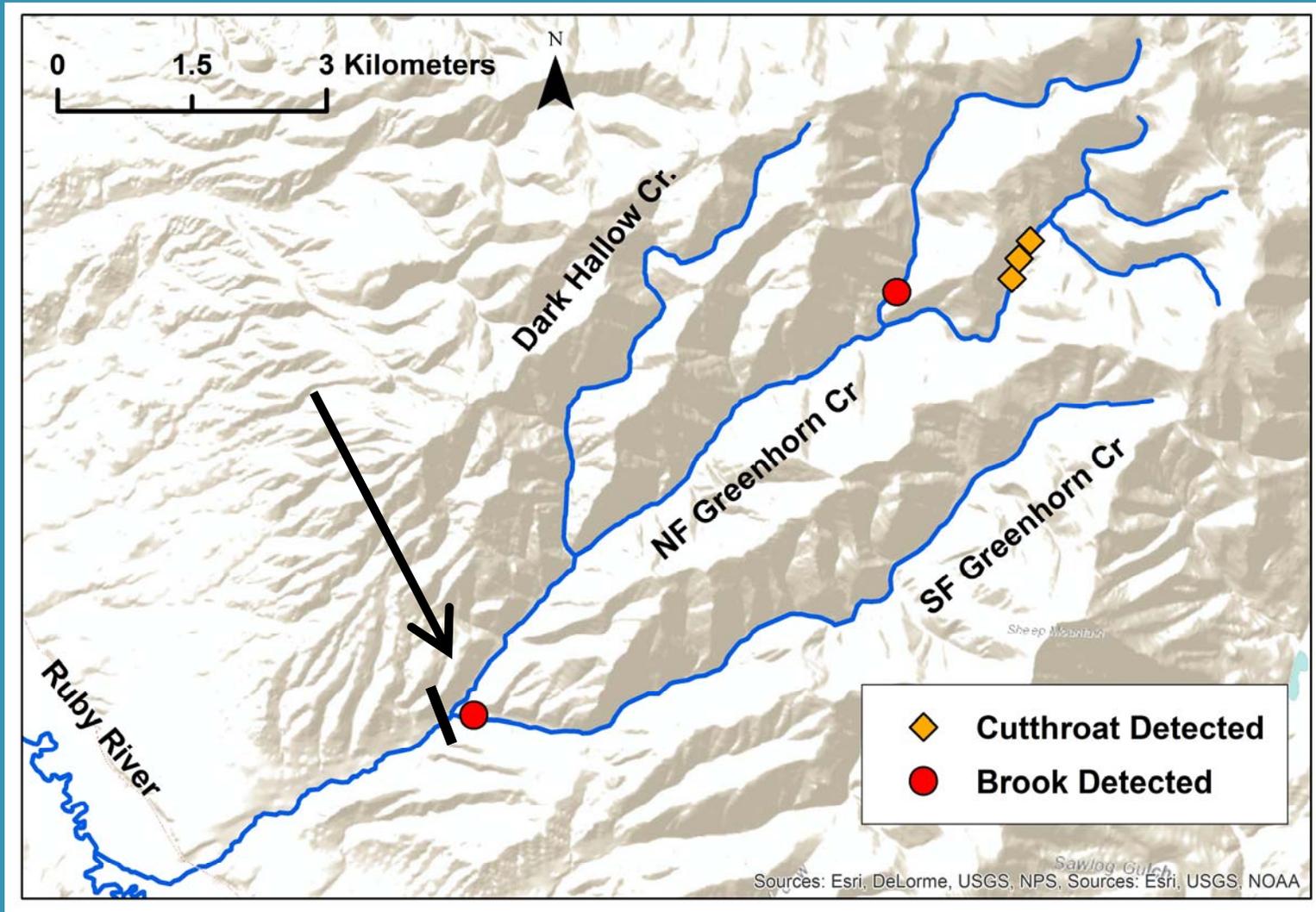
Locations of eDNA Detections



North Fork and Meadow Fork detections consistent with electrofishing



South Fork detection had a low level of DNA, no fish recovered



Validation of Results



Google Earth image showing barrier in Greenhorn Creek

Conclusions and Sampling Recommendations

eDNA sampling is highly efficient and sensitive for assessing eradication efforts

eDNA is highly sensitive to contamination

Unexpected results must be interpreted in context

Additional sampling should be used to validate results

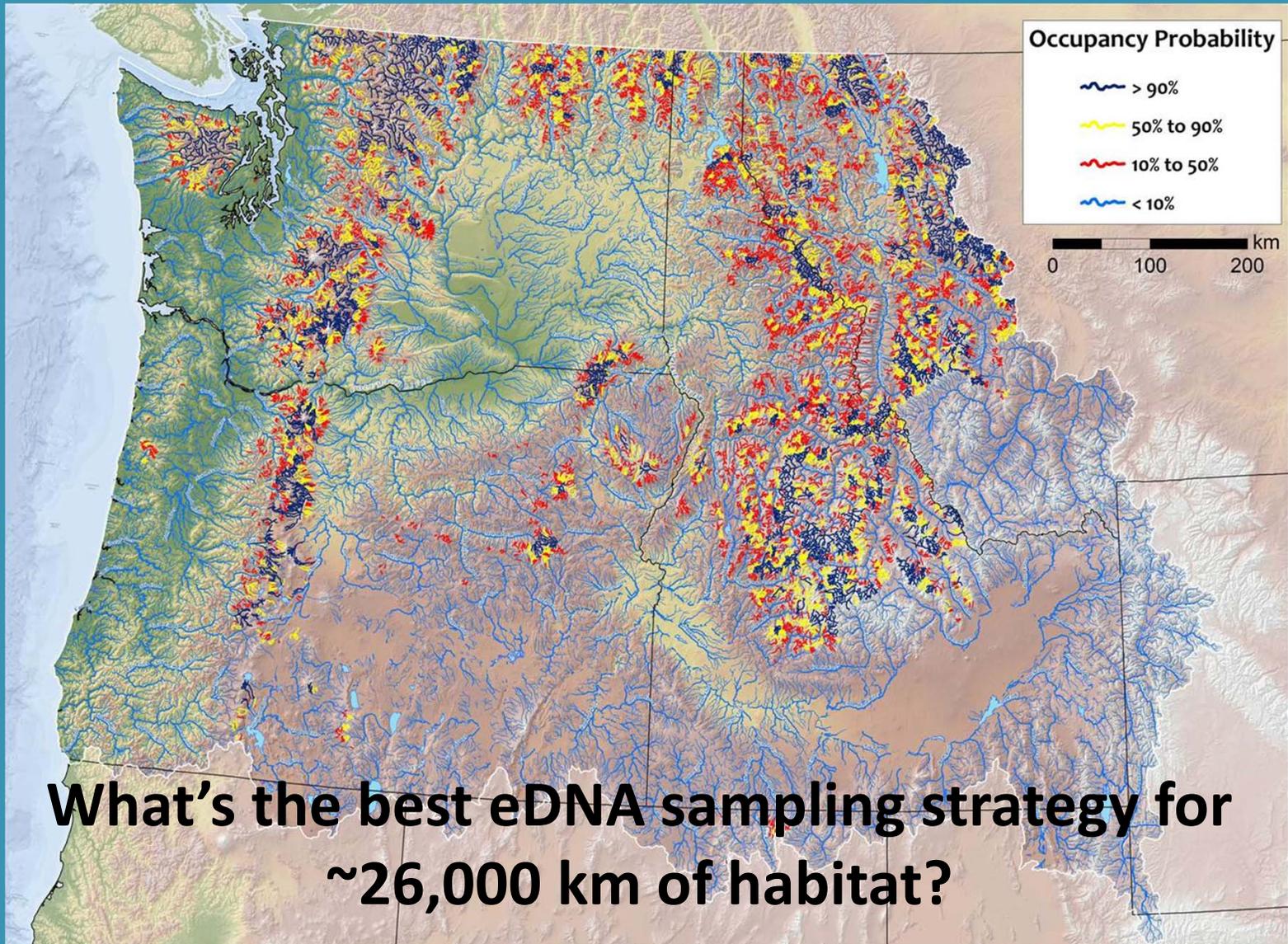


eDNA for Inventory and Distribution



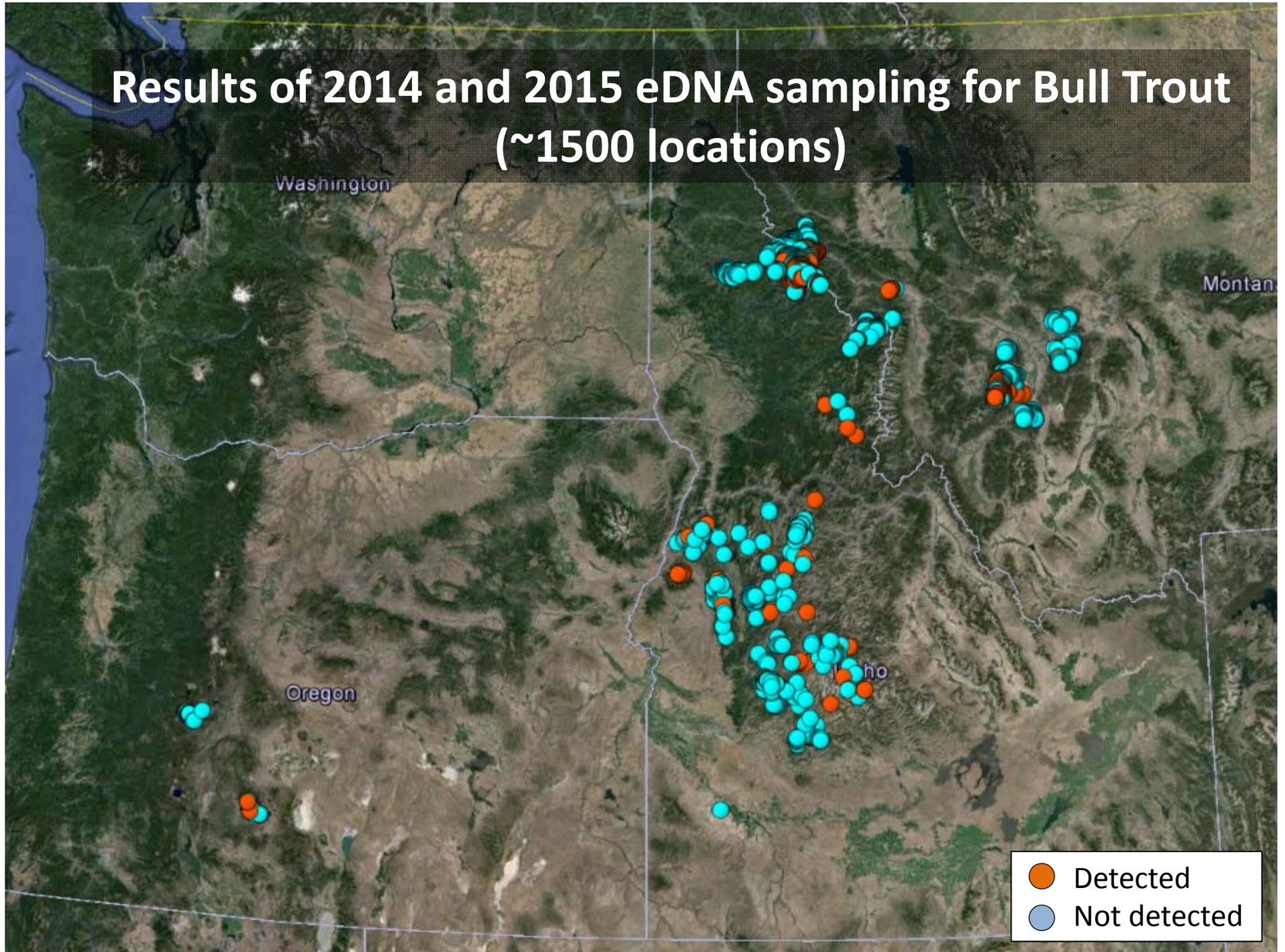
**Assessing Bull Trout Occupancy in the
Columbia River Basin**

Climate Shields Model: Identifying Suitable Bull Trout Habitat



**What's the best eDNA sampling strategy for
~26,000 km of habitat?**

Results of 2014 and 2015 eDNA sampling for Bull Trout (~1500 locations)

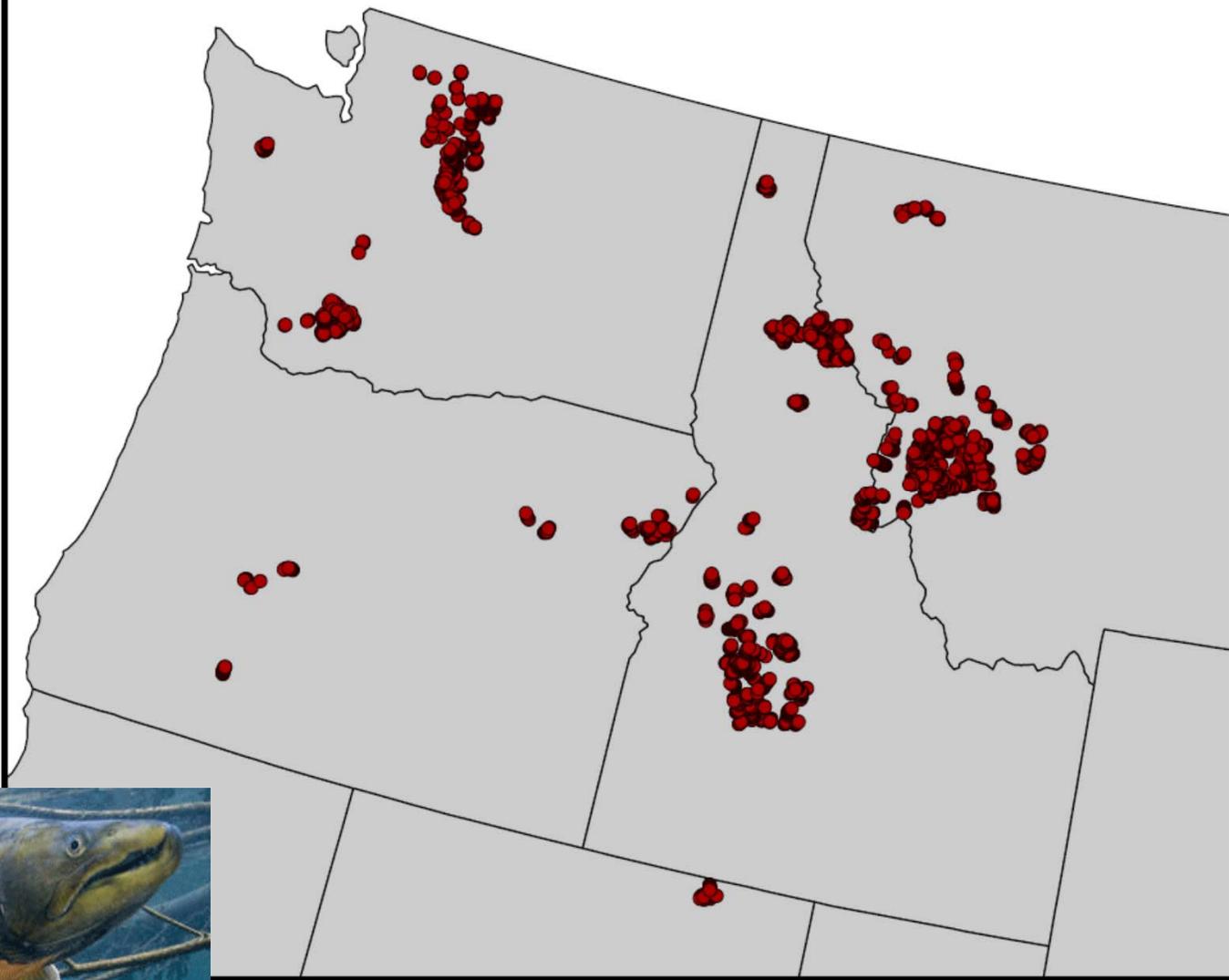


Bull Trout Inventory in the Columbia River Basin

Federal, State, Tribal and Non-profit Collaborative Effort



14 National Forests
3 USFS Regions



**> 3,500 locations sampled
since 2014**

Reintroduction Efforts



Eradication Efforts

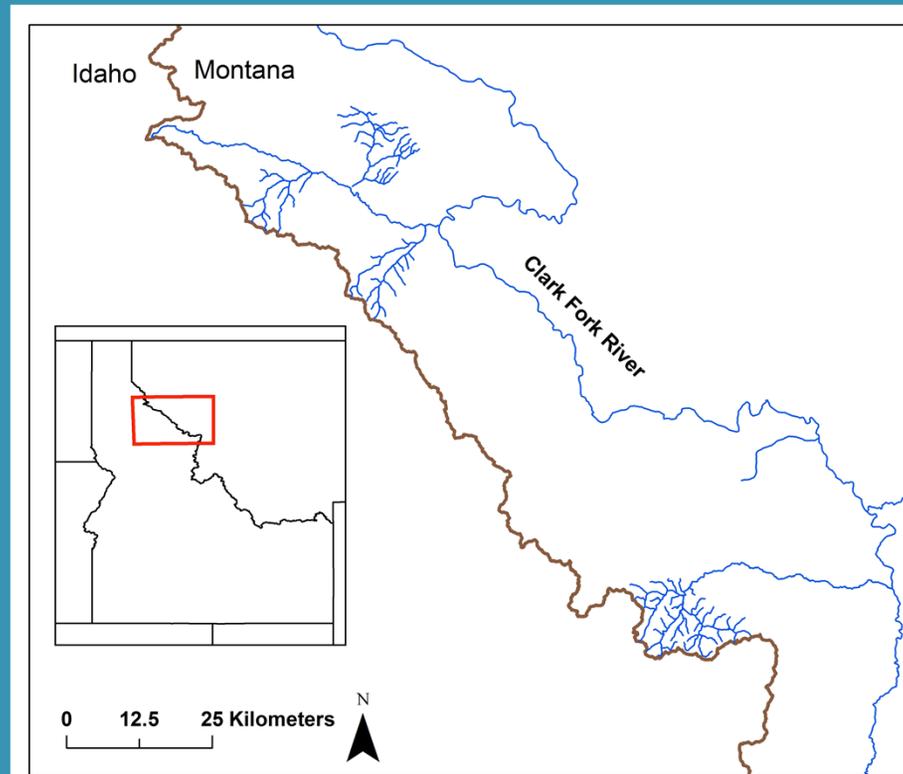


Inventory & Distribution



Spread and emergence of invasive species
Community composition
Monitoring seasonal movements
And many more...

Pilot Study: eDNA vs. Electrofishing for Detection of Bull Trout Populations



5 drainages in southwestern Montana
76 eDNA samples with 1.5 km spacing
47 sites with paired electrofishing data (1999-2014)

Results: eDNA vs. Electrofishing

McKelvey et al. 2016

eDNA

Electrofishing

Absent

Present

Absent

24

Present

Total= 47 sites with paired data

Results: eDNA vs. Electrofishing

McKelvey et al. 2016

eDNA

Electrofishing

	Absent	Present
Absent	24	
Present	0	

Total= 47 sites with paired data

Results: eDNA vs. Electrofishing

McKelvey et al. 2016

eDNA

Electrofishing

	Absent	Present
Absent	24	
Present	0	16

Total= 47 sites with paired data

Results: eDNA vs. Electrofishing

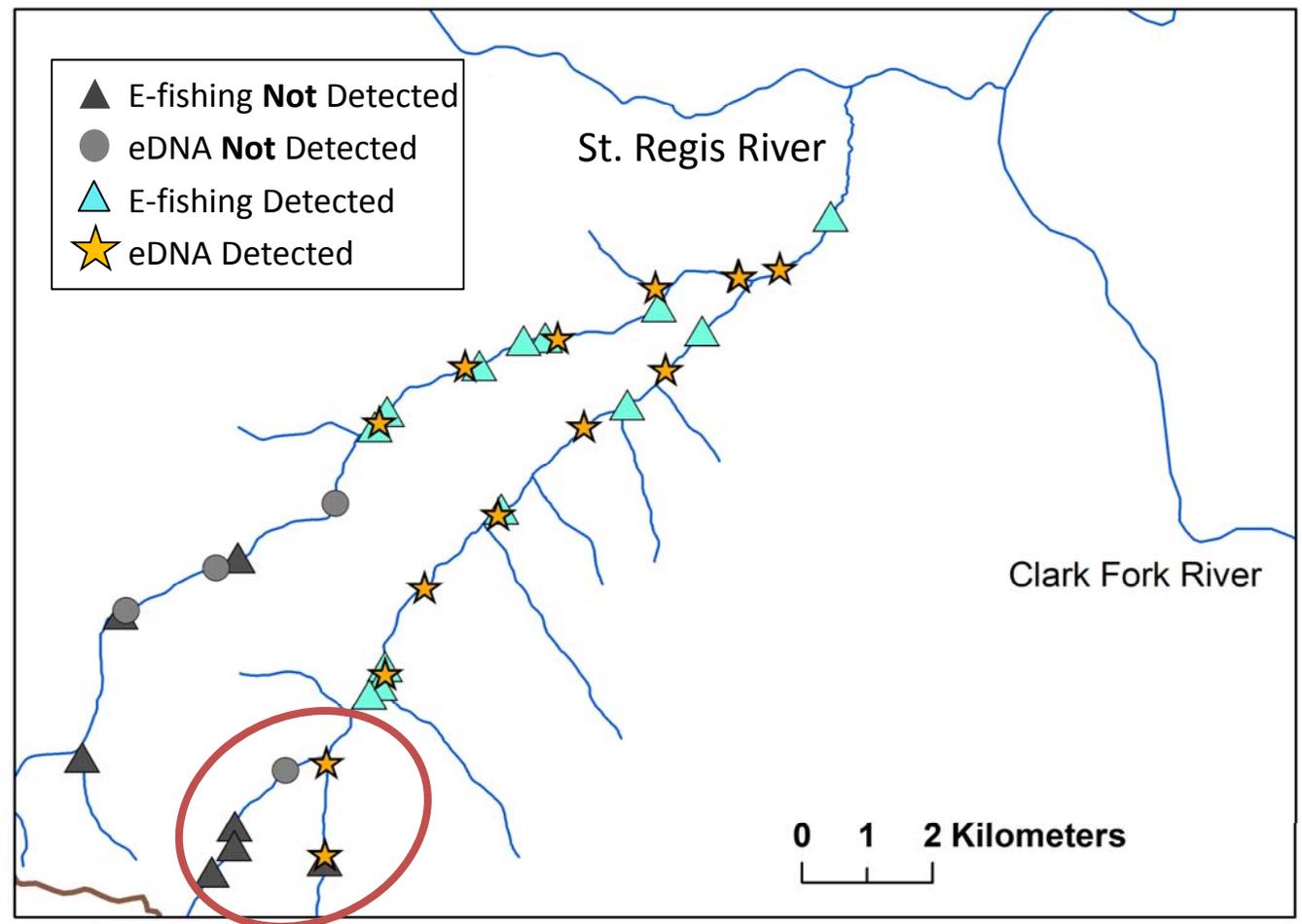
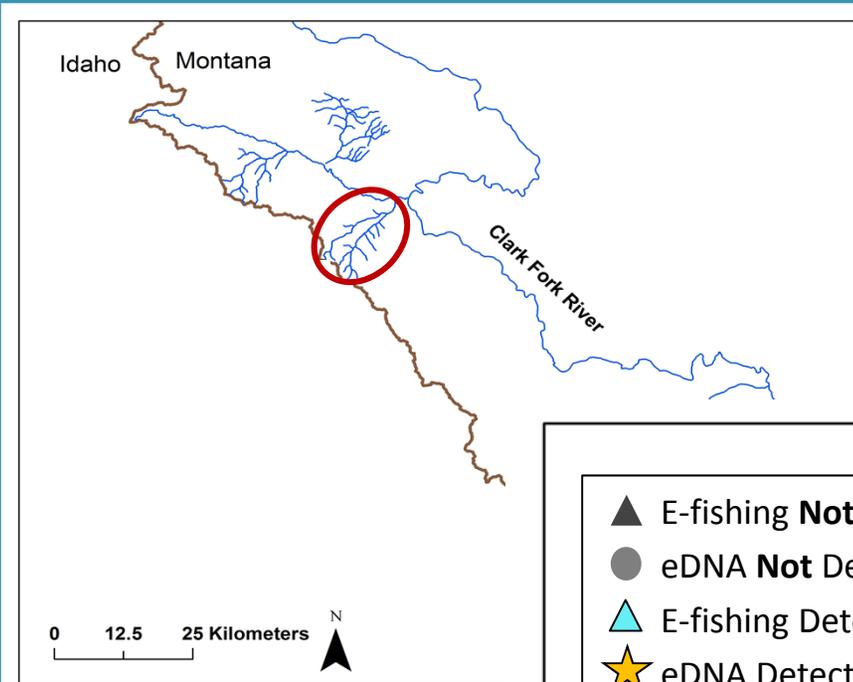
McKelvey et al. 2016

		eDNA	
		Absent	Present
Electrofishing	Absent	24	7
	Present	0	16

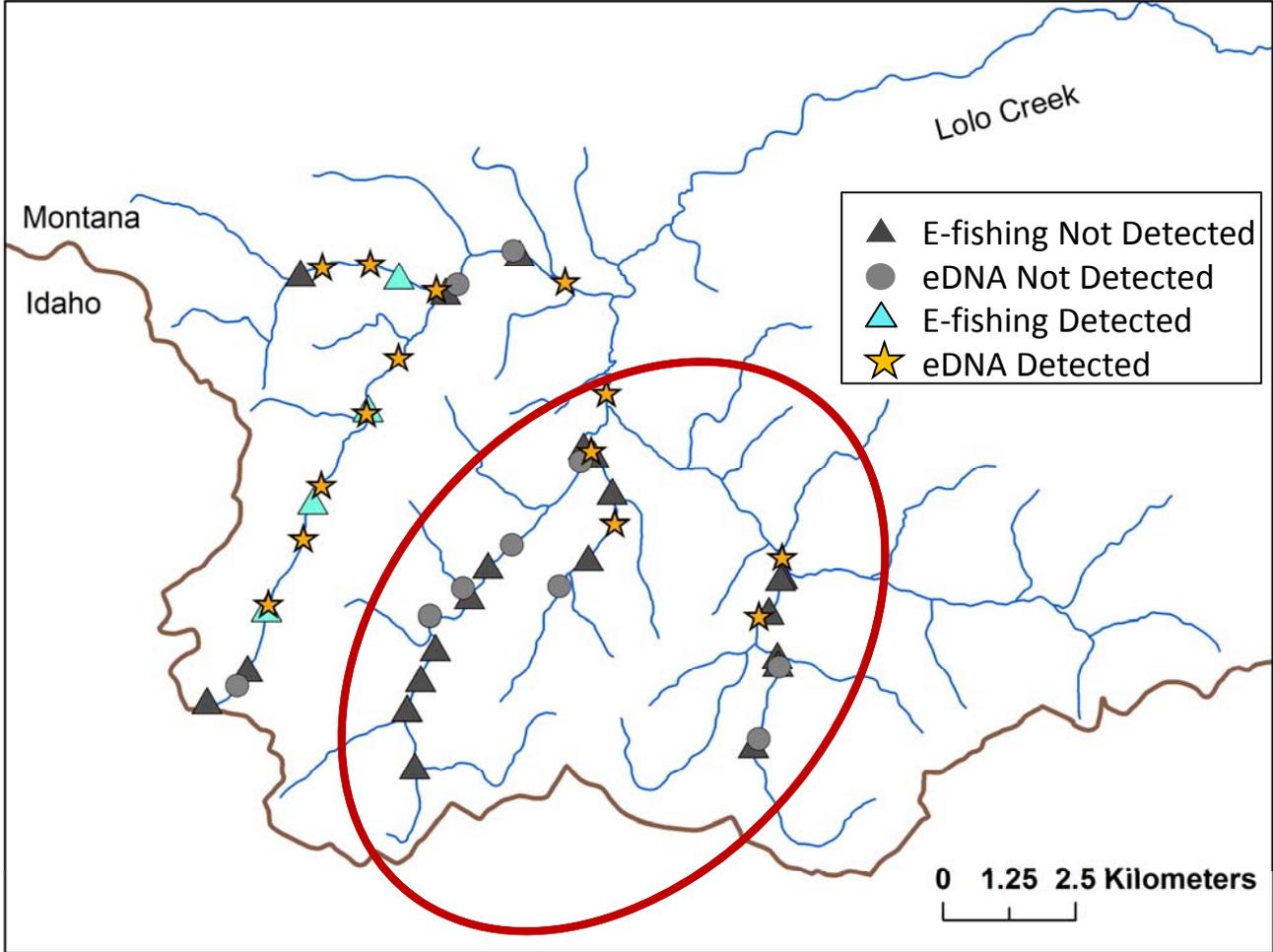
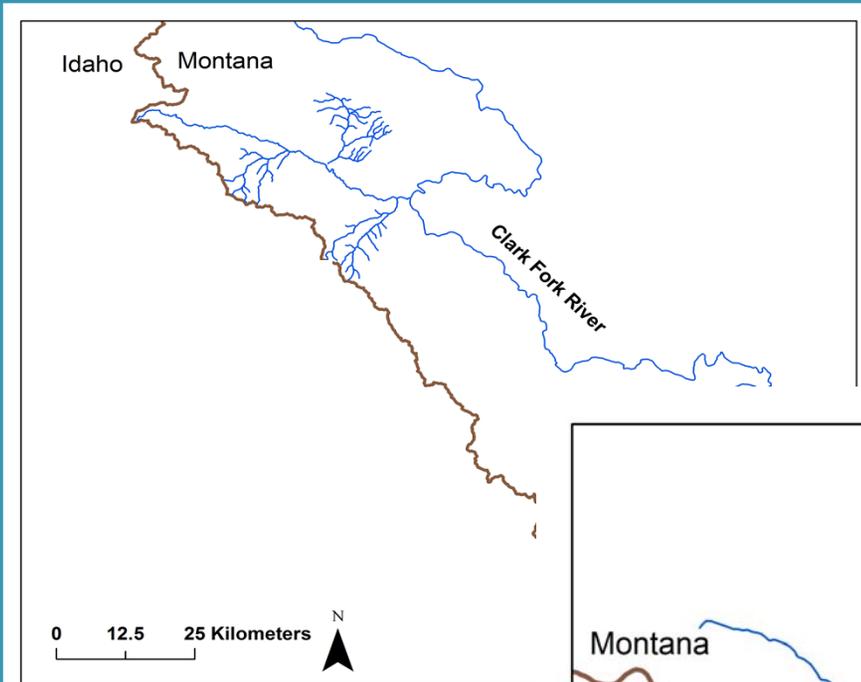
Total= 47 sites with paired data

Little Joe Drainage

Upper extent of occupied habitat



Lolo Creek Basin: Discovery of unknown populations



McKelvey et al. 2016

Conclusions and Sampling Recommendations

Faster and more sensitive than electrofishing

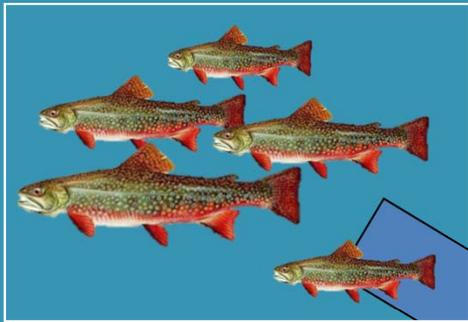
1km intervals good for population level detection

Sampling at fixed intervals helps delineate length of occupied habitat



How close do you need to be to an animal to detect it?

In headwater systems, 100 g of brook trout were consistently detected 240m downstream



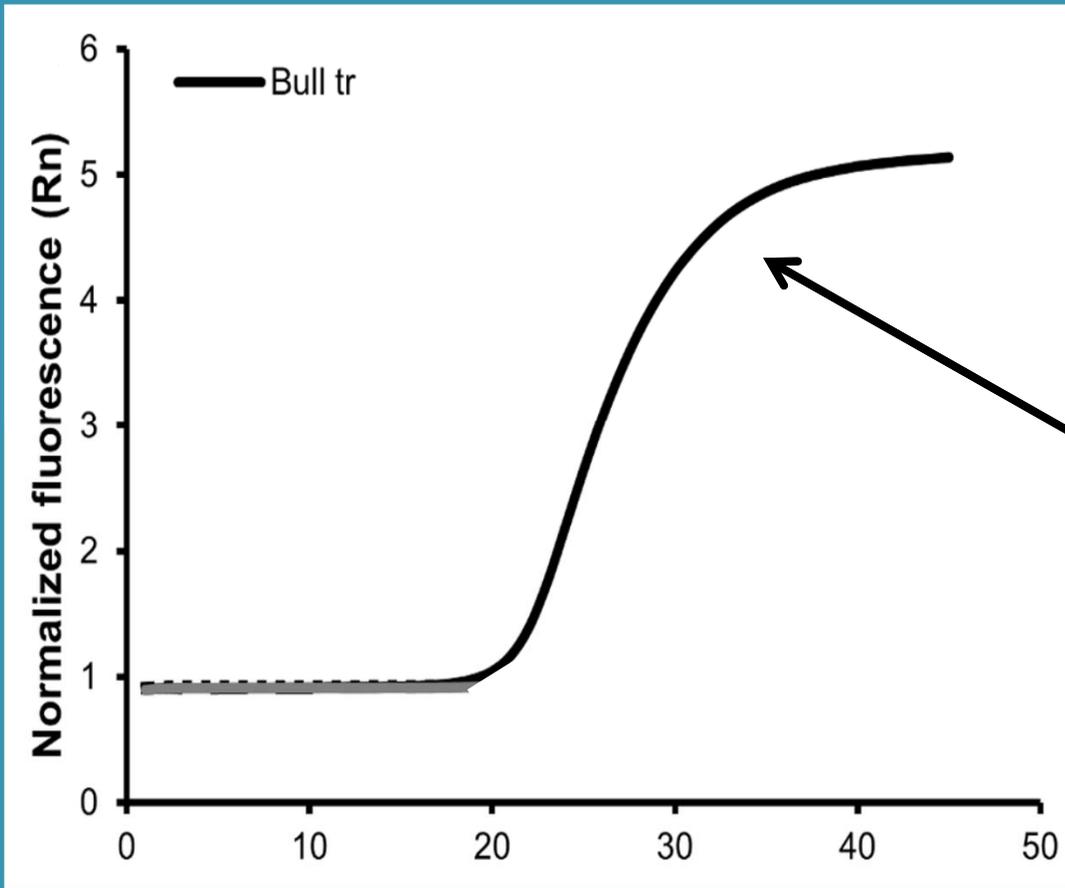
240 meters

DNA



Jane et al. 2015

Specificity of eDNA markers - detecting ONLY the species of interest

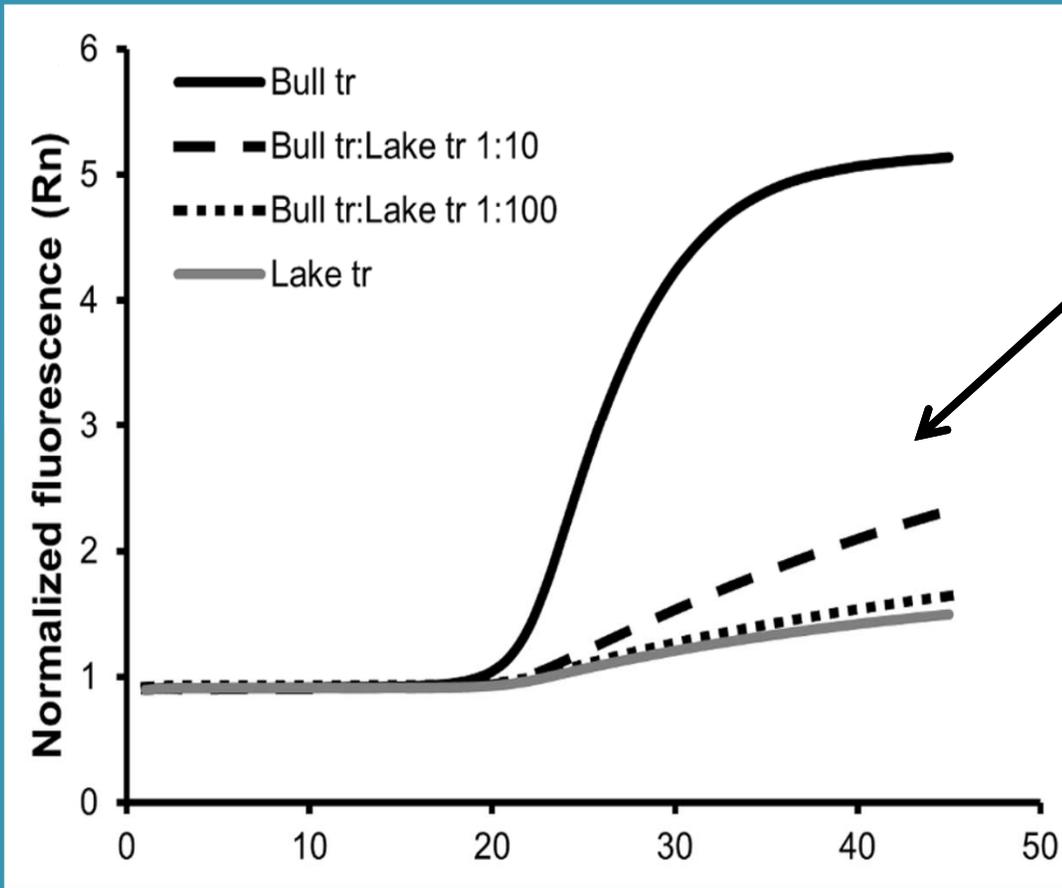


Detection of bull trout DNA with the bull trout marker



Bull Trout
(*Salvelinus confluentus*)

Specificity of eDNA markers- detecting ONLY the species of interest



The lake trout DNA competes for the marker, reducing detection of bull trout

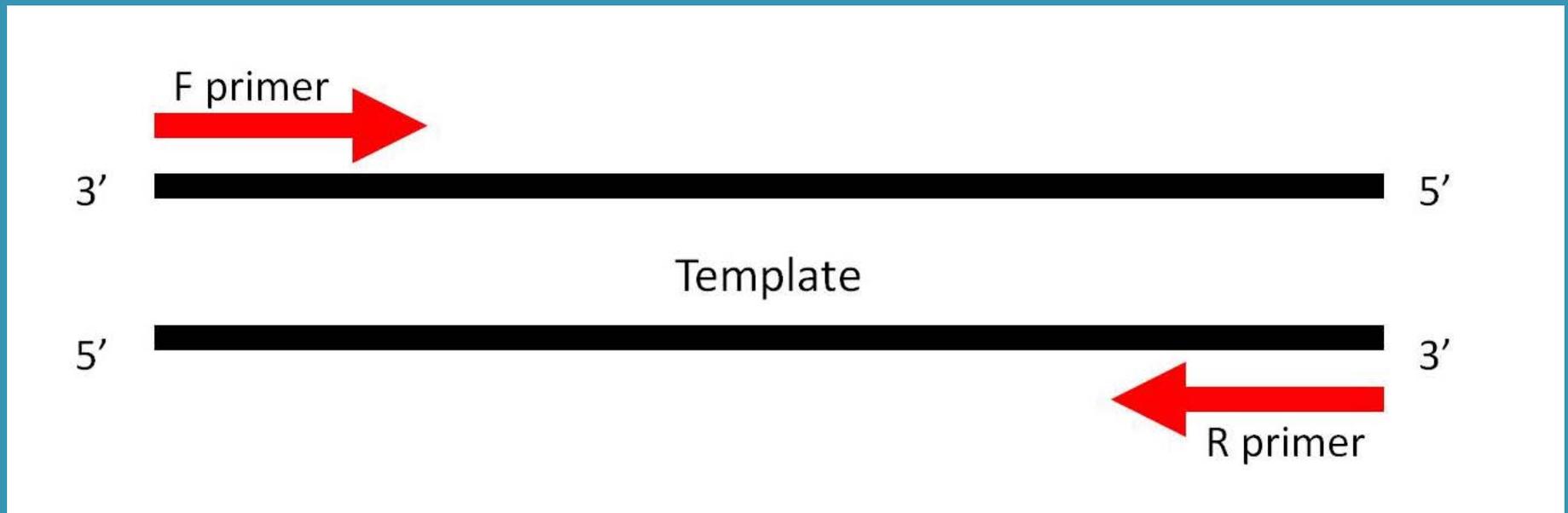


Bull Trout
(*Salvelinus confluentus*)



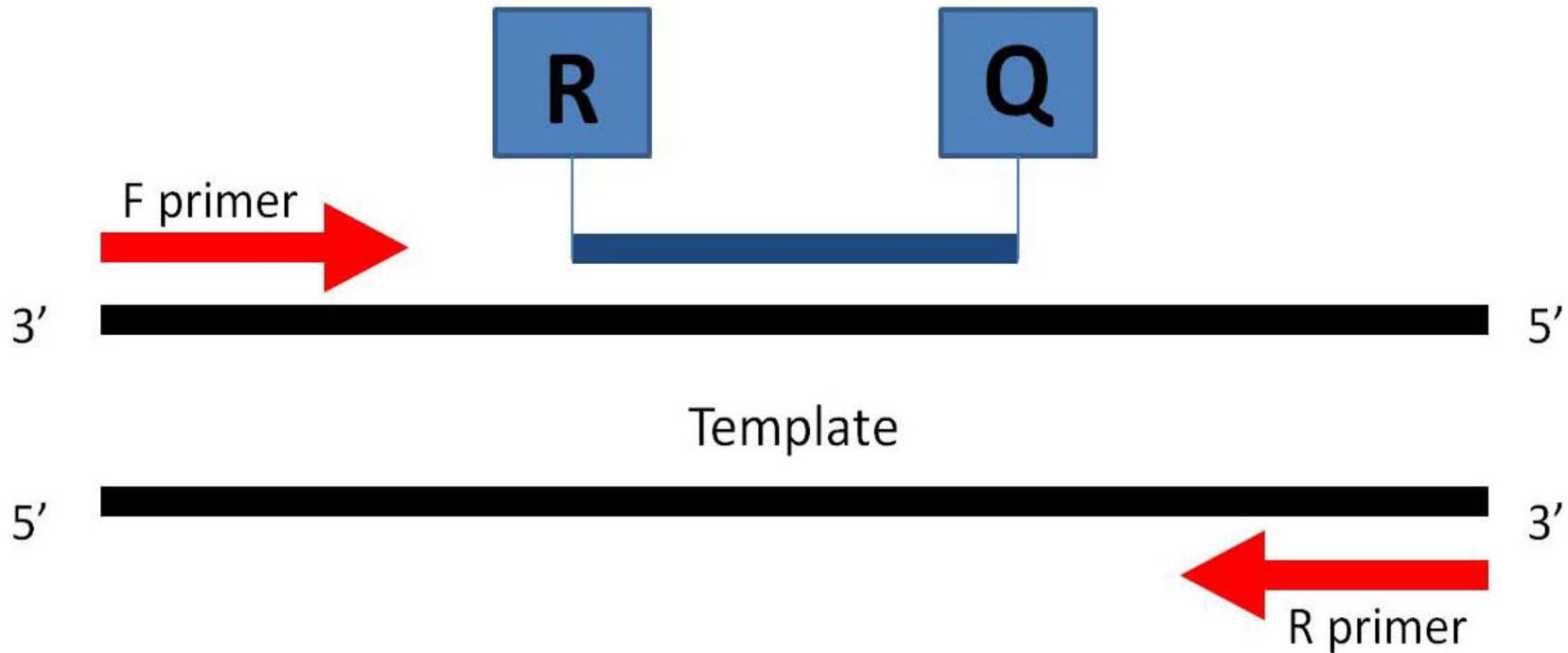
Lake Trout
(*Salvelinus namaycush*)

Traditional PCR



Quantitative PCR

Probe consists of a reporter and a quencher



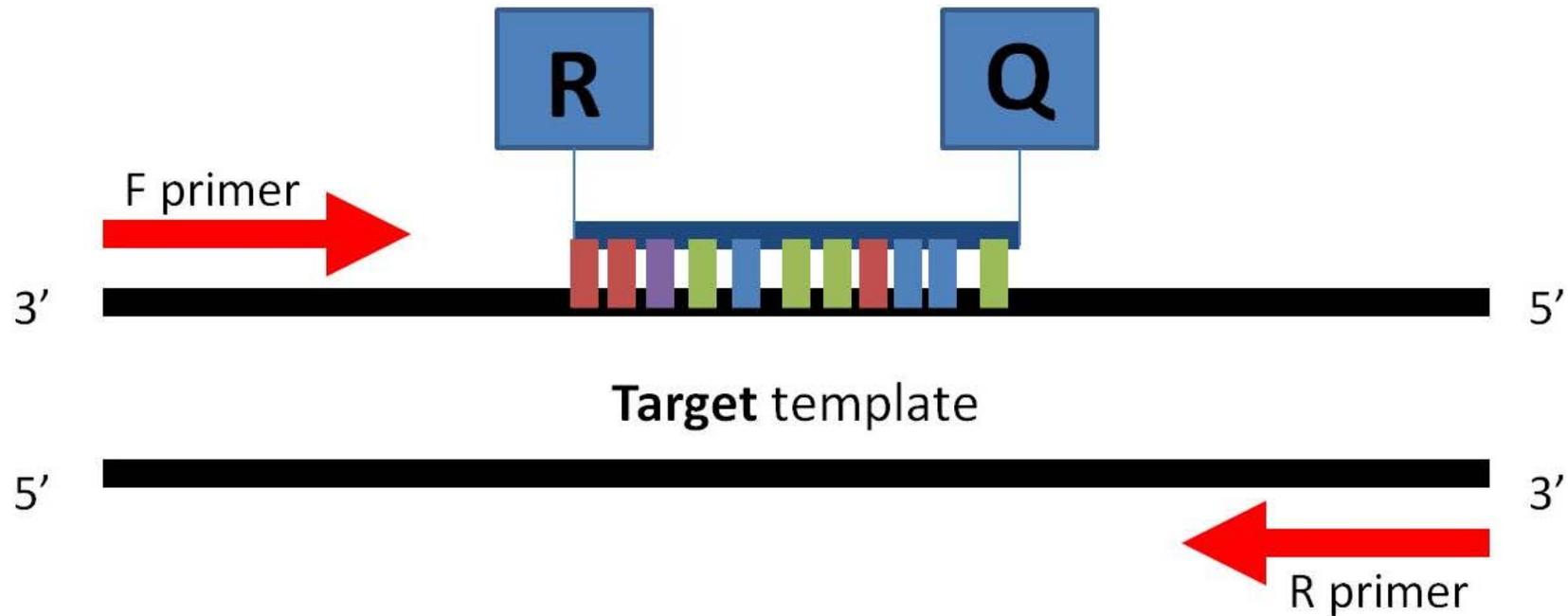
When the probe is intact, there is no fluorescence

Probe is a perfect match to target template

Bull Trout
Salvelinus confluentus



ATTCCTGC

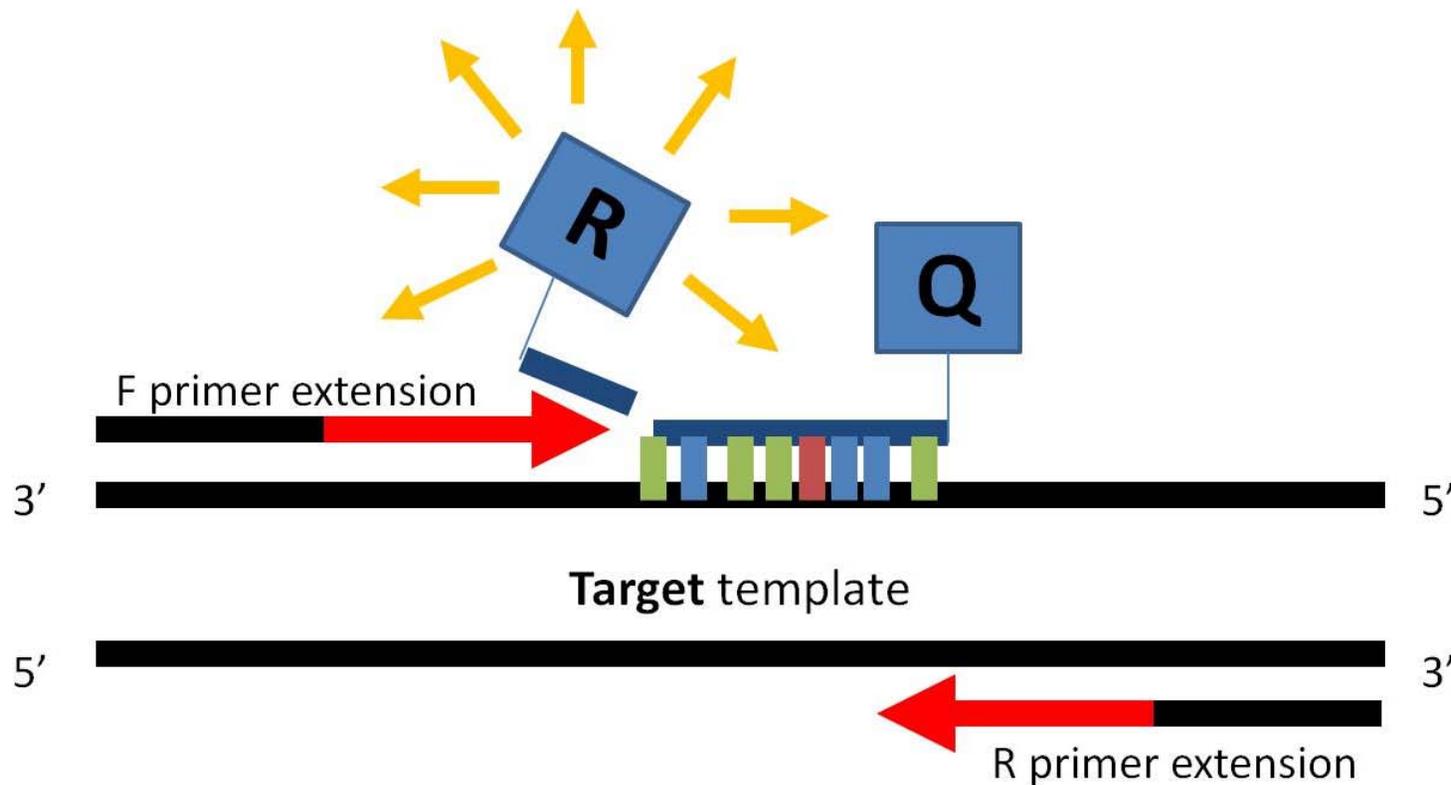


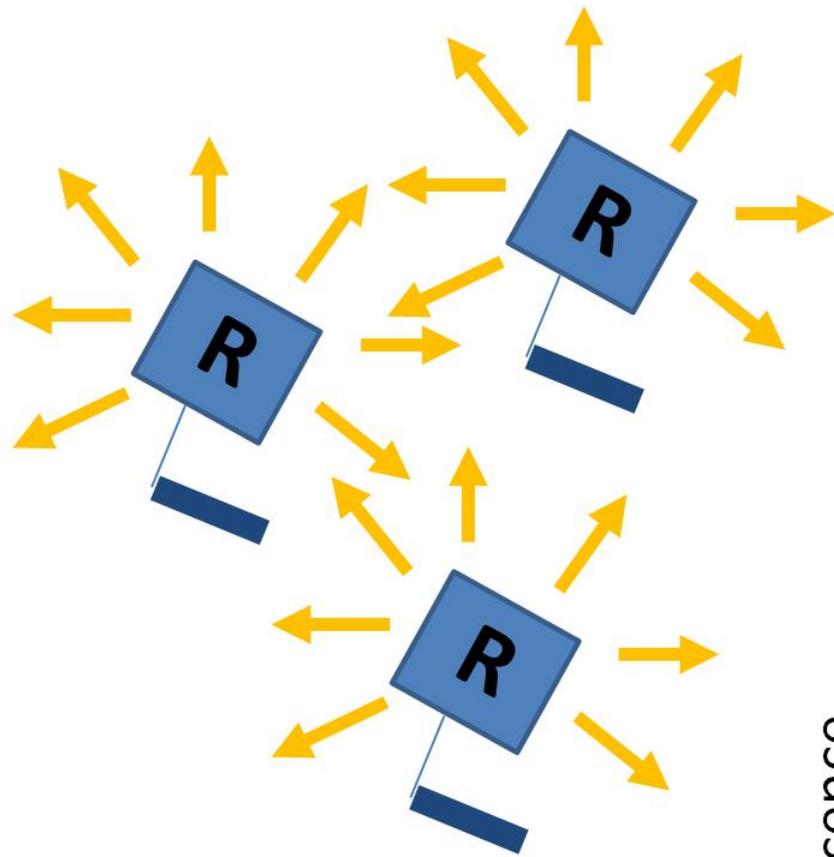
Probe is broken with primer extension and the “reporter” fluoresces

Bull Trout
Salvelinus confluentus

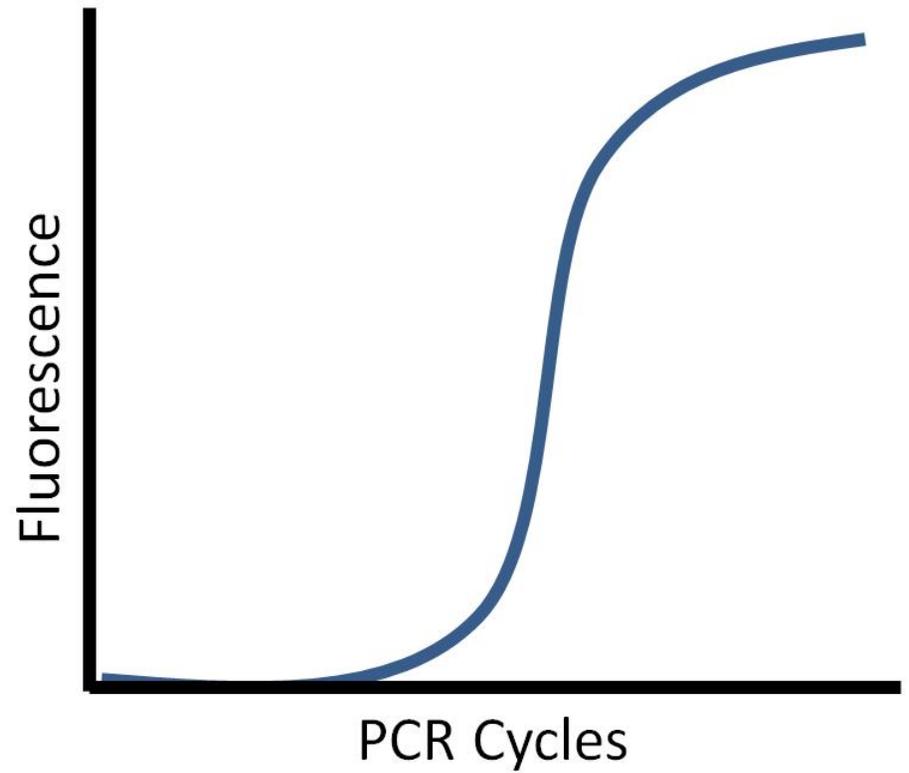


ATTCCTGC





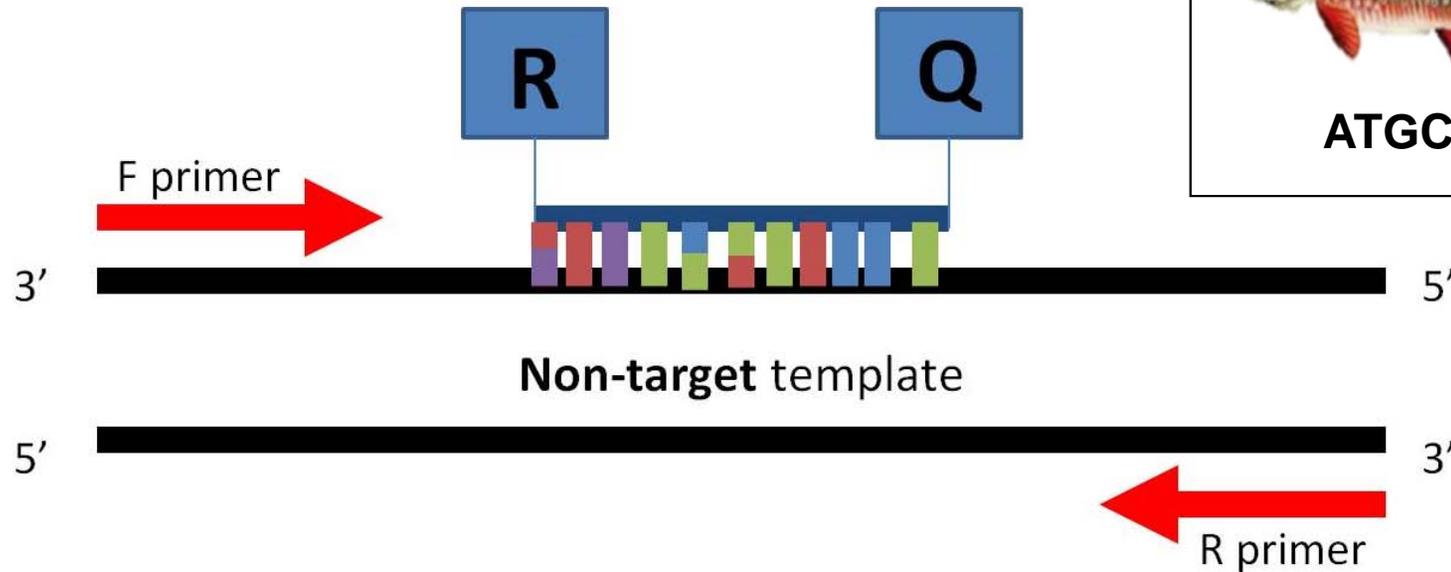
As PCR products increase, so does fluorescence



Brook Trout
Salvelinus fontinalis



ATGCGTGC



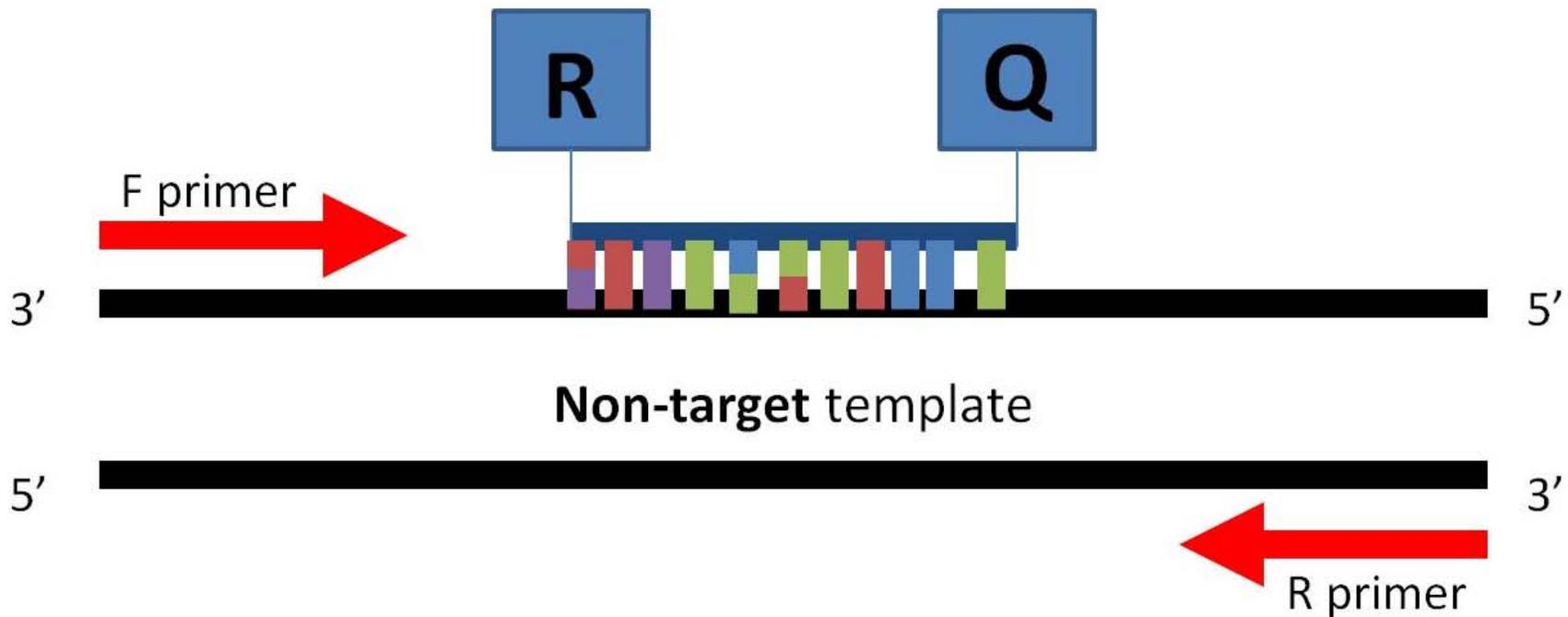
Probe is NOT a perfect match
to target template

Probe is NOT a perfect match to target template

Brook Trout
Salvelinus fontinalis



ATGCGTGC

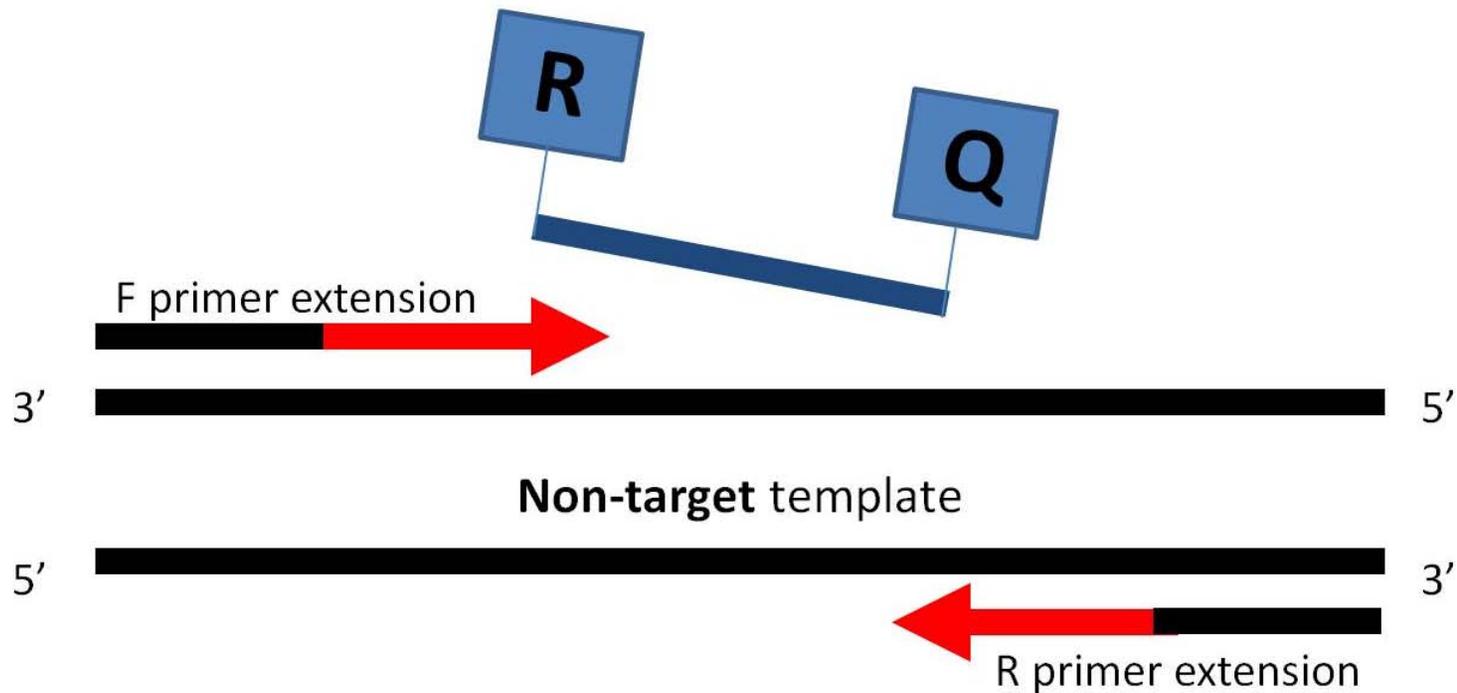


Entire probe is displaced and
there is no fluorescence

Brook Trout
Salvelinus fontinalis



ATGCGTGC



Detecting DNA of a Target Species

Bull Trout

Salvelinus confluentus



ATCCTGC

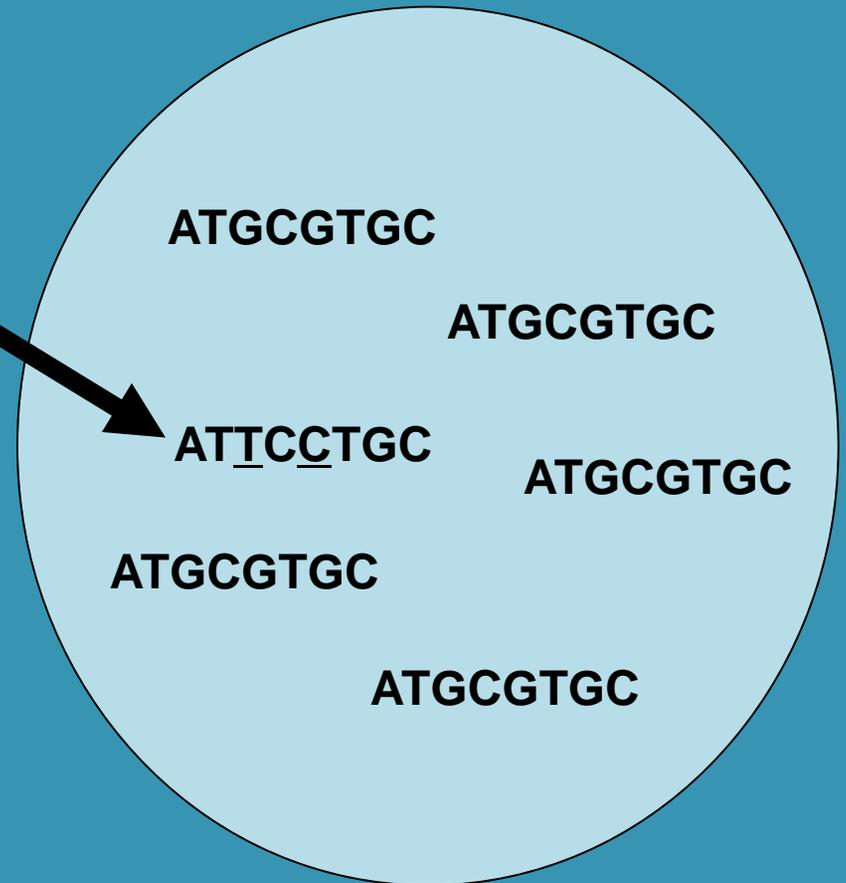
Bull Trout DNA
(Target)

Lake Trout

Salvelinus namaycush



ATGCGTGC



ATGCGTGC

ATGCGTGC

ATCCTGC

ATGCGTGC

ATGCGTGC

ATGCGTGC