# USING ENVIRONMENTAL DNA IN MONITORING PROGRAMS FOR FISH AND AMPHIBIANS

ALEXANDER K. FREMIER, KATHERINE STRICKLER AND CAREN GOLDBERG

WASHINGTON STATE UNIVERSITY, PULLMAN

# WHAT IS ENVIRONMENTAL DNA?

Sample

*Extract and identify DNA from water sample*


qPCR

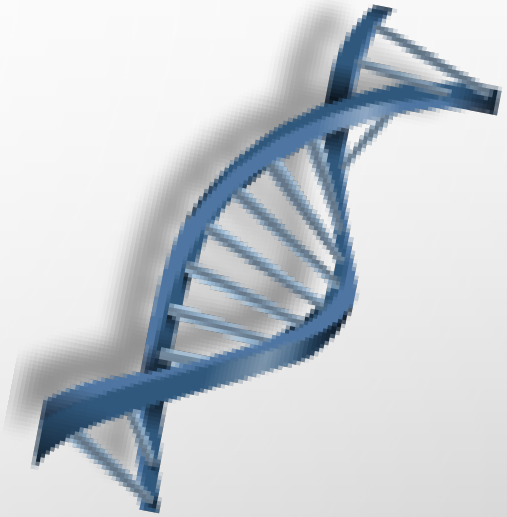
**Absence**

Presence

# DNA

# E-DNA APPLICATIONS IN CONSERVATION BIOLOGY

- INITIAL CONFIRMATION OF E-DNA (FICETOLA ET AL. 2008)
- SINCE THEN…
  - PONDS, LAKES, RIVERS, STREAMS, LAGOONS, WETLANDS, OCEANS
  - FISH, AMPHIBIANS, CRUSTACEANS, INSECTS, VERTEBRATES, REPTILES
- FAMOUSLY – ASIAN CARP (JERDE ET AL. 2011)




Goldberg et al. 2011, Dejean et al. 2012, Thomsen et al. 2012, Takahara et al. 2012, Foote et al. 2012, Olson et al. 2012, Wilcox et al. 2013, Laramie et al. 2013, Piaggio et al 2013, among others…

# A DIFFERENT SAMPLING METHODOLOGY

- UV
- water temperature
- water pH
- microbial activity

Degradation

Production

Detection

- species
- season
- density
- diet

Probability of Detection

Diffusion/Transport

- water discharge
- eDNA diffusion

# STUDY OBJECTIVES

TO IMPROVE METHODS FOR MONITORING SPECIES

> *"… TO PRODUCE AN EFFICIENT, BROADLY APPLICABLE SET OF PROTOCOLS FOR THE USE OF E-DNA TECHNIQUES FOR MONITORING SENSITIVE AQUATIC VERTEBRATE SPECIES AND THEIR INVASIVE THREATS AT DEPARTMENT OF DEFENSE INSTALLATIONS."*

SPECIFIC OBJECTIVES:

1. IMPROVE AND STREAMLINE E-DNA FIELD AND LAB METHODS FOR WIDESPREAD APPLICATION
2. COMPARE E-DNA TO TRADITIONAL METHODS – COST EFFICIENCY ANALYSIS
3. QUANTIFY THE SPATIAL AND TEMPORAL PATTERNS OF E-DNA TO ADVISE SAMPLING

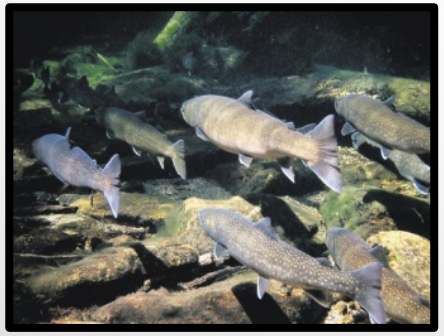
# E-DNA PROJECTS - DOD

- FORT HUACHUCA
  - SONORAN TIGER SALAMANDER
  - CHIRICAHUA LEOPARD FROG
  - ARIZONA TREEFROG
  - NORTHERN MEXICAN GARTERSNAKE
  - THREATS (INCLUDING PATHOGENS)

- EGLIN AIR FORCE BASE
  - RETICULATED FLATWOODS SALAMANDER
  - ORNATE CHORUS FROG

# EDNA PROJECTS - DOD

- YAKIMA TRAINING CENTER
  - BULL TROUT, SPRING AND FALL CHINOOK, BROOK TROUT



- RELATED WORK
  - PACIFIC LAMPREY, LAKE TROUT, NORTHERN LEOPARD FROGS, NEW ZEALAND MUDSNAILS, TORRENT SALAMANDERS, WESTERN TOADS…

# TRADITIONAL & E-DNA METHODS: ALL SPECIES

**Traditional Field Method**

| eDNA Method | Presence | Absence |
|---|---|---|
| Presence | 36 | 5 |
| Absence | 4 | 99 |

**Sensitivity of eDNA method** 3.6%

~~**Inferential Error**~~

**Sampling Error** 2.9%

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# TRADITIONAL & E-DNA METHODS: FISH ONLY

| | | Traditional Field Method | |
|---|---|---|---|
| | | Presence | Absence |
| eDNA Method | Presence | 28 | 1 |
| | Absence | 1 | 62 |

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# FISH RESULTS – BULL, CHINOOK, BROOK

- FIELD VALIDATION OVER A RANGE OF FISH SPECIES AND DENSITIES
  - BULL TROUT, CHINOOK (F & S) AND BROOK TROUT
  - HIGH, LOW AND EXTREMELY LOW DENSITIES

- SPECIFIC QUESTIONS…
  - DOES E-DNA WORK FOR THESE SPECIES?
  - DOES E-DNA WORK FOR DIFFERENT DENSITIES OF BULL TROUT REDDS?
  - CAN E-DNA DETECT EXTREMELY LOW DENSITIES OF BULL TROUT?

    (POTENTIALLY EXTIRPATED BULL TROUT IN NF TEANAWAY)

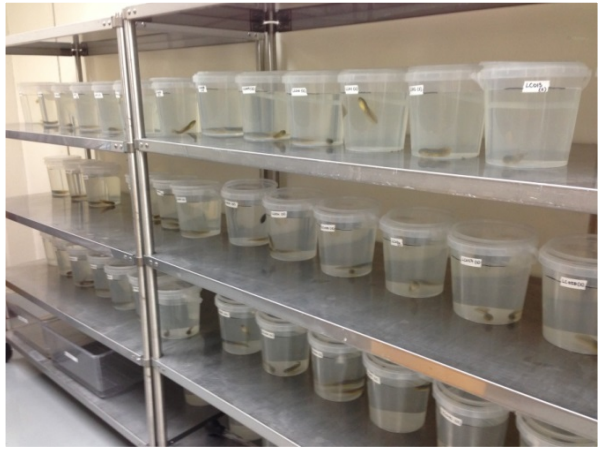
# STUDY LOCATIONS

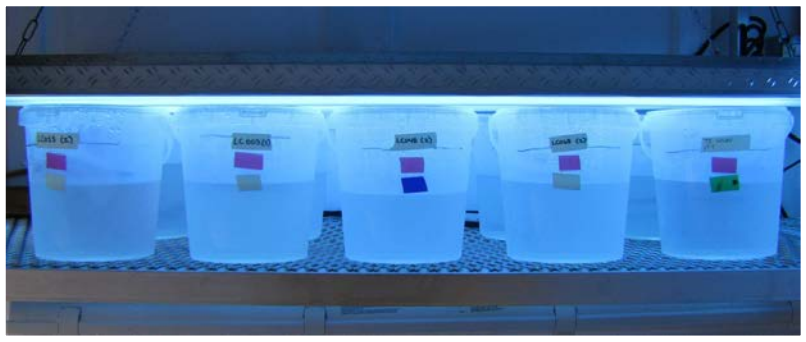
- HIGH DENSITY BULL TROUT REDDS: SF TIETON, SPRUCE CREEK, UNION CREEK (4 SITES, REDDS)
- HIGH DENSITY SPRING CHINOOK: AMERICAN RIVER, LOWER YAKIMA (4 SITES, REDDS + CARCASSES)
- LOW DENSITY BULL TROUT: PEND OREILLE TRIBUTARIES (2 STREAMS, 4 SITES)
- EXTREME LOW DENSITY BULL TROUT: NF TEANAWAY: DE ROUX CREEK (2 SITES 2012, 2013), MAINSTEM ABOVE DE ROUX (2 SITES 2012, 2013), MAINSTEM BELOW DE ROUX (8 SITES 2013)
  - 12 SITES, 7 SITES 10-800M DOWNSTREAM OF PROBABLE REDD
- EXTREME LOW: YAKIMA TRAINING CENTER: ALKALI CREEK (4 SITES), LMUMA CREEK (5 SITES)
  - BULL TROUT, CHINOOK (EXTIRPATED?), BROOK TROUT

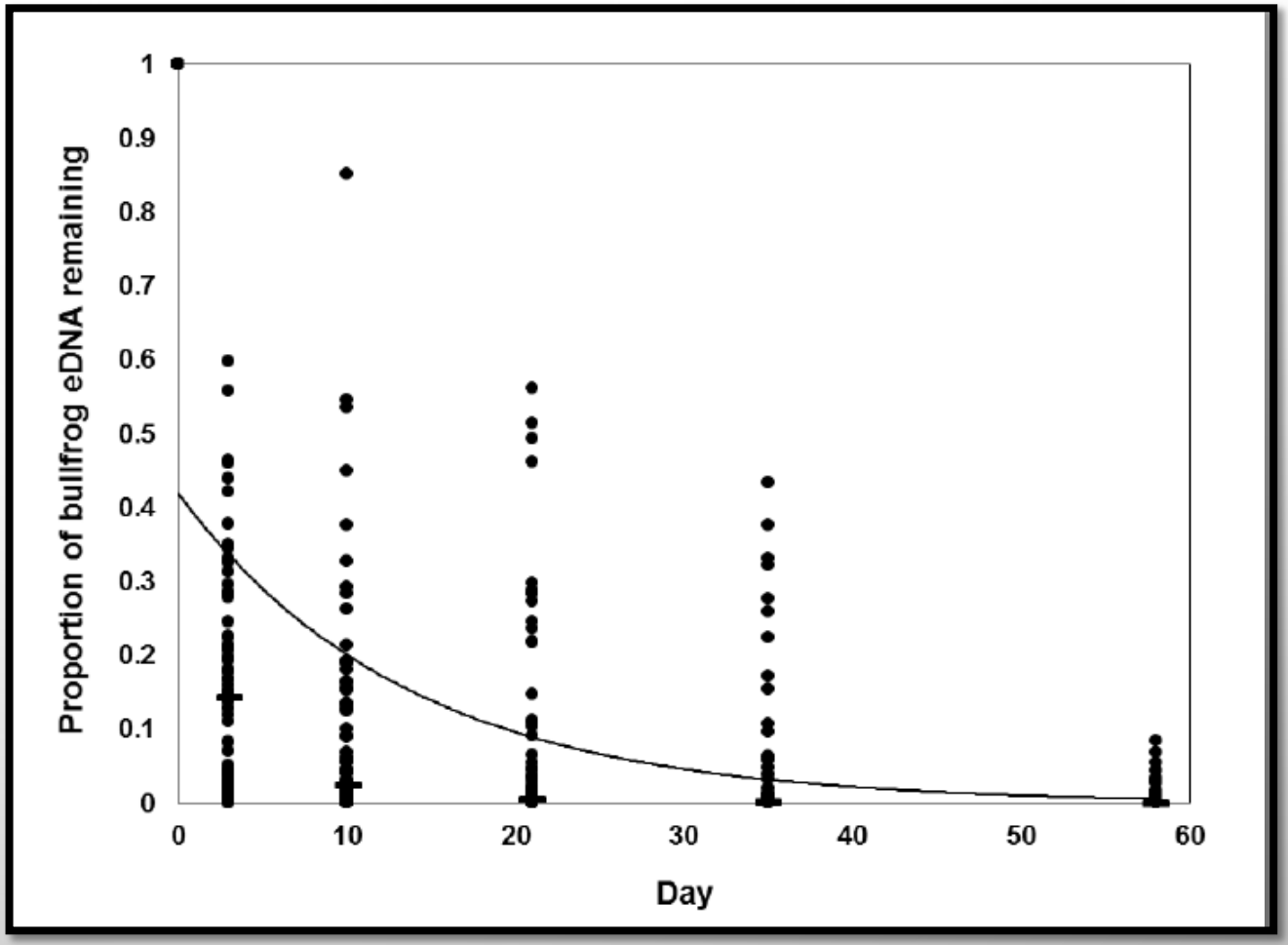
# RESULTS… SO FAR

- DOES E-DNA WORK FOR THESE SPECIES?
  - YES,  FALL AND SPRING DISCRIMINATION TEST IN PROGRESS
- DOES E-DNA WORK FOR DIFFERENCE DENSITIES OF FISH POPULATIONS?
  - YES, IN LOW DENSITY EXAMPLE IT WAS MORE SENSITIVE
- CAN E-DNA DETECT EXTREMELY LOW DENSITIES OF FISH POPULATIONS?
  - MAYBE

# HOW FAST DOES E-DNA DEGRADE?







# DEGRADATION OF E-DNA

| pH4 | UV-B (kJ/m²/day) 2 | 25 | 50 |
|---|---|---|---|
| Temperature (°C) 5 | 0.05 / 53 | 0.13 / 22 | 0.13 / 17 |
| 20 | 0.14 / 14 | 0.17 / 9 | 0.14 / 1 |
| 35 | 0.20 / 10 | 0.15 / 1 | 0.34 / <1 |

Degradation rate / Days until <0.05 eDNA remaining

| pH7 | UV-B (kJ/m²/day) 2 | 25 | 50 |
|---|---|---|---|
| Temperature (°C) 5 | 0.07 / 23 | 0.12 / 12 | 0.12 / 12 |
| 20 | 0.14 / 7 | 0.12 / 1 | 0.12 / 1 |
| 35 | 0.11 / 8 | 0.13 / 1 | 0.15 / 3 |

| pH10 | UV-B (kJ/m²/day) 2 | 25 | 50 |
|---|---|---|---|
| Temperature (°C) 5 | 0.05 / 53 | 0.05 / 54 | 0.05 / 41 |
| 20 | 0.06 / 39 | 0.12 / 13 | 0.14 / 13 |
| 35 | 0.09 / 27 | 0.15 / 11 | 0.14 / 8 |

# WHERE TO SAMPLE IN THE STREAM?

- HIGH DENSITY SAMPLING:
- 3 BULL TROUT STREAMS, 5 TRANSECTS PER STREAM

# SPATIAL SAMPLING IN STREAMS

- 5 TRANSECTS PER STREAM
- SAMPLE IN LOW VELOCITY (0-0.4 M/S) VS HIGH VELOCITY (0.3-0.9 M/S)


More eDNA in slow than fast water ($P<0.01$)

# FAQ 1: WILL IT WORK FOR MY SPECIES?


## Probably, but…

- **COST EFFICIENCY** AND **QUESTION** MATTER MOST
- E-DNA DETECTION VARIES BY SPECIES

**How cryptic is the species?**
**What is the scale of the question?**

- SAMPLING PROTOCOLS NEED TO MAXIMIZE DETECTION

| | |
|---|---|
| • Season for sampling | • Volume sampled |
| • Number of replicates | • Preservation method |
| • Spatial distribution of replicates | • Extraction method |
| | • Analysis method |

- PILOT STUDY IS IMPORTANT
- EDNA SAMPLING CAN COMPLEMENT FIELD SURVEYS

# FAQ 2: CAN YOU ESTIMATE ABUNDANCE?


# FAQ 3: WHAT ARE THE CHANCES OF A FALSE POSITIVE?


## 2 SOURCES OF FALSE POSITIVES:

1. CROSS-CONTAMINATION
   - FOLLOW BEST PRACTICES TO AVOID CONTAMINATION
   - USE NEGATIVE CONTROLS AT ALL STEPS OF SAMPLE COLLECTION AND LAB ANALYSIS

2. DEMONIC INTRUSION (EXTRANEOUS SOURCES)
   - FOLLOW UP WITH ADDITIONAL SURVEYS

# FAQ 4: HOW MUCH DOES IT COST?

## It depends… on data and the ecology

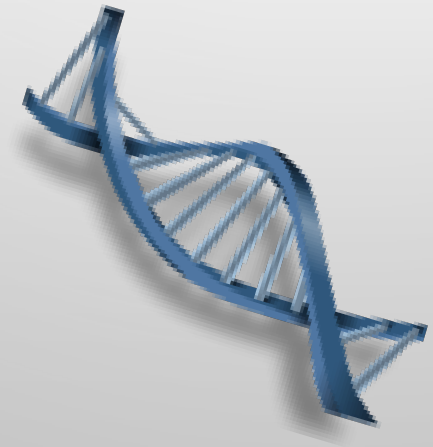
- $35 - $60/SAMPLE
  - IF E-DNA ASSAY EXISTS: LAKE TROUT, BROOK TROUT, BULL TROUT, PACIFIC LAMPREY, CHINOOK, NEW ZEALAND MUDSNAILS, MOST NW AMPHIBIANS
- $3-10K/PROJECT FOR ASSAY DEVELOPMENT
  - IF PHYLOGENETIC DATA EXISTS BUT NO ASSAY: DEPENDING ON HOW DIFFERENT THE TARGET SPECIES IS FROM CO-OCCURRING SPECIES
- $10K+/PROJECT FOR ASSAY DEVELOPMENT
  - NO PHYLOGENETIC DATA EXISTS: REQUIRES EXTENSIVE SAMPLING FOR PHYLOGENETIC DESCRIPTION

# SUMMARY

1. IMPROVE AND STREAMLINE E-DNA FIELD AND LAB METHODS FOR WIDESPREAD APPLICATION
    - IMPROVE METHODS READY FOR APPLICATION WITH SOME EXCEPTIONS
    - PRELIMINARY STUDIES NECESSARY TO ADVISE SAMPLING SCHEMES
2. COMPARE E-DNA TO TRADITIONAL METHODS – COST EFFICIENCY ANALYSIS
    - MOST LIKELY MORE EFFECTIVE WHEN EFFORT TO DETECT IS HIGH
    - COMPLEMENTARY SAMPLING APPROACH TO TRADITIONAL METHODS
3. QUANTIFY THE SPATIAL AND TEMPORAL PATTERNS OF E-DNA TO ADVISE SAMPLING
    - DEGRADATION RATES ARE HIGHLY VARIABLE – 1 -53 DAYS
    - HIGHER CONCENTRATIONS OF E-DNA IN SLOWER WATERS IN STREAMS

# THANKS TO MANY.

- DOD – ESTCP program
- DOD – Legacy program
- Yakima Training Center
- Washington Department of Fish and Wildlife
- Yakama Nation
- Virginia Tech
- Arizona Department of Game and Fish
- Eglin Air Force Base
- Fort Huachuca
- University of Idaho – LEECG
- Idaho Department of Fish and Game



# Thanks for listening…