

Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park
Standard Operating Procedure (SOP) No. 1
Routine Amphibian Survey Field Methods
Version 1.0 (August 2009)

Revision History Log

Previous Version #	Revision Date	Author	Changes Made	Reasons for Change	New Version #

1.1 Routine Amphibian Survey

Routine Amphibian Surveys are the main structural component of the Amphibian Monitoring Program at Klondike Gold Rush National Historical Park. Survey methods described in the SOP pertain to all field monitoring efforts at “core” and “non-core” sites within the monitoring strategy. Routine amphibian surveys include visual encounter surveys and individual measurements of adults and juveniles.

1.1.1 Time Commitment

Time commitment for each monitoring site survey varies, depending on the breeding activity at the site, the number of adult toads to be captured, chytrid fungus sampling schedules, and the site location. Approximate time commitments for each activity of a routine amphibian survey:

Equipment preparation: 30 minutes
Driving to Dyea monitoring sites from office: 30-40 minutes
Walking to sites from vehicle parking: 5-10 minutes
Equipment disinfecting between sites: 10-20 minutes
Amphibian survey at site: 1-2 hours
Driving to office from Dyea monitoring sites: 20-25 minutes
Data entry and editing: 30-60 minutes
Data management: 60-90 minutes

Typically four to five core site visits can be completed in a nine or ten-hour monitoring day. Core sampling can be completed by one person, but may be easier with two people if there are many individuals to be captured. For extensive non-core monitoring sites, extra time is needed for travel to the sites. Chilkoot Trail sites can be logistically divided into two monitoring expeditions. A two-day, one-night trip is suitable to cover the five monitoring sites between the trailhead and Canyon City. A longer three-day, two-night trip may be necessary to visit sites between Pleasant Camp and Chilkoot Pass. See Appendix C for site descriptions and maps.

Equipment should be assembled into backpacks and vehicles for the day. An equipment list is included in SOP 2: Field Preparation and Equipment. Navigation to the survey site can be

accomplished using site maps (Appendix B). Upon arriving at each monitoring site, data recording begins using the Routine Amphibian Survey Form (Figure 1). If data is being collected with the PDA, the appropriate Pendragon Form is used (See SOP 9: Using Pendragon Forms).

1.1.2 Seasonal monitoring schedule

Core sites are visited once a week from late April to early May, or until toads start gathering at the sites for breeding. Additional searches specifically targeting adult toads are made once the period of breeding and egg laying starts, at sites with prior records of breeding. Core monitoring sites are visited at least twice-weekly, and daily if needed during the egg-laying period because toads will be concentrated at the ponds during this time. Ideally two or more full visual encounter searches occur at each core site per week, in each of the egg period, larval period, and metamorphosis period for western toads (Chelgren 2005). When several weeks have passed without new detection of adult or juvenile toads, monitoring can be reduced to once-weekly visits to the sites, with continued monitoring of larvae development. Monitoring activities end for the season when new toadlets start leaving the breeding ponds for upland forest sites.

Non-core sites within the high intensity stratum are visited at least twice in the season, at times when detection is predicted to be higher such as time of breeding or metamorphosis. Late June is an appropriate time to visit sites within the Chilkoot Trail Monitoring Panel and early July can be a suitable time to search sites monitoring panels in the White Pass area.

1.2 Survey Procedures

1.2.2 Visual Encounter Survey

Upon arrival at the site, a Routine Amphibian Survey is initiated. All Routine Amphibian Survey data is recorded on the Routine Amphibian Survey Form (Appendix 1) and is organized into the following categories:

General site information: observer, date, arrival time, start time, end time, and pond ID.

Routine Amphibian Survey Form		Observer(s)
Date	Time Start	<u>Time at Site</u> Arrive: Leave:
Pond ID	Time End	

Fields in the general site information category are defined as follows (see Routine Amphibian Survey Field Reference Sheet):

Observer(s)- the three-letter initials of individuals conducting the survey at the site

Date- date the survey is being conducted. Use MM-DD-YY format (e.g. 4/09/09 for April 9 of 2009)

Pond ID- the site number, name (e.g. “Dyea3), or 4-digit code (DY03) assigned to the pond site. If the site was not pre-assigned a number, document it as a new site and assign it a four-digit code based.

Time Start- start time of Visual Encounter Survey

Time End- end time of Visual Encounter Survey. Time between start and end is meant to represent time spent searching and net sweeping the site for amphibians, excluding time spent taking habit, weather, or amphibian measurements and recording data. Make a note or adjust end time to account for other activities. Record in 24-hour format (e.g. 16:35 for 4:35 pm)

Time at Site Arrive: time of arrival at the monitoring site recorded in 24-hour format (e.g. 16:35 for 4:35pm)

Time at Site Leave: time of departure from the monitoring site

Weather: Air temperature, precipitation, wind, cloud cover, surface glare, and ripples are recorded.

Air Temp.	Ripples	none	some	significant
Precip	dry	lt rain	heavy rain	snow/sleet
Clouds	clear	partially cloudy	overcast	
Precip last 2 days?	none,	fog,	light rain,	medium rain,
	heavy rain,	snow		

Fields in the weather category are defined as follows:

Air Temp- air temperature in the shade, approximately 1 meter off the ground, in Celcius

Ripples- circle “none” if the surface of the water is perfectly flat and smooth. Circle “some” if there is some disturbance in the surface, but there is still good visibility beneath the water. Circle “significant” if visibility below the surface is significantly affected by ripples or surface disturbance.

Precip- circle “dry” if no precipitation falls during all or most of the survey. Circle “lt rain” if there is light rain falling during all or most of the survey. Circle “heavy rain” if a heavy rain is falling during all or most of the survey. Circle “snow/sleet” if snow or sleet is falling during all or most of the survey.

Wind- circle “calm” if there is no wind. Circle “light” if the wind is 0-10 knots (0-12mph, 0-19kph, Beaufort 1-3). Circle “mod” when the wind speed is 10-20 knots (13-24mph, 20-38kph, Beaufort 4-5), circle “heavy” if the wind speed is over 20 knots (>24mph, >38kph, >Beaufort 5). Clouds- circle “clear” when cloud cover is 0-35%, “partially cloudy” when cloud cover is 35-70%, “overcast” when cloud cover is >70%

Surface Glare- circle “none” if there is no visible glare off the surface of the water, “some” if there is noticeable glare but still some visibility below the surface, or “significant” if below-surface visibility is significantly affected by glare. Polarized glasses should be used during field surveys and when recording surface glare data.

Precip last 2 days?- circle the condition most dominant in the past two days. Selected precipitation rating should represent conditions for a minimum of ½ day during the past two days.

Habitat information: Water level, clarity, turbidity, iron floc, sheen, vegetation, and water temperatures are recorded.

Habitat	Water level high average low dry		
Clarity clear stained	Turbidity clear cloudy	iron floc absent present severe	
Sheen none organic petro	Pictures? Yes No Picture #'s:		
%Shallow	% surface area w/ emergent vegetation		
% bottom cover by submergent veg	% surface area w/ floating vegetation		
% shoreline covered by snow	% surface area covered by snow		
Water Temp	loc'n	Temp Logger deployed collected	
Water Temp	loc'n	Under water? Yes No Light?	
Water Temp	loc'n	Temp Time	
Water Temp	loc'n		
Water Temp	loc'n		
Water Temp	loc'n		

Fields in the Habitat Information category are defined as follows:

Water level- circle the best description of the water level relative to the water level at the site throughout the season. For an initial rating, refer to pictures from the previous year

Clarity- select the true color of the water, represented by the materials dissolved in the water and not by pond floor material

Turbidity- how cloudy the water is, represented by the mixture of dissolved or suspended sediments in the water column

Iron floc- iron flocculent is formed when highly mineralized, iron-rich groundwater surfaces, comes into contact with air and oxidizes, forming an orange scum that coats rocks and vegetation (Hocker 2003). Record the presence of rusty orange to red color in the water or on the bottom of the pond.



Figure 1.1 Iron flock forms a rusty-orange colored scum on rocks and vegetation in wetlands.

Sheen- sheen is a shiny, rainbow-colored smear across the surface of stagnant water. To test whether the sheen is organic or inorganic, stir through it with a finger or a stick. If it swirls, it is likely inorganic and petroleum-based, such as oil or gas. If it breaks apart into plates, it's most likely organic in origin (Hocker 2003). Note whether or not sheen is detected on the surface of the water and whether the source is petroleum or organic.

Pictures- Indicate if pictures are taken of the site and record photo numbers assigned to the image by the digital camera. Do not include pictures taken of individual amphibians in this field.

%Shallow- record the percentage of the site area that is covered in water less than 0.5 meters deep

%Shoreline w/ emergent vegetation- record the percentage of the site area that is covered by emergent vegetation

%Bottom covered by submergent vegetation- record the percentage of the site area that is covered by submerged vegetation

%Shoreline covered by snow- record the percentage of the shoreline that is covered by snow

%Surface area covered by snow- record the percentage of the pond and site surface area that is covered by snow

Water temperature- collect water temperatures in different locations of the pond site. Submerge thermometer until temperature reading stabilizes and record the number in Celsius.

Location- describe the location of water temperature measurements taken at the site in relation to general compass direction and the depth (e.g. NW edge of pond, at surface)

Amphibian Search: The entire perimeter of the wetland is scanned for embryos and larvae by having one person wading and the other walking along the shoreline. Polarized sunglasses must be worn to reduce glare and increase visibility below the surface of the water. Early in the season, eggs and tadpoles can be very small and difficult to see. Care is taken to closely examine the path ahead to ensure eggs and larvae are not trampled. If moveable cover items are present, they can be moved to see if larvae are hiding beneath them. Vegetated areas and other habitats likely to harbor embryos and larvae should also be searched. Record each species-stage detected and total numbers of each life-stage of each species encountered are recorded on the datasheet. If toadlets (metamorphs) or adult amphibians are found, they are captured using a dipnet and are placed in individual containers if there are too many to keep track of at the site (see SOP No. 7: Amphibian handling guidelines).

Amphibian Search					Full? Yes No		% Shore Searched	
Connection inlet outlet both neither						Recent disturbances?		
% bottom visible					Fish Present? Yes No			
Fish Species						Other Predators?		
Survey Method		visual	hand ID	boards	traps			
net sweep					hand ID	boards	traps	net sweep
Amphibian Species Seen (if any seen, complete back of form also)							Where?	

Fields in the amphibian search data are defined as follows:

Full?- indicates whether the entire length of the shoreline is searched (yes or no)

%Shore searched- percentage of shoreline searched

Connection- indicates whether the water body is visibly connected to the water source (inlet) or outlet. Bubbles in the substrate may indicate underground inlets.

Recent disturbances?- note any human disturbances such as trash, foot prints, vehicle tracks, natural disturbances such as flooding, landslides, windfall, or any significant changes to the pond/site structure

%Bottom visible- percent of the bottom up to 2m depth that is visible, excluding areas that are covered with vegetation, obscured by stained or turbid water, or other obstructed by anything

Fish present?- were any fish observed during the survey?

Fish species- record fish species observed, if known

Other predators?- note signs or presence of other predators. Common predators are predaceous diving beetles, damselfly or dragonfly nymphs, other aquatic beetles and insects, domestic dogs, coyotes, foxes, weasels, minks, martens, badgers, black bears, owls, magpies, ravens, crows, jays, shrikes, mallards, and spotted sandpipers.

Survey method- circle methods used to search for amphibians at the site during the survey
 Amphibian Species Seen- For each species record the first two letters of the scientific genus and species names (e.g. BUBO for Bufo boreas)

Where?- describe where amphibians were generally found at the site (e.g. E shore of pond in emergent veg <1m water depth)

Egg Masses and Larvae: If egg masses are distinct, they are counted. If egg masses cannot be counted, their number is estimated based on the average size of isolated egg masses. For larvae, ocular estimation is used to determine total numbers (e.g., a crew member counts all the larvae found within a small area and then counts the number of those size areas which have the same density). Median body length of larvae is determined by measuring the largest, smallest, and a few in the middle range (Appendix B. Body Measurement of Tadpoles). Median Gosner stage development should be determined with the least amount of disturbance to larvae or larval mass (Appendix C. Gosner Developmental Stages). Care is taken to search the area in a manner that prevents double-counting individuals.

Egg Masses and Larvae							
Location within Site:							
Individual ID (EM1, L1)							
Estimated # in egg mass							
Estimated total # larvae							
Tot. Length							
Body Length							
Gosner stage							

If egg masses and/or larvae are encountered, data is recorded based on the following definitions:

Location within site- description of where egg masses and larvae were found at the survey site

Individual ID- assigned alpha-numerical identification number for the day and site of capture (e.g. EM1 for the first egg mass identified at the site that day, or L3 for the third larvae measured).

Estimated # in egg mass- estimate number of individual eggs within an egg mass

Estimated total # of larvae- visually estimate total number of tadpoles, counting by 10's or 100's

Tot. Length- average total length of tadpoles at the site. Measure the length from the tip of the snout, not including any fleshy projections from the oral disk, to the end of the tail.

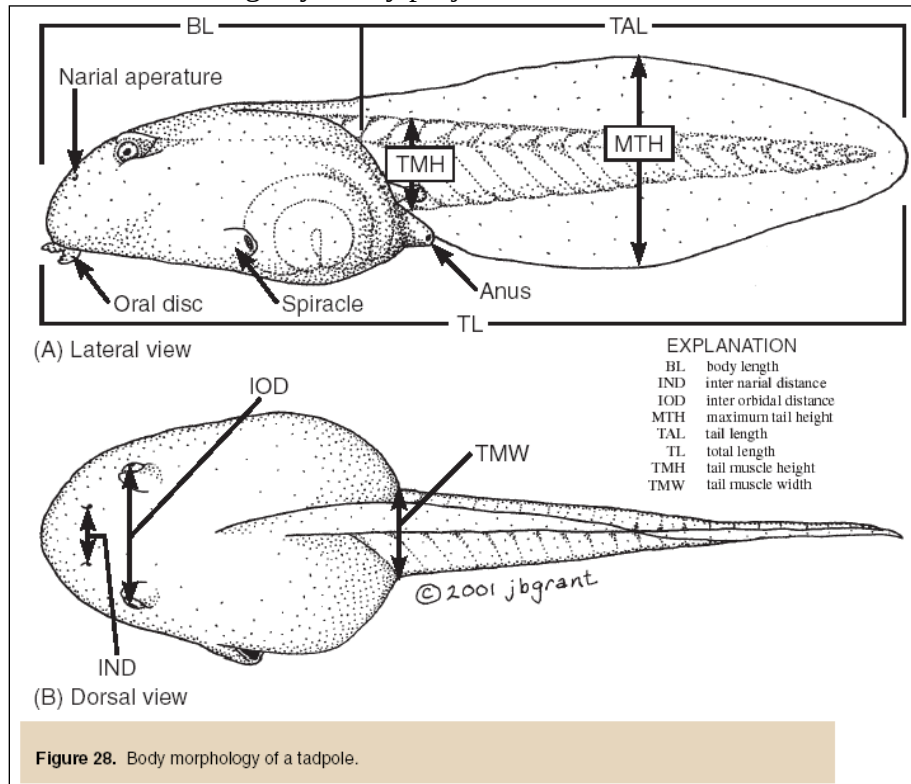


Figure 1.2 Body length (BL) and Total Length (TAL) measurement locations for tadpoles.

Body Length- average body length of tadpoles at the site. Measure the length from the tip of the snout, not including any fleshy projections from the oral disk, to the junction of the posterior body wall with the axis of the tail myotomes. The axis is defined by an imaginary line connecting the apices of the tail myotemes (Altig 2005). Measure 6 tadpoles at the site to calculate an average length and length range.

Gosner stage- record the average Gosner stage of larvae at the site

1.2.3 Adult and Juvenile toad measurements.

Capturing adult and juvenile toads is conducted according to the handling considerations and instructions outlined in SOP 7: Amphibian Handling Guidelines. A net may be used to facilitate capture at monitoring sites.

Measurements are collected and recorded for each captured adult amphibian: SVL, Gape, Forearm, Weight, Nuptial pads, Calling, Location within site, and Individual ID (Appendix 2. Field Reference Sheet)

Location within Site:							
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Individual ID (e.g. A1,J2)							
SVL (mm)							
Gape (mm)							
Forearm (mm)							
Weight (g)							
Nuptial pads?							
Calling?							
Tag? (Y/N)							
Swab?(Y/N)							

If juvenile or adult toads are captured during the Visual Encounter Survey, measurement data is recorded according to the following definitions:

Location within site- general area where the amphibian was found at the site (e.g. SE shore of pond in submerged veg <0.5 m water depth)

Individual ID- assigned alpha-numerical identification number for the day and site of capture (e.g. A1 for adult 1 observed and/or captured at the site that day, or J3 for the juvenile observed and/or captured at the site that day)

SVL (mm)- snout-to-vent length of the amphibian

Gape (mm)- head width at the point of jaw articulation

Forearm (mm)- length of the right forearm from the end of the bent elbow (including the elbow) to the tip of the longest toe. Straighten the limb gently so the elbow is at 90 degrees before measuring.

Weight (g)- weight of toad after subtracting empty weight of the bag

Nuptial pads?- note the presence or absence of darkened nuptial pads on the thumbs and first 2 fingers (Figure)

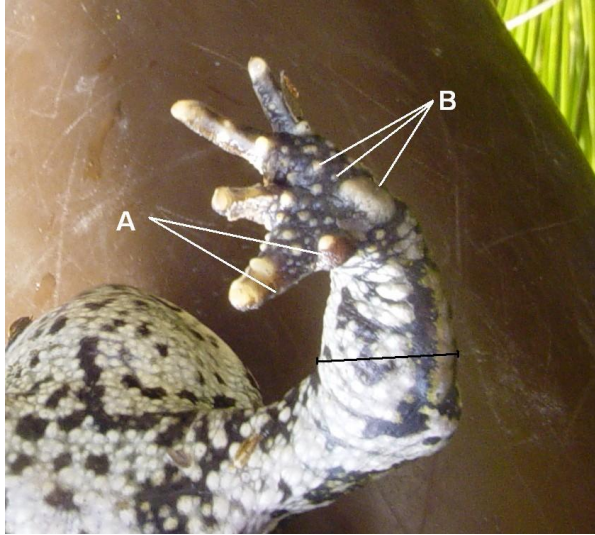


Figure 1.3 Tubercles (B, light in color) are present on all toads. Darkened nuptial pads (A), effectively calluses, are present only on males and are most developed during the breeding season.

Calling?- note whether a chirp-like release call is made by the individual when handled

Tag?- Is a PIT tag detected? Any adult toad >45 mm SVL that is encountered in the study area is scanned for an existing PIT tag. If a PIT tag is identified, the toad is considered a “recapture”.



Figure 1.4 PIT tag reader scanning an adult toad for a tag number.

Swab?- was a swab taken to test for chytrid fungus? If sampling is being conducted for chytrid fungus (*Batrachochytrium dendrobatidis*), a swab is collected for each adult amphibian and the swab number is recorded (see SOP No.4 Chytrid Fungus Testing Protocol).

During Visual Encounter Surveys, amphibian pairs in amplexus should not be separated, but should be captured after they separate naturally. Sites of known amplexing pairs are frequented throughout the day to check on their status. Amphibians are released as close to the spot of capture as possible. While conducting adult and juvenile measurements, it is helpful to keep individuals in plastic containers (see SOP 7: Amphibian Handling Guidelines) to avoid recapture and repeat sampling until the site survey is completed.

1.2.4 Completion of Routine Amphibian Surveys

Comments and notes pertaining to the habitat, amphibians, data collected or general activities are recorded on the amphibian survey form or in a separate field notebook. At the end of each site survey the “end time” and “site leave time” must be recorded.

1.3 Cleaning Field Equipment

All field equipment that has come in contact with stream water, including all footwear, must be cleaned and disinfected between any sites that are not “water-connected”, or that amphibians don’t freely move between. At the end of each sampling day all field equipment must be thoroughly cleaned and sterilized. Procedures for cleaning field equipment exposed to stream water during sampling are found in SOP No.3: Hygiene Protocol.

References

- Altig, R. (2006). "Comments on the descriptions and evaluations of tadpole mouthpart anomalies." *Herpetological Conservation and Biology* 2(1): 1-4.
- Chelgren, N. (2005). Some Thoughts on Toad Monitoring. B. Kevin Payne, KLGO. Skagway, Alaska: 10.
- Hocker, K., T. Schwarz (2003). *Streamwalker's Companion*. Juneau, Discovery Southeast.

Appendix A. Routine Amphibian Survey Form and Field Reference Guide

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KLGO Routine Amphibian Survey Field Reference Sheet

General site data

Observers- initials of individuals

Date- MM-DD-YY

Pond ID- site number (e.g. Dyea3)

Time Start- start time of amphib. search

Time End- end time of amphib. search

Arrive- time of arrival at site

Leave- time of departure from site

Weather data

Air Temp- deg C in shade, 1 m off ground

Ripples- **none-** water is flat and smooth
some- some disturbance in the surface, but still good visibility beneath the water.
significant- visibility below the surface is affected by ripples

Precip- **dry-** no precipitation during survey
lt rain- light rain falling during survey
heavy rain- heavy rain falling
snow/sleet- snow or sleet falling

Wind- **calm-** no wind.
light- wind 0-10 knots (0-12mph, 0-19kph, Beaufort 1-3).
mod- wind 10-20 knots (13-24mph, 20-38kph, Beaufort 4-5),
heavy- wind over 20 knots (>24mph, >38kph, >Beaufort 5).

Clouds- **clear-** cloud cover 0-35%,
partially cloudy- cloud cover 35-70%,
overcast- cloud cover >70%

Surface Glare- (when polarized sunglasses are used) **none-** no visible glare off water
some- noticeable glare but still some visibility below the surface,
significant- below-surface visibility affected by glare.

Precip last 2 days?- condition most dominant in the past two days

Habitat data

Water level- relative to water level at the site throughout the season

Clarity- true color of the water (not pond floor)

Turbidity- how cloudy the water is from the mixture of dissolved or suspended sediments in the water column

Iron floc- presence of rusty orange to red color in the water or on the bottom of the pond.

Sheen- shiny, rainbow-colored smear across the surface of stagnant water. If it swirls when stirred with a stick, it is likely inorganic and petroleum-based, such as oil or gas. If it breaks apart into plates, it's most likely organic in origin.

Pictures- photo numbers assigned to the image by the digital camera.

%Shallow- % of site covered in water <0.5 meters deep

%Shoreline w/ emergent vegetation- % of shoreline covered by emergent vegetation

%Bottom covered by submergent vegetation- % of site covered by submerged vegetation

%Shoreline covered by snow- % of shoreline covered by snow

%Surface area covered by snow- % of site covered by snow

Water temp (C)- from different locations in pond. Submerge thermometer until temperature reading stabilizes

Location- compass direction and depth of water temps (e.g. NW edge at surface)

Temp Logger- condition of temp logger
Under water?- is the logger under water?
Light?- is the light on?
Temp- record temp reading with thermometer w/in 1 inch of temp logger
Time- time of water temp reading

Amphibian Search Data

Full?- all of the shoreline searched?

%Shore searched- % shoreline searched

Connection- how water body is visibly connected to the water source (inlet) or outlet.

KLGO Routine Amphibian Survey Field Reference Sheet

Recent disturbances?- any human or natural disturbances (i.e trash, foot prints, vehicle tracks, landslides, flood)

%Bottom visible- % of bottom < 2m depth that is visible, excluding areas that are obstructed, covered with vegetation, or obscured by stained or turbid water

Fish present?- fish observed during the survey?

Fish species- fish species observed

Other predators?- signs or presence of predators: predaceous diving beetles, damselfly or dragonfly nymphs, other aquatic beetles and insects, domestic dogs, coyotes, foxes, weasels, minks, martens, badgers, black bears, owls, magpies, ravens, crows, jays, shrikes, mallards, and spotted sandpipers.

Survey method- methods used to search for amphibians at the site

Amphibian Species Seen- first two letters of the scientific genus and species names
BUFO- Western toad
RALU- Columbia spotted frog
UNID- Unidentified

Where?- where amphibians were generally found at site (e.g. E shore of pond in emergent veg <1m water depth)

Amphibian Capture Data

Location within site- general area where amphibian was found at the site (e.g. SE shore of pond in submerged veg <0.5 m water depth)

Individual ID- assign an alpha-numeric ID # (eg. A1, A2 or J1, J2) A=adult, J=juvenile.

SVL (mm)- snout-to-vent length

Gape (mm)- head width at point of jaw articulation

Forearm (mm)- right forearm length from the end of the bent elbow to the tip of the longest toe

Weight (g)- weight of toad after subtracting empty weight of bag

Nuptial pads?- presence or absence of darkened nuptial pads on the thumbs and first 2 fingers

Calling?- a chirp-like release call is made by the individual when handled

Tag?- is a PIT tag detected

Swab?- was a swab taken to test for chytrid fungus?

Egg Masses and Larvae Data

Location within site- where egg masses and larvae were found at the survey site (eg. NW edge under logs)

Individual ID- assign an alpha-numeric ID# (eg. EM1, EM2 or L1) EM=egg mass, L=larva

Estimated # in egg mass- estimate number of individual eggs within an egg mass

Estimated total # of larvae- estimate total number of tadpoles, counting by 10's or 100's

Tot. Length- average total length of tadpoles at the site from the tip of the snout (excluding fleshy projections from the oral disk) to the end of the tail

Body Length- average body length of tadpoles at the site from the tip of the snout (excluding fleshy projections from the oral disk) to the junction of the posterior body wall with the axis of the tail myotomes.

Gosner stage- average Gosner stage of larvae at the site

Recaptures

Individual ID- assigned alpha numeric #

Tag #- tag number identified by the PIT tag scanner

Swab ID- swab number taken from the individual for chytrid fungus testing

Appendix B. Chart of Gosner Development Stages

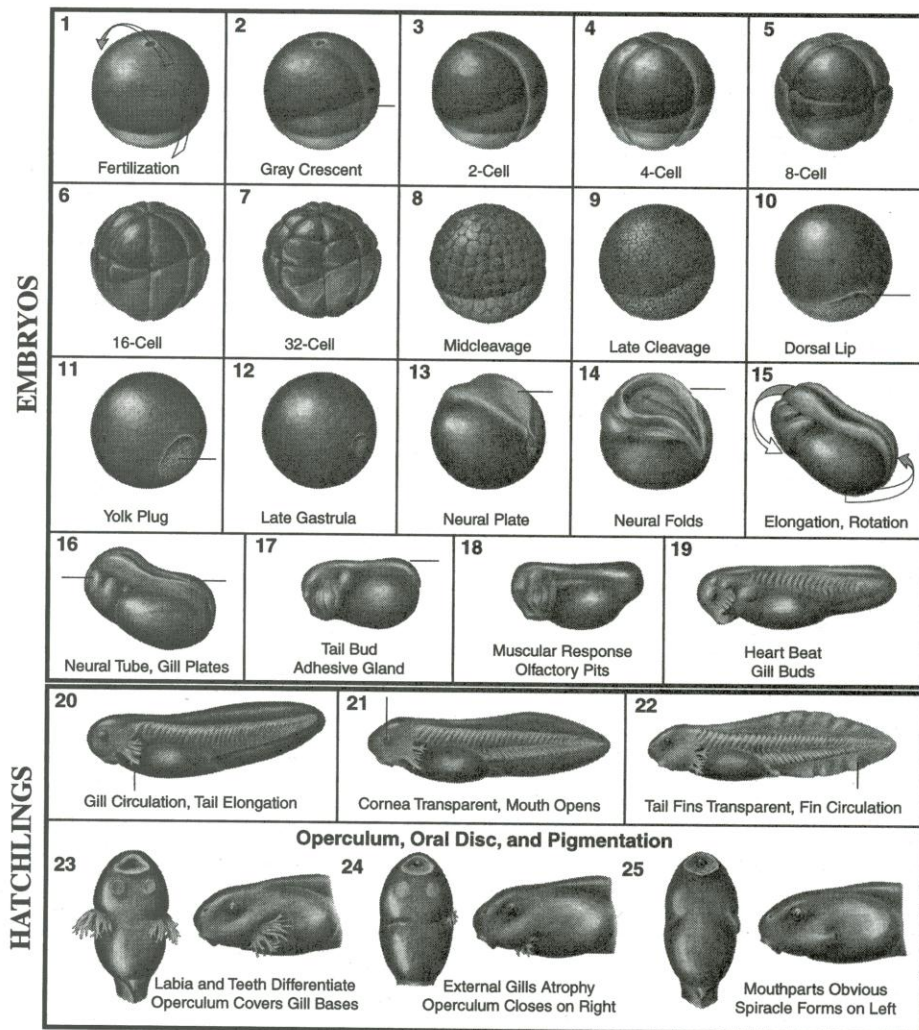


Fig. 2.1. The Gosner (1960) staging system recommended for use with exotrophic tadpoles; developmental stages are based on *Bufo variiceps* raised at 25° C. Translations to other staging systems are shown in table 2.1. Embryos: stages 1–19, 41.5 h (6.2%) developmental time. After fertilization (stage 1) and release of the second polar body (2), the zygote undergoes cleavage to stage 9 without an increase in size. Germ layers begin to form during gastrulation (10–12), which is followed by neural tube formation (13–15). Sensory structures appear during stages 16–19 and somitogenesis occurs in stages 18–19. Hatching may occur as early as stage 16. Hatchlings: stages 20–25, 73.5 h (11.0%) developmental time. This period represents the transition from a relatively immobile embryo to an active, feeding tadpole; external gills atrophy and the spiracle forms. Structures associated with feeding and swimming appear and pigments begin to form larval color

patterns. Tadpoles: stages 26–41, 384 h (57.6%) developmental time. This is the longest part of the larval period and is marked by growth and limb development. Metamorphosis: stages 41–46, 168 h (25.2%) developmental time. During this crucial period the tadpole loses its larval characteristics and takes on adult structures; the tail begins to atrophy (43), larval feeding structures are replaced by adult jaws and tongue (41–43), and forelimbs and hind limbs become functional. This period typically is marked by a passage from the aquatic to the terrestrial environment. Metamorphosis is complete (46) when the tadpole has become a froglet. Original drawings of stages 1–25 were provided by Linda Trueb (Duellman and Trueb 1986); depictions of stages 26–46 were redrawn by Kate Spencer; additional drawings were prepared by Ty Thierry.

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