



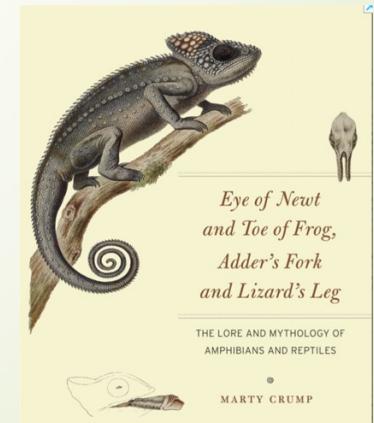
Survey Methods

James Bettaso, Don Ashton,
Ryan Peek, Sarah Kupferberg

FYLF - TWS
Workshop
Arcata CA
May 2018

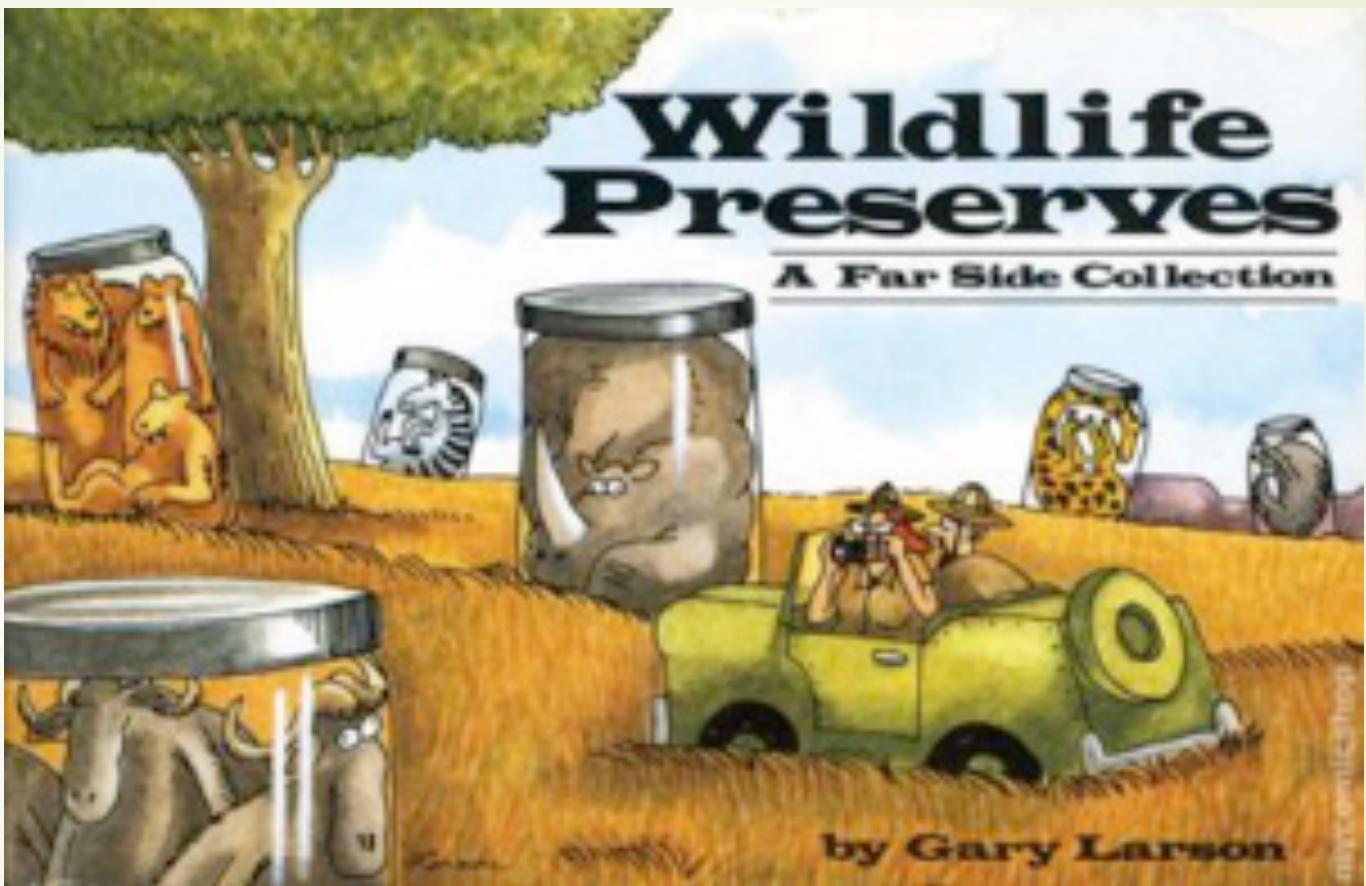
“ One thing to remember is to talk to the animals. If you do, they will talk back to you. But if you don't talk to them, they won't talk back to you, then you won't understand. And when you don't understand, you will fear, and when you fear, you will destroy the animals, and if you destroy the animals you will destroy yourself.”

Chief Dan George,
Tseil-Waututh Nation, North Vancouver
1899-1981



Overview

- ▶ Adult Surveys (comparison of VES to radio-telemetry)
- ▶ Underwater calling
- ▶ Tadpole surveys
- ▶ Egg Mass (EM) surveys
- ▶ eDNA monitoring (methodology & application)
- ▶ How survey techniques changes with size of river
- ▶ Detection Inhibitors
- ▶ Equipment
- ▶ Decontamination

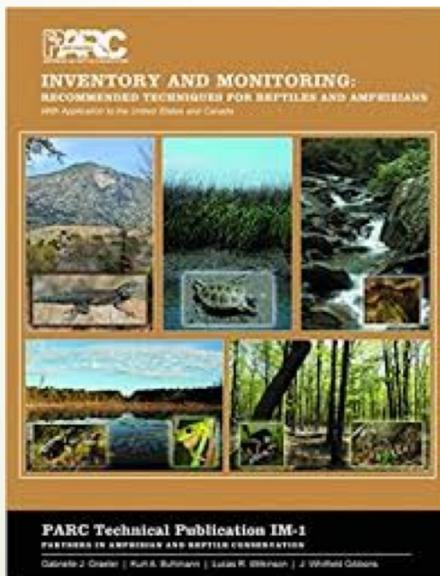


by Gary Larson

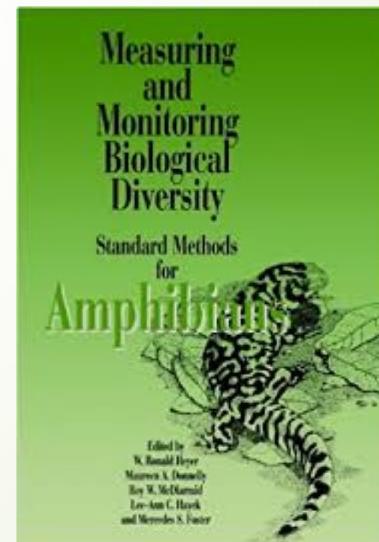
www.garylarson.com

Suggested Reference Material

PARC 2013
Gabrielle J. Graeter,
Kurt A. Buhlmann,
Lucas R. Wilkinson,
J. Whitfield Gibbons



Smithsonian 1994
Heyer, W. R.,
M. D. Donnelly,
R. W. McDiarmid,
L. C. Hayek,
M. S. Foster

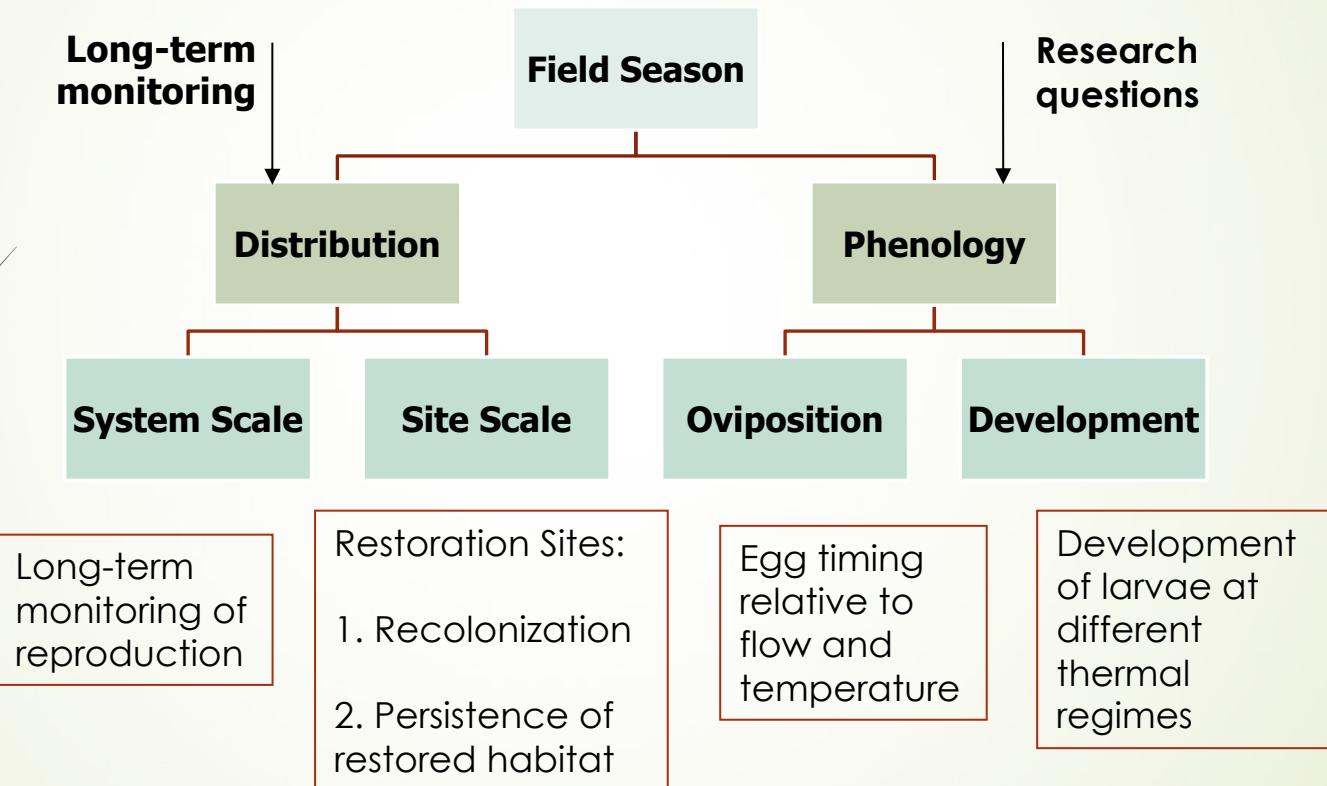


Definitions---

Measuring and Monitoring Biological Diversity:
Standard Methods for Amphibians. 1994. Eds,
Heyer et al.

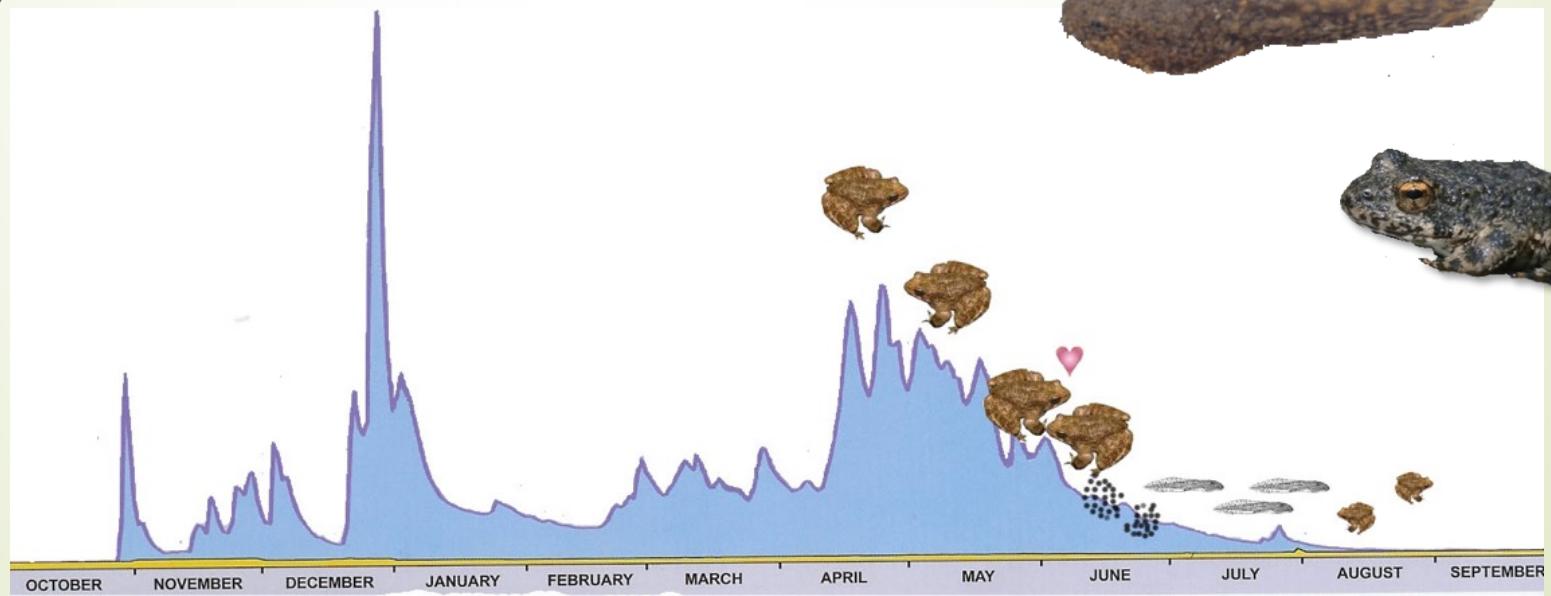
- ▶ **Relative abundance:** proportional representation of species in a sample
- ▶ **Inventory:** study of a specific area, site, or habitat to determine the number of species present (i.e. species richness)
- ▶ **Monitoring:** study of the abundance of individuals in one or more populations of a species at a site through time
- ▶ **Mark-recapture techniques:** methods for determining population size that involve capturing, marking, and releasing animals, and subsequently recapturing or re-sighting them one or more times.
- ▶ **Visual Encounter Survey (VES):** estimate the diversity (number of different species) and abundance (number of different individuals) of animals at a particular location

What question(s) will surveys address?



Example conceptual diagram

Life Cycle



Timing of Surveys

- ▶ Monitor:
 - ▶ River stage
 - ▶ Storm forecasts
 - ▶ Water temperature

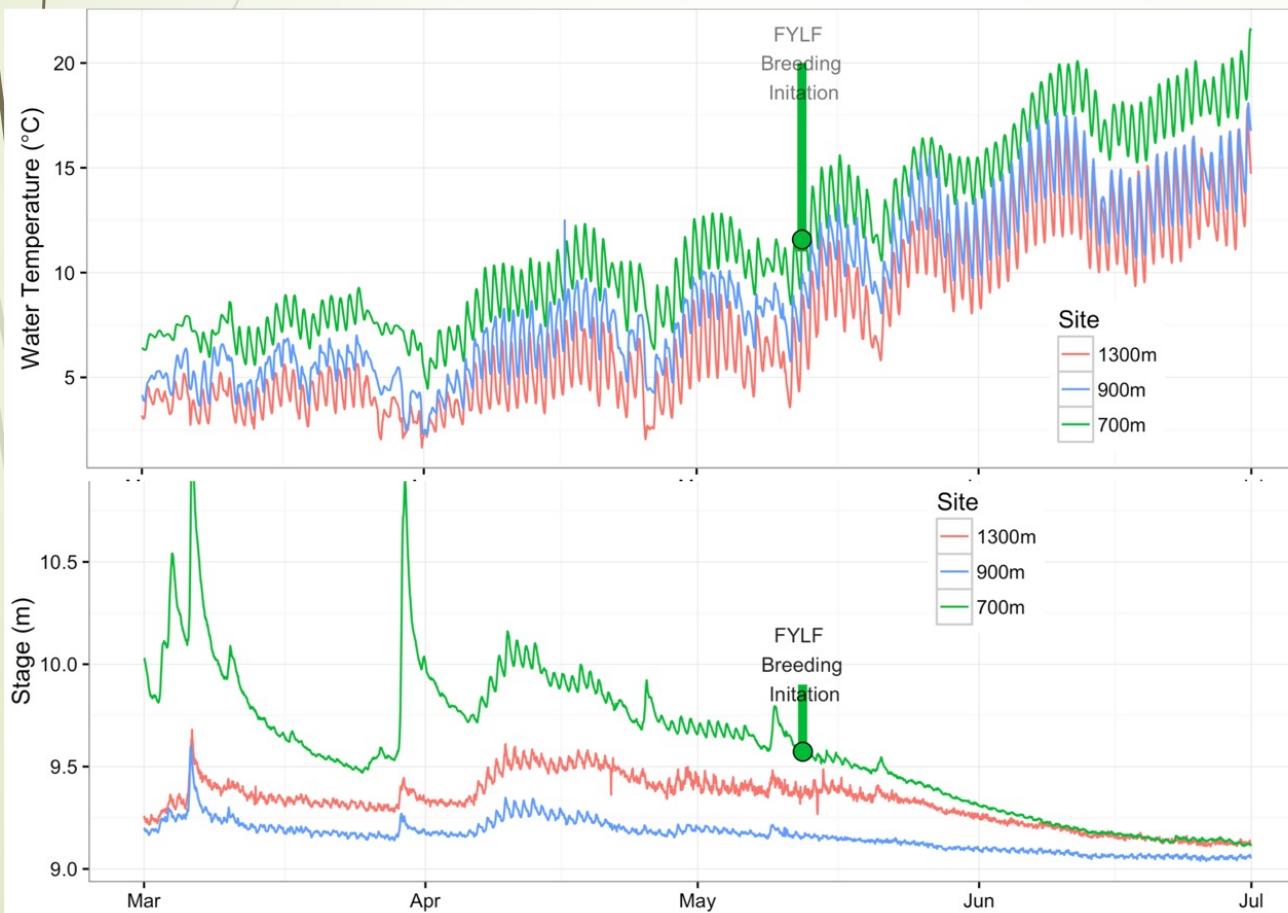
- ▶ Check activity at reference sites



Timing of Surveys: Webdata

► Useful websites for monitoring flow/temp:

- USGS (<https://waterdata.usgs.gov/ca/nwis/rt>)
- CDEC (<http://cdec.water.ca.gov/cdecstation2/>)
- Dreamflows (<http://www.dreamflows.com/realtime.php>)



Survey Considerations/Gear

- ▶ Polarized glasses
- ▶ Search time
 - ▶ 9:00-19:00
- ▶ Search duration
- ▶ Search area
- ▶ Weather
 - ▶ Warm, sunny
 - ▶ Calm or light wind

Breeding dates are variable;
timing of surveys is critical



Environmental/habitat variables



► Stream Habitat – Physical Attributes

- Water Temperature
- Water Velocity
- Water Depth
- Distance to Shore

► Attachment Substrate

- Pebble (33-64 mm)
- Cobble (65-256 mm)
- Boulder (>256 mm)
- Bedrock
- Woody material
- Live riparian vegetation

► Orientation of Egg Mass on Substrate

- Upstream
- Shore side
- Thalweg side
- Top of substrate

► Flow Direction Relative to Egg Mass

- No Flow
- With Flow
 - Away
 - Alongside
 - Backflow
 - Flow over top

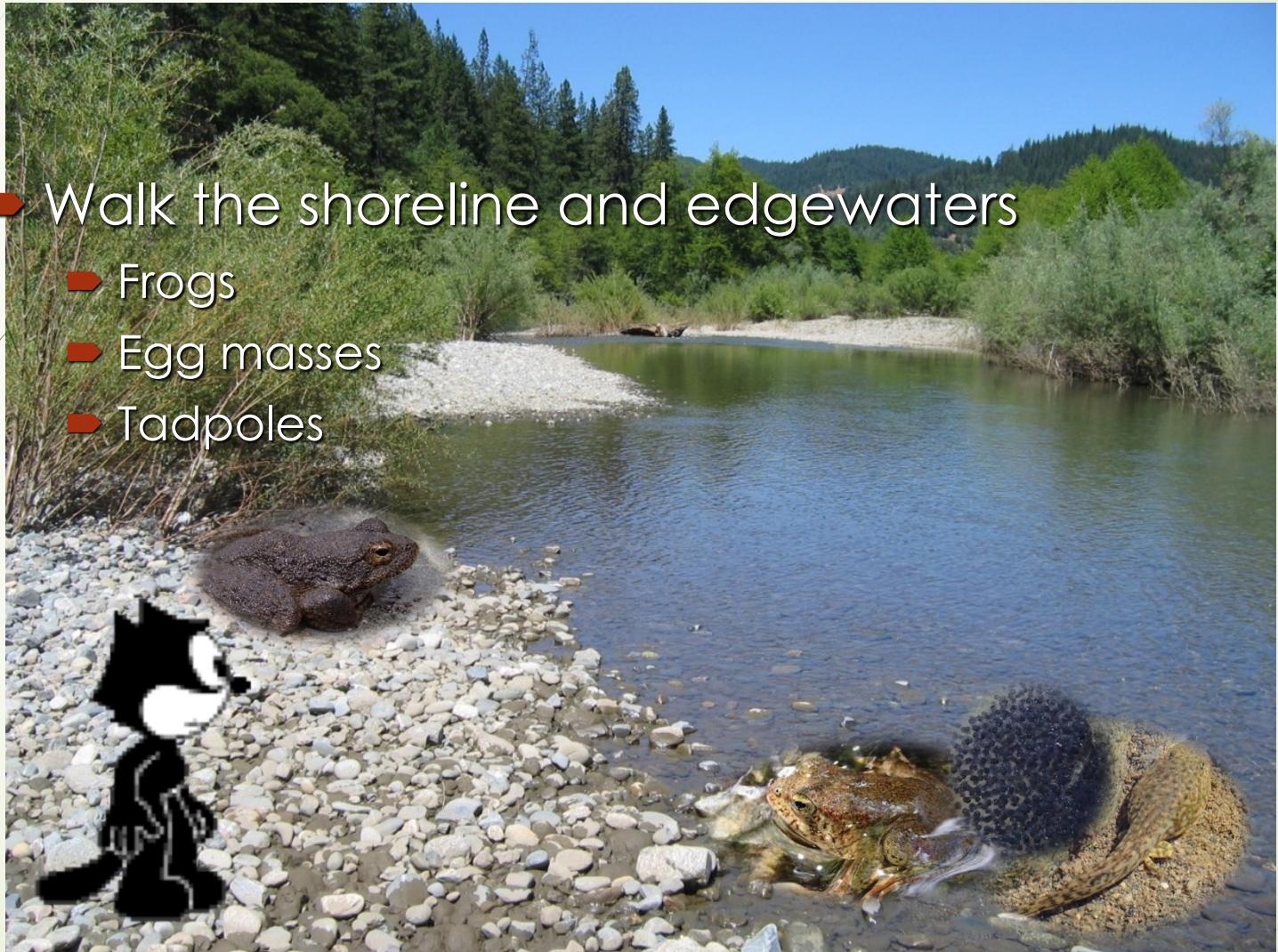
Oviposition Habitat

Nate Nieto measuring egg mass



Visual Encounter Surveys (VES)

- ▶ Walk the shoreline and edgewaters
 - ▶ Frogs
 - ▶ Egg masses
 - ▶ Tadpoles



Visual Encounter Surveys (VES)



Check edgewaters & dry shoreline



VES: CWS Protocol

<https://watershed.ucdavis.edu/project/long-term-river-monitoring>

- ▶ VES-based, see website for datasheet/details
- ▶ Snorkeling highly recommended/mandatory for spring egg mass surveys
- ▶ Suitable for assessing/monitoring the abundance, distribution and habitat associations of *R. boylii*



VISUAL ENCOUNTER SURVEY PROTOCOL FOR *RANA BOYLII* IN LOTIC ENVIRONMENTS

June, 2017

R.A. Peek¹, S.M. Yarnell¹, A.J. Lind²

¹ Center for Watershed Sciences, John Muir Institute of the Environment,
One Shields Avenue, University of California, Davis, CA 95616

² USDA Forest Service, Tahoe & Plumas National Forests, 631 Coyote Street, Nevada City, CA 95959

NOTE: This field survey protocol can be used in support of instream flow studies or as part of a monitoring program, but it should not be implemented without a study design and associated state or federal permits. This protocol does not substitute for a well-designed field study, but is a suitable methodology for collection of field data.

VES OVERVIEW

This Visual Encounter Survey (VES) protocol and associated datasheet are for use in river and stream reaches up several kilometers in length where information on all life stages and the habitat associations of each individual is desired. The data from this survey protocol is intended to 1) describe the abundance, distribution and habitat associations of *R. boylii* (Foothill yellow-legged frog or FYLF), and 2) provide the data necessary to coordinate with other stream reach study efforts, such as instream flow studies where hydrodynamic modeling will be used.

VES: Snorkeling

- ▶ Egg masses can be very cryptic
- ▶ Snorkeling for egg masses can be highly effective
- ▶ Site conditions can greatly impact where egg masses are located (e.g., didymo mats)



Didymo/rocksnot!

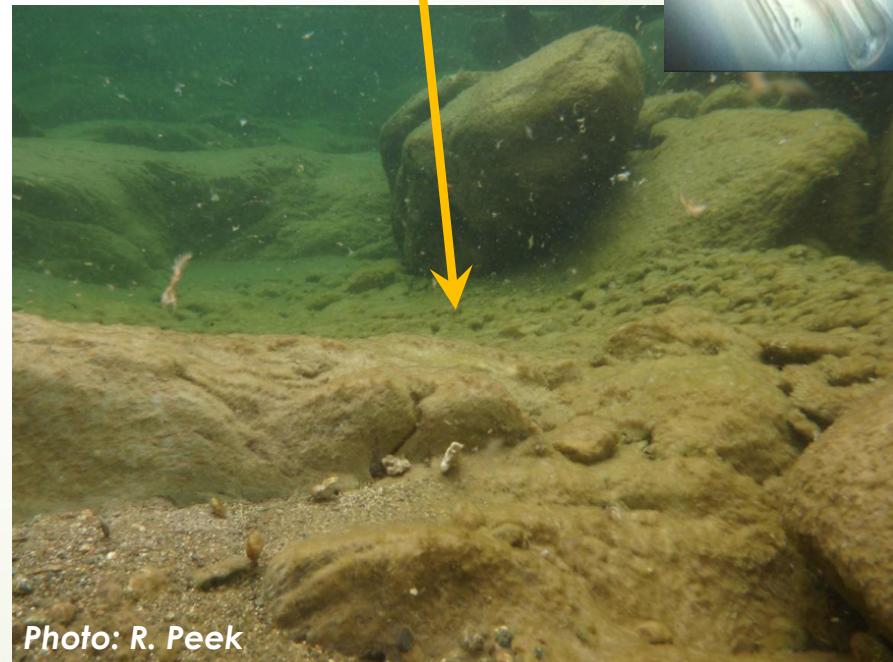


Photo: R. Peek

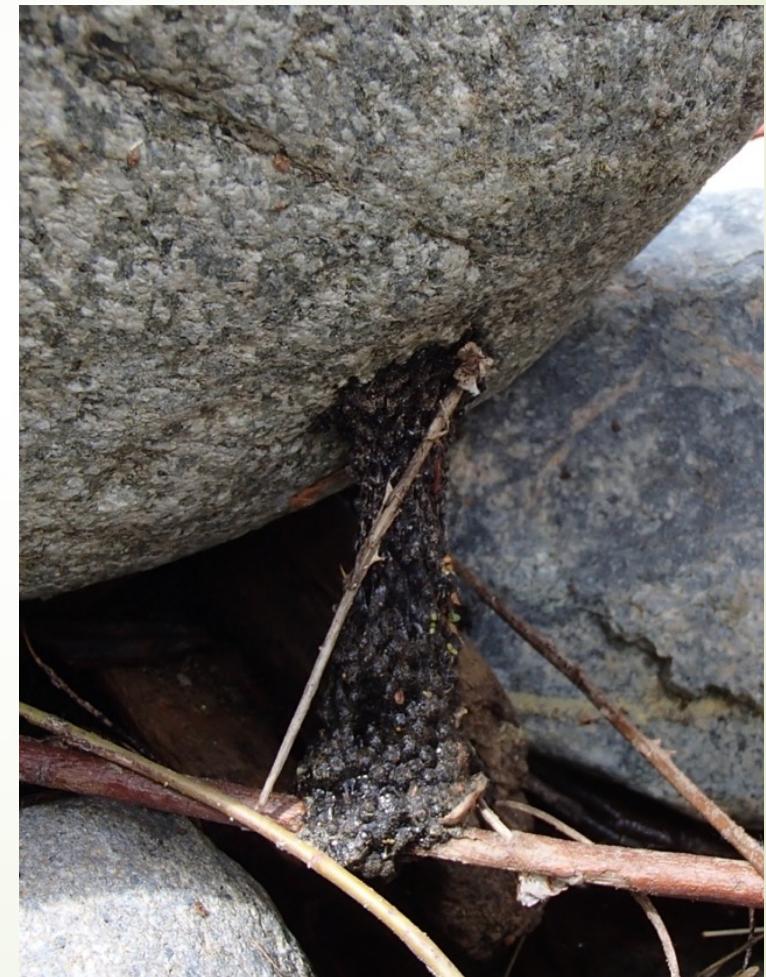


Larger substrates

VES: Float Surveys For Egg Masses



Egg Mass Desiccation



Tracking Fate of Individual Egg Masses

17 JULY 2006 965 cfs



Egg mass hatching

24 JULY 2006 508 cfs



Young tadpoles trapped

31 JULY 2006 489 cfs



100% mortality (6 egg masses)

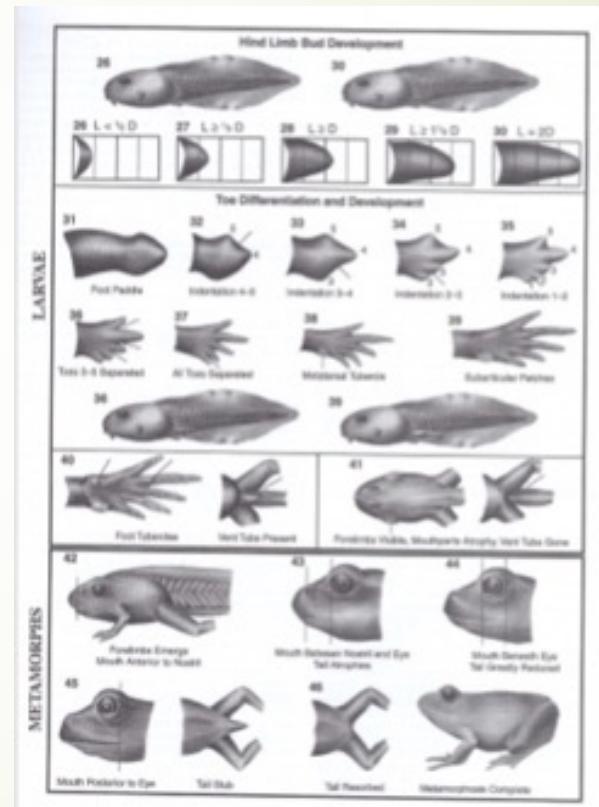
Eggs:
Viewed
from
below

Gosner Stage
Ken Gosner (1960)



Gosner Stage

Kenneth L. Gosner (1960)



Gosner App (beta)

http://shiny.cws.ucdavis.edu/shiny/rapeek/Gosner_photos/



Gosner Stages of *Rana boylii*

Select or type a Gosner Stage (1-46) to view a corresponding photo of Foothill yellow-legged frog (*R. boylii*) development. Not all stages currently have photos. See Github for code: (https://github.com/ryan-ucd/gosner_boylii)

Select a Gosner Stage

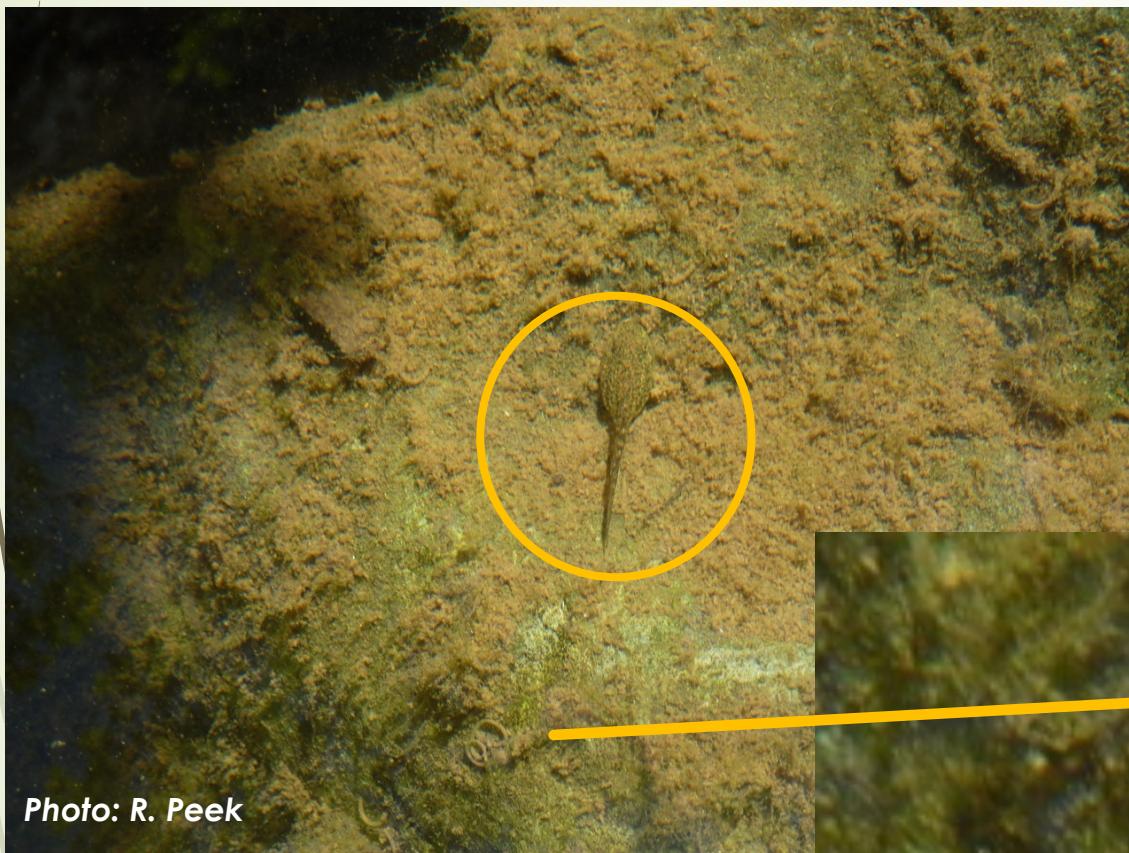
From Gosner 1960. *Herpetologica*, Vol. 16, No. 3 (Sep. 23, 1960), pp. 183-190.



- ▶ Shows photos of various Gosner Stages



Tadpole Spirals (poop)



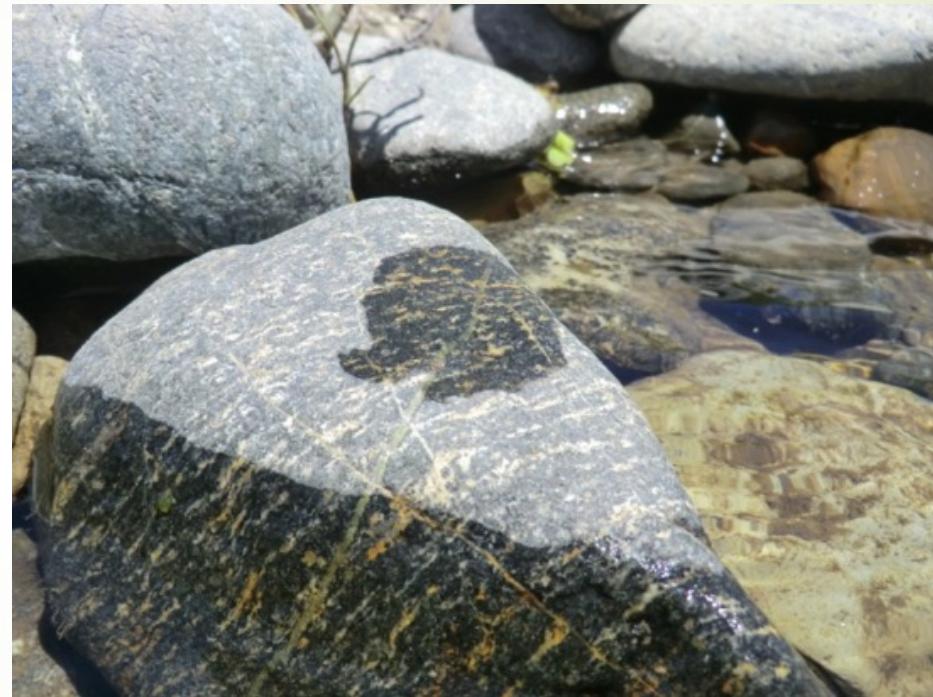
- ▶ Helpful hint that tadpoles may be nearby
- ▶ Spirals/semi-circles, same color as algae
- ▶ Flip some rocks!



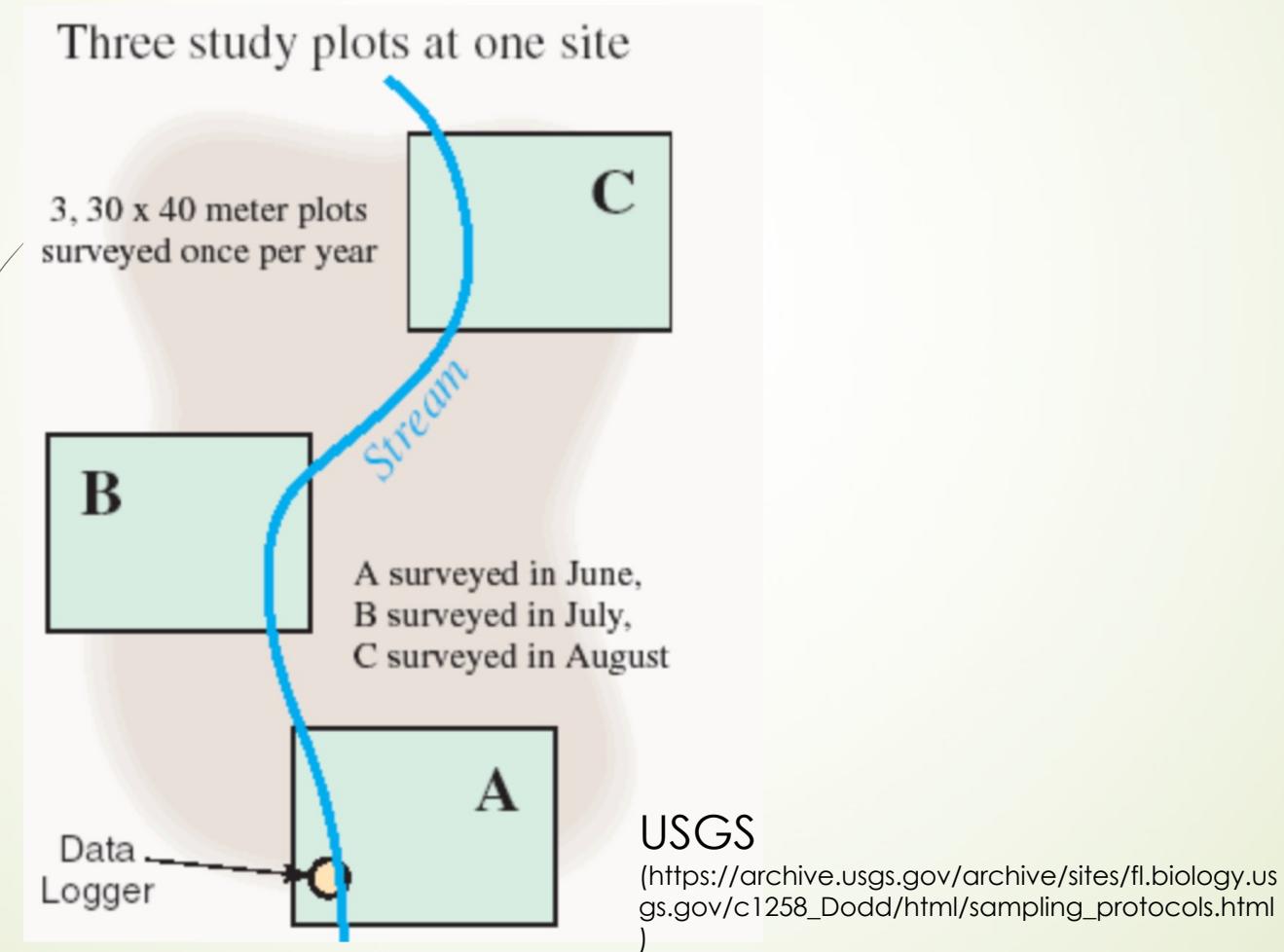
Additional Survey Methods



Photo: D. Ashton



Time-Constraint / Area-Constraint Searches (TCS/ACS)



PIT-Tagging

PIT tag: a marking tag made of a passive integrated transponder (PIT) that relies on passive radio-frequency identification of a 10-digit hexadecimal number, read with a scanner and portable reader.



Photo ID



Chin-spot photo recognition

- ▶ Koen Breedveld
 - ▶ Pit River
- ▶ Clara Wheeler
 - ▶ Smith River

eDNA (Environmental DNA)



Photo: Mallory Bedwell

- ▶ Goldberg Lab (M. Bedwell and C. Goldberg)
 - ▶ <https://labs.wsu.edu/edna/>
- ▶ Developed assay for *Rana boylii* and *Rana sierrae*
- ▶ Successfully deployed in Feather, Merced, and a few other watersheds
- ▶ Active work by other labs/consulting firms to get labs up and running

eDNA

FROM THE COVER

Monitoring endangered freshwater biodiversity using environmental DNA

PHILIP FRANCIS THOMSEN,^{1,*} JOS KIELGAST,^{1,*} LARS L. IVERSEN,[†] CARSTEN WIUF,[‡] MORTEN RASMUSSEN,^{*} M. THOMAS P. GILBERT,^{*} LUDOVIC ORLANDO^{*} and ESKE WILLERSLEV^{*}

^{*}Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, DK-1350 Copenhagen, Denmark, [†]Freshwater Biology Section, Department of Biology, University of Copenhagen, Helsingørsgade 51, DK-3400 Hillerød, Denmark, [‡]Bioinformatics Research Center (BiRC), Aarhus University, C. F. Möllers Alle 8, DK-8000 Århus, Denmark

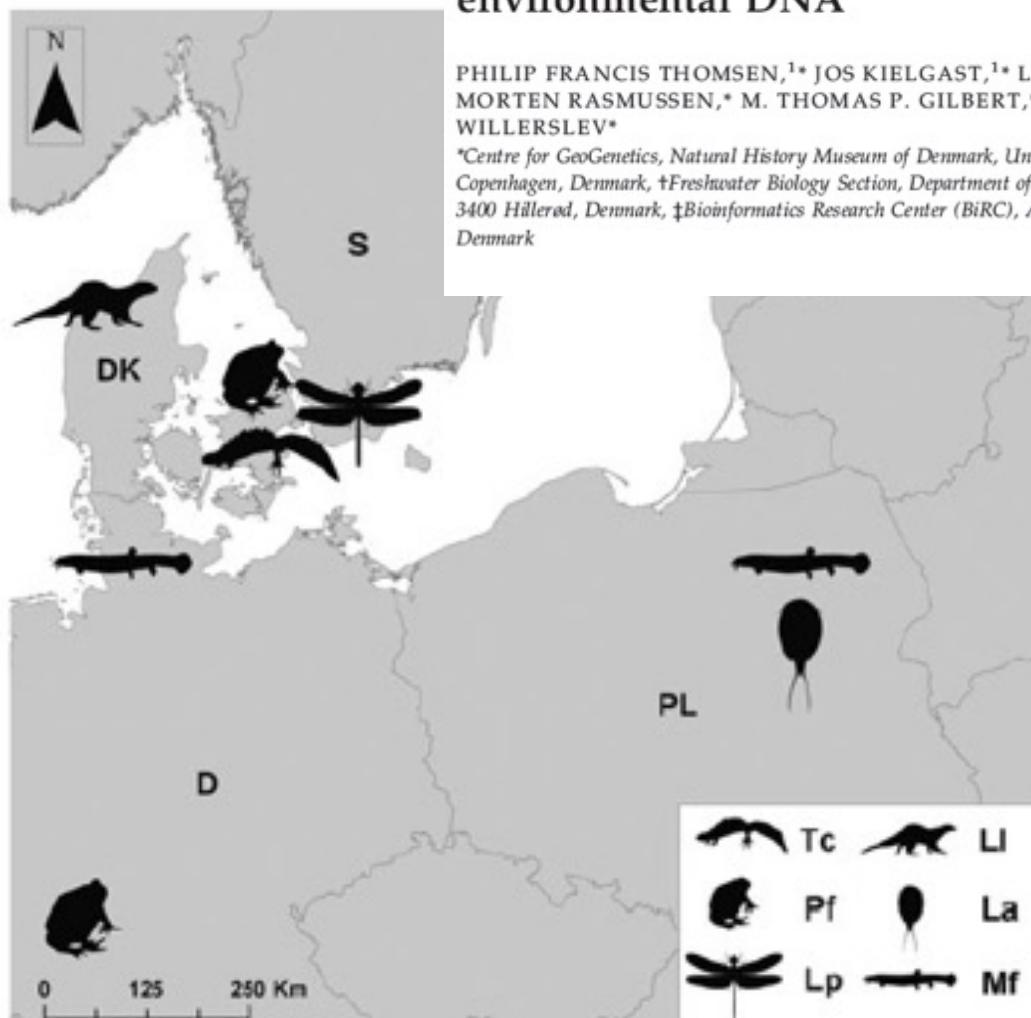
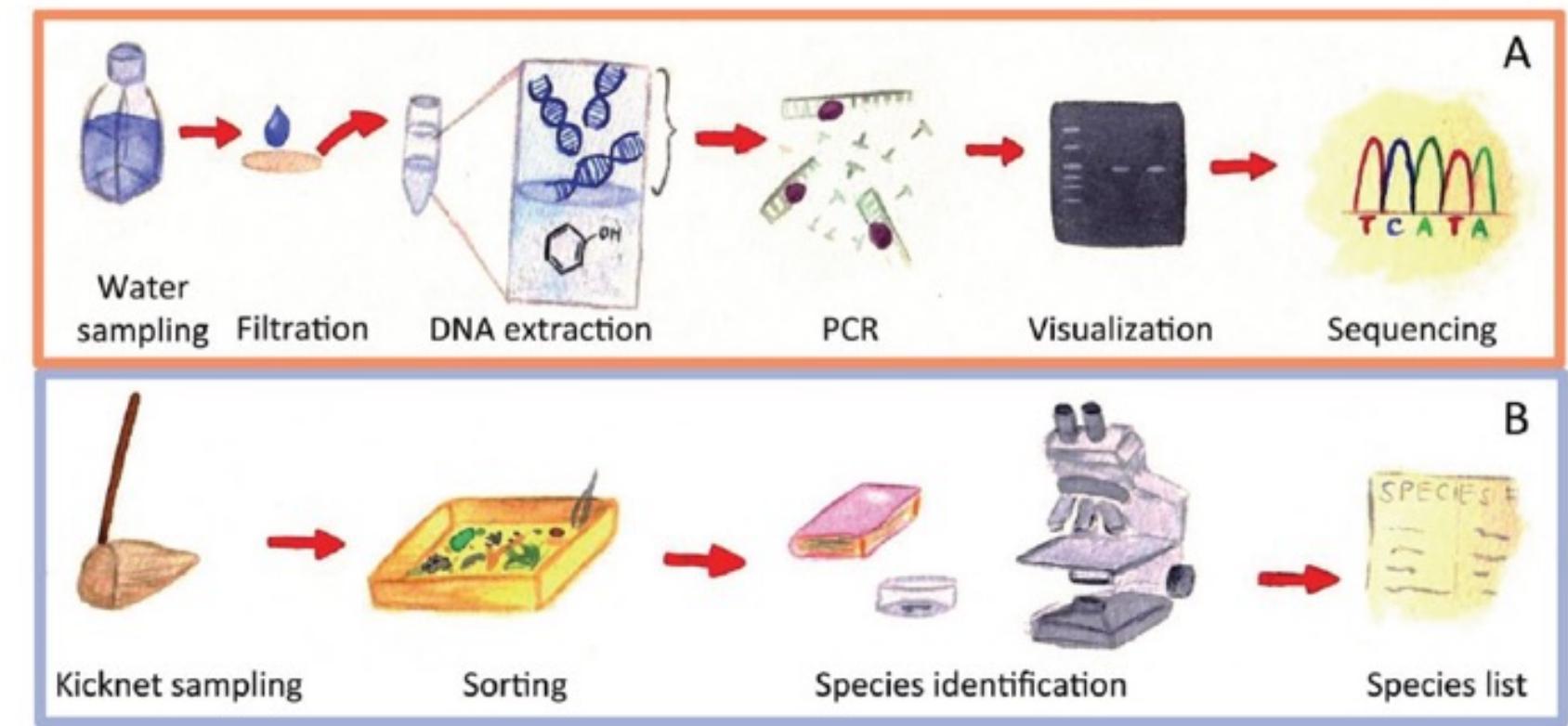


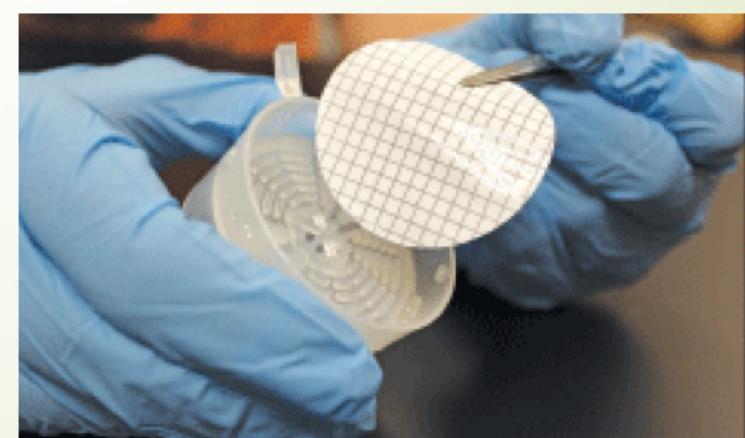
Fig. 1 Sampling locations of the 90 European natural freshwater systems targeted in this study. Samples were taken from Denmark (DK), Sweden (S), Germany (D), Poland (PL) and Estonia (EST) and covers *Tc* (*Triturus cristatus*, 11 ponds), *Pf* (fish, 11 ponds), *Lp* (*Leucorhinia pectoralis*, 11 ponds), *Li* (*Lutra lutra*, 15 streams and lakes), *Mf* (*Misgurnus fossilis*, 11 ponds).

eDNA



from Machler et al. 2014. Freshwater Science

- ▶ Filter a lot of water
- ▶ Clean/sterile replicates
- ▶ Cover more ground
- ▶ Not definitive!



Telemetry

Movement studies, habitat use



Photo: D. Ashton

Decontamination

- ▶ <http://www.ccadc.us/docs/DeconForProfessionals.pdf>
- ▶ <http://www.cwhc-rcsf.ca/docs/HHWG%20Decontamination%20Protocol%202017-05-30.pdf>

Decontamination

<http://www.cwhc-rccsf.ca/docs/HHWG%20Decontamination%20Protocol%202017-05-30.pdf>

Appendix 1: Tested efficiency of decontamination products

Product (% active ingredient)	Tested concentration	Exposure time (min)	Effective against	Notes	Source* (see references)
Bleach (6% Sodium Hypochlorite)	1:32 (0.2% Sodium Hypochlorite)	1-10	<i>Bd</i> <i>RV</i> <i>SFD</i>	Vapor may cause severe irritation or damage to eyes and skin; harmful if swallowed. Fatal to amphibians at high concentrations. Corrodes metals; fades colours and breaks down cloth fibers.	1, 5, 9, 13
70% Ethanol	70% ethyl alcohol	2	<i>Bd</i> <i>RV</i> <i>SFD</i>	May be fatal if swallowed or inhaled; can damage liver, kidneys and nervous system by repeated or prolonged exposure; may be absorbed through skin; repeated or prolonged contact can cause eye irritation or dermatitis. May harm amphibians by damaging epidermal coating. May damage rubber and plastics; may cause deterioration of glues.	1, 5, 9, 13
Benzalkonium chloride	1mg/ml	10	<i>Bd</i> <i>RV</i> <i>SFD</i>	Requires extreme care in handling (see M.S.D.S.). Very toxic to aquatic organisms. Essential to ensure this chemical does not enter the environment.	5, 13, 15
Virkon S® (20.4% Potassium peroxymonosulfate)	1mg/ml	1	<i>Bd</i> <i>RV</i>	Not tested against SFD. Harmful if swallowed; irritating to respiratory system and skin; may cause serious eye damage. Non-toxic to amphibians. Safe for fabric; may cause pitting on galvanized or soft metal if not rinsed with water.	1, 5, 8, 9, 15, 18
Nolvasan® (2% Chlorohexidine)	1:127	1	<i>RV</i>	Not effective against SFD. May be fatal if inhaled; causes irreversible eye damage; harmful if swallowed. Safe for amphibians for short durations. No reported effects on equipment.	1, 9, 13
Lysol® Power Bathroom Cleaner	100%	10	<i>SFD</i>	Not tested against <i>Bd</i> , <i>RV</i> .	13
Lysol® All Purpose Cleaner	100%	10	<i>SFD</i>	Not tested against <i>Bd</i> , <i>RV</i> .	13
NPD®	100%	10	<i>SFD</i>	Not tested against <i>Bd</i> , <i>RV</i> .	13
CLR® Bath & Kitchen Cleaner	100%	10	<i>SFD</i>	Not tested against <i>Bd</i> , <i>RV</i> .	13
409®	100%	10	<i>SFD</i>	Not tested against <i>Bd</i> , <i>RV</i> .	13
Didecyldimethyl ammonium chloride	2mL/L	1	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 15, 18
Quaternary ammonium compound 128	Full strength to 1×10^{-3}	5	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 8
F10® Super Concentrate Disinfectant	0.7 mL/litre	1	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 18
TriGene® Virucidal Surface Disinfectant Cleaner	0.2 mL/L	1	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 18
10% sodium chloride	10%	5	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 8
2% potassium permanganate	2%	10	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 8
Sterilizing UV light	1000 mW m^{-2} wavelength 254nm	1	<i>RV</i>	Not effective against <i>Bd</i> . Not tested against SFD.	5, 8, 15

Incidental Observations

- ▶ Make note of other things at site
- ▶ May not see relevant at the time, important later



Photo: R. Peek



Photo: R. Peek



Questions?

- ▶ skupferberg@gmail.com
- ▶ rapeek@ucdavis.edu
- ▶ ashton.don@gmail.com
- ▶ jbettaso@fs.fed.us