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

June, 2017

R.A. Peek[1], S.M. Yarnell[1], A.J. Lind[2]

[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 lifestages 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.

The basic VES protocol follows Heyer et al. (1994) and Lind (1997), and is summarized for stream habitats as follows. Surveys will be conducted by a minimum of two surveyors but three or more surveyors may be needed in larger streams. Wherever possible, surveys should be conducted walking upstream so individual frogs seeking cover in the stream (often swimming downstream) are not counted twice. However, surveys could proceed in the downstream direction if surveyors are well-practiced in identification, are manually feeling and checking behind cobbles and boulders for egg masses, and can adequately keep track of any downstream migrating individuals. Surveyors may wade or walk the shoreline and shallow-water habitats where possible, scanning ahead for approximately 10 meters at a time, searching for all lifestages (eggs, larvae, juveniles, adults). If conducting surveys for egg masses, one or more snorkelers should be used, moving upstream adjacent to surveyors in shore and shallow water habitats. If binoculars are available they may be used to scan upstream for post-metamorphic individuals (juveniles and adults) basking on exposed substrate or partially hiding under cover, in an attempt to identify animals before they hide or escape. Aquatic habitats are visually and manually searched along a several-meter wide transect parallel to the stream at the water's edge, and the number of animals encountered over a known period of time and distance are recorded. When wading in the near-shore habitat, surveyors will use a carefully gauged zig-zag pattern to search the shallows in one pass. A stopwatch or wristwatch with a timer (HH:MM) will be used starting at the beginning of the survey and stopped for any time surveyors are not

actively searching for frogs. This is the "*Survey Time*" field in the header of the datasheet, and it should be calculated and recorded for each survey site and visit to provide an estimate of relative effort. Any additional herpetofauna observed, particularly potential predators, invasive species, etc. (*e.g.*, smallmouth bass, crayfish, bullfrogs), should be documented in the notes.

FYLF EGG MASSES

FYLF may prefer lower gradient stream segments, often associated with pool tail-outs and gravel/cobble bar complexes, for oviposition habitat. Breeding can occur as early as March to as late as mid-May in coastal systems (generally April) and as early as late April through early July in the Sierra Nevada (generally mid-May) depending on the seasonal precipitation patterns and water year type. Most egg masses are attached to rocky substrates ranging from small gravel to large boulders and bedrock, however, in some locales egg masses may be attached to roots or large pieces of wood. While the majority of egg masses are located in shallow (< 1 m) water, eggs have been observed in water >2 m. As such, where safe and possible, snorkeling in deeper water (0.5-3 m deep) in and adjacent to suitable breeding habitat (e.g., edges of cobble bars, pool tail outs) is highly recommended for increasing detection of egg masses, and is mandatory for spring egg mass surveys. Non-snorkeling surveyors should carefully use their hands to feel under bedrock and boulder ledges as well as in deep pockets beneath large cobble in low velocity areas where they cannot see, as eggs may be located in highly cryptic and well concealed locations only accessible by touch. Gently brush the entire surface of the rock substrate with a bare hand, following the surface of the rock until the junction between the rock and the stream bed can be felt to ensure the entire surface has been surveyed. This process is time-consuming but effective, and care should be taken to thoroughly survey suitable habitat (typically open canopy areas with low-flow, low-gradient habitat such as runs, pool tails, and edgewater of riffles, however sites and regions may differ, plunge-pool complexes in small tributaries are frequently used for breeding).

FYLF TADPOLES

In shallow water habitats, dip-nets may be used to seine the channel bottom to collect tadpoles. This search effort in shallow water habitats should be balanced to minimize habitat disturbance, but adequately sift through any silt, gravel or vegetation where individuals may be hiding. Use of a viewing box in shallow, wadeable areas to aid in detection of tadpole lifestages is helpful but not required. Polarized glasses help reduce glare and permit more effective inspection beneath the surface of the water, which can be particularly helpful during tadpole surveys. Tadpoles often are well concealed and highly cryptic, but they leave telltale signs of recent presence via the spiral shaped feces that are often uniform in color and highly prevalent in areas where tadpoles occur. During later stages of tadpole development (Gosner 31 and greater), tadpoles may hide under rocky substrates, so carefully lifting and moving cobbles may aid in detecting movement. Minimize handling and disturbance time of tadpoles where possible.

POST-METAMORPHIC INDIVIDUALS (YOUNG-OF-YEAR, JUVENILES, & ADULTS)

For post-metamorphic individuals, a moderate level of search effort is used to survey for individuals active on the surface of the ground, on rocks, or at the water's edge. All individuals are identified, and if possible, captured and photographed ("chin photo") for species and individual identification and measurement. Chin photographs have been successfully used as a tool for non-invasive mark recapture methodology for this species (Marlow et al. 2016), and can be utilized depending on project goals. Subsequent survey observations can be used for comparison and confirmation purposes. Individuals observed in amplexus or gravid adult females should not be captured unless absolutely necessary to minimize stress during critical breeding periods, however notes should be taken on condition and location. All substrate and other surface cover objects that may be overturned while searching for individuals should be returned to its original position to minimize disturbance to the habitat. Habitats are not systematically destroyed in order to find animals, and voucher specimens are not collected unless absolutely necessary for identification. In shallow water habitats, hand dip nets may be used to capture individual adults and sub-adults.

DISINFECTION PROTOCOLS

Field gear (i.e., wading boots, topset rods, waders) should be sterilized using standard protocols (Johnson et al. 2003, CCDAC 2007). When sampling between multiple rivers and watersheds in the same day, prioritize the order of site visits to minimize the spread of pathogens (i.e. move from most pristine to least pristine sites or from upstream to downstream), and ensure appropriate time is permitted to treat gear between sites. All equipment that has been in contact with an amphibian or in the water needs to be disinfected, including rulers, dipnets, plastic bags, or other field gear. Be sure to avoid taking wet equipment saturated with disinfectant into aquatic habitats. Quat 128 (1 oz per gallon of water) or bleach that can be diluted to approximately a 5% solution (granulated chlorine bleach has better disinfectant efficacy over time, CCADC 2007), are standard disinfectants. Submerge and immerse all items that need disinfecting in the solution for 10 minutes. A large plastic tub with a tight lid can function as a sanitization station at the office or field site, but should be completely switched with new solution every few days, particularly with heavy use. Bleach can be discarded on site, well away from any water source.

EQUIPMENT & DATASHEETS

A list of field equipment required to complete the surveys is attached at the end of this protocol. In general, equipment should be selected to be lightweight, waterproof or water resistant, and compact enough to fit within a daypack so that surveyors can be fully mobile.

The associated datasheet was developed to document the data needed at each observation. It is similar to datasheets used in previous academic research and hydropower relicensing studies (Lind 1997, Seltenrich and Pool 2002, Yarnell 2005). In order to simplify the complications and potential errors associated with multiple datasheets for different lifestages, a single datasheet is used for each survey, regardless of time of year and focus of survey (i.e., breeding surveys in spring vs. tadpole surveys in summer). The data for each lifestage observed during the survey is recorded on a single row. The habitat data collected for each lifestage may differ and as a result, some fields in the row may be marked as N/A (i.e., total depth for an adult observation). Small modifications to the datasheet may be made to accommodate unique survey situations (such as associated habitat number rather than associated GPS point when identifying observation location), but these should be kept to a minimum.

Note that the datasheet is designed to be printed in landscape format on 8 x 11.5 inch paper (see **FYLF VES Survey Datasheet.xlsx** file). Details on recording data are provided below. The datasheet is designed for collection of data in metric units, so use of English US units must be explicitly noted.

DATASHEET: GENERAL SITE DATA

Date:	Month, Day, Year
River/SiteID:	Name of river/stream/creek and site to be surveyed. If sub-reaches are used, clarify which sections are to be surveyed. For example: North Yuba River, Reach A-1, River Mile 12.5-13.5.
Surveyors:	Names or Initials of all participating surveyors. The initials of the team member filling out the datasheet should be noted first.
Start/End Time:	Record start/end times of survey. This should reflect the total time, not the actual survey/search time.
Survey Time:	Record the total active time spent surveying (HH:MM). This represents the time spent between the start and end time that is exclusively expended searching for FYLF. Time spent filling out VES data sheets, capturing or identifying animals, or taking breaks is included within the start and end times, but is excluded from Survey Time. A stopwatch should be used as this variable should not be estimated.

Start/End GPS: Coordinates of start and end survey locations on the stream. Average waypoints if possible.

Datum, Metric/US, Accy: Indicate coordinate datum (i.e., NAD83, WGS84) and circle either Metric or US if relevant. If possible record Accuracy of GPS (in same units as circled).

Air/Water Temp (˚C): Record temperature of air (in the shade) and water (well-mixed/moving flow) at start and end of survey or mid-day when possible, in degrees Celsius.

%Right/Left Bank: The proportion of the right bank or left bank (facing downstream) bank that was surveyed of the total survey reach, to the nearest 10 or 25%.

Weather: Circle the appropriate variable that describes the current survey conditions. If weather in the last 24 hours may have significance on survey, write a brief note explaining why.

Invasives? Note presence/absence of non-native predators/invasives (i.e., bullfrogs, smallmouth bass, crayfish) anywhere in survey reach. If needed, add notes on back of page. If possible provide lifestage and estimates of relative abundance for observations.

Photo numbers: Record digital photo ID numbers for photos taken throughout survey. Include photos of the start and end locations, chin photos of frogs, typical mid-channel habitat, typical edgewater and backwater habitats, examples of breeding habitat (occupied or otherwise), etc. If needed, add notes on back of page.

DATASHEET: DETAILED OBSERVATION DATA

**Note: Some fields are applicable only to certain lifestages. Be sure to record NA or "–" in the datasheet for fields not appropriate to the observation. Do not leave fields blank.*

GPS X / Y: Coordinates of observation location. Average waypoints and provide accuracy if possible. (X = Easting = Longitude, Y = Northing = Latitude). Make sure your datum and units are reflected in the header of the datasheet.

Lifestage/Sex: Note life stage of individual; enter code from list:

AF Adult Female
AM Adult Male

AU	Adult Unknown
J	Juvenile
Y	Young-of-Year (newly metamorphosed, typically fall only)
L	Larvae/Tadpole
E	Egg mass

 # Observed: Number of individuals noted in a single location (e.g., 1 AM on boulder in a riffle vs. 50 tadpoles in a backwater pool)

Gosner Stage: If possible, collect Gosner stage for egg masses and tadpoles following Gosner (1960). If categorized, then note categories on back of datasheet. If possible, take a macrophoto of the egg mass (underwater) to use for validation/verification at a later time. For comparison a web app with example photos for a variety of stages for this species can be found at *http://shiny.cws.ucdavis.edu/shiny/rapeek/Gosner_photos/*

Length (mm): For post-metamorphic individuals (A, J, Y) measure **SUL** (**Snout to urostyle length**). If necessary, (project dependent), measure tadpole **Total length** (tip of nose to tip of tail).

Total Depth (m): For all egg masses and representative number of tadpole groups, record **total** depth at the location of the observation. If egg masses are at significantly different depths than total depth make a note.

Mid-Col Velocity (m/s): For egg masses and representative number of tadpole groups, record average local **mid-column** flow velocity (0.6 of the total depth from the top) of the microhabitat using a flow meter. For egg masses, this should be immediately adjacent to the oviposition location at the midpoint of the egg mass.

EM/Perch Substrate: Substrate size of perch for adults/juveniles/YOY or attachment site for egg masses (N/A for tadpoles)

SLT	Silt
SND	Sand (< 2mm)
GRV	Gravel (2 – 64 mm)
COB	Cobble (64 – 256 mm)
BLD	Boulder (> 256 mm)
BDX	Bedrock
WOOD	Woody debris, treeroots, logs
VEG	Vegetation

Dominant Riparian Type: Describe dominant riparian/adjacent channel vegetation based on Lind (1997) to provide data on vegetation encroachment; enter code from list:

1 Gravel/Cobble Bar (side or mid channel, clear of veg)
2 Pure Willow
3 Willow/Alder Mix
4 Alder Dominant
5 Mature Riparian
6 Bedrock (clear of veg)

Geomorphic Unit: Identify the geomorphic unit at location of observation (Frissell et al. 1986, Montgomery and Buffington 1997). Categories used:

RIF (Riffle): swift flow, rippled water surface, shallow depth
RUN: slow gently moving flow, faster than a pool, slower than a riffle, moderate depth
POOL: includes flatwater, dammed pools, confluence pools, mid-channel pools and pool tail-outs
STEP: boulders transverse to the flow creating short pour-overs and plunge pools on the downstream side
RAP (Rapid): swift water with breaking waves, rapids of high gradient
BDX (Bedrock): chutes or sheet flow over bedrock

Nearest Bank: Record the bank nearest the observation, when looking downstream. RB (Right Bank), LB (Left Bank), MC (Mid-Channel).

Photo Numbers: Photos of individual or habitat at location. For Adults, Juveniles and YOY, chin photos can be very helpful for future surveys. Use white background and try to take picture with little/no angle (i.e., plane of camera is parallel to plane of frog chin, from the same distance for each frog.

Notes: Include here any relevant information. Description of local habitat, species condition, presence of non-natives, photo description, etc.

REFERENCES

CCDAC. 2007. Decontamination Protocol to Reduce the Risk of Spreading Infectious Amphibian Diseases in Freshwater Systems. California Center for Amphibian Disease Control, http://www.ccadc.us/docs/DeconForProfessionals.pdf.

Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A Hierarchical Framework for Stream Habitat Classification - Viewing Streams in a Watershed Context. Environmental Management **10**:199-214.

Gosner, K. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica:183-190.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster. 1994. Measuring and monitoring biological diversity. Standard methods for amphibians. Smithsonian Institution Press, Washington DC.

Johnson, M. L., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid Batrachochytrium dendrobatidis. Dis Aquat Organ **57**:255-260.

Lind, A. J. 1997. Survey Protocol for Foothill Yellow-legged Frogs (*Rana boylii*) in Streams. USDA Forest Service, Pacific Southwest Research Station, Arcata, CA.

Marlow, K. R., K. D. Wiseman, C. A. Wheeler, J. E. Drennan, and R. E. Jackman. 2016. Identification of Individual Foothill Yellow-Legged Frogs (*Rana boylii*) using Chin Pattern Photographs: a Non-Invasive and Effective Method for Small Population Studies. Herpetological Review **47**:193-198.

Montgomery, D., and J. Buffington. 1997. Channel-reach morphology in mountain drainage basins. Bulletin of the Geological Society of America **109**:596-611.

Seltenrich, C. P., and A. C. Pool. 2002. A Standardized Approach for Habitat Assessments and VES for the Foothill Yellow-legged Frog (*Rana boylii*). Pacific Gas & Electric Company.

Yarnell, S. M. 2005. Spatial heterogeneity of *Rana boylii* habitat: Physical processes, quantification and ecological meaningfulness. Dissertation. UC Davis.

FIELD EQUIPMENT LIST

Required:

Field notebook
Datasheets (w/copy of survey protocol) and clipboard
Clean copies of study site aerial/topo maps (for sketching habitats, survey area, etc)
Pencil, pen, sharpie
Stopwatch
Flagging
Thermometer
Binoculars
Dip net or small handheld net for scooping tadpoles and catching individuals
Clear see-thru rulers (marked in metric) to measure individual length
Small clear plastic vial or wide-mouth bottle to capture tads for identification
Camera – extra batteries, memory card
Background for chin photo pictures (white piece of paper, or something of solid color)
Handheld GPS – extra batteries
Velocity meter w/wading rod or other stick/device to measure depth – Marsh McBirney recommended – need accuracy in low velocities - +/- 0.01 m/s ideal.
Waders
Snorkeling gear – drysuit, mask/snorkel, shoes – **for spring egg mass surveys**
First Aid kit
Personal – water, food, sunscreen, bug juice, etc

Recommended:

Viewing box (ideal if made of plexiglass, but could be lightweight plastic with clear plastic affixed to hole in bottom)
Snorkeling gear – drysuit, mask/snorkel, shoes
Throw rope for use in swift water
Lifejackets
Hand lens (aid in Gosner staging and identification of limb buds and mouth parts on tadpoles)
30m tape – w/metric markings

Optional:

Range finder – to record large scale distances (river width, length of bar, etc)
Compass
Walkie talkies
Inflatable kayak, inner tube, or some means of floating river if needed – includes lifejackets, drybags, paddles, ropes, etc.