Herps on the FERP
Monitoring Protocol

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Version 1.0: June 2015
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1. Introduction

1.1. Purpose

The goal of this project is to track long term spatial and temporal patterns in the populations of herpetofauna, a group of animals comprised of amphibians and reptiles, on the UC Santa Cruz Forest Ecology Research Plot (UCSC FERP). Herpetofauna biodiversity is a good indicator for ecosystem health, and establishing long term monitoring systems can provide important insights into the drivers behind local population changes and worldwide declines (Heyer et al. 1994). At the time of this writing, there is no other long term monitoring system for herpetofauna on the UCSC campus. The monitoring system described in this document involves both terrestrial and aquatic sampling using a network of coverboards combined with periodic aquatic dip netting. The monitoring system will be a resource for future student research projects.

1.2. Study Area

The 16 ha FERP was established in 2007 to study the dynamics of woody vegetation in a mapped forest plot and to provide a resource on campus for other ecological research (Gilbert et al. 2010). The abundance of spatial and temporal information available on the FERP, such as meteorological, topographical, soils, and vegetation data will allow for comparative analyses of herpetofauna monitoring data (G. Gilbert et al., unpublished data). The plot extends 400m east and north of a 0,0 post placed at NAD 1983 UTM Zone 10 latitude, longitude coordinates (582297.5,4096646.1) deg. offset by (-7.7981,-3.7478) m with local east and north coordinates aligned to a declination of -14.67 deg. The western half of the plot is mixed evergreen forest dominated by Douglas fir (Pseudotsuga menziesii), and several species of oaks (Quercus agrifolia, Q. parvula, and Notholithocarpus densiflorus) and is bounded by Empire Grade Road and a small rural community (Haff et al. 2008; Gilbert et al. 2010). On the eastern half, the forest transitions into second growth coastal redwood forest (Sequoia sempervirens; see Appendix C.3. Three streambeds converge in the southeast corner to form the intermittent headwaters of Cave Gulch Creek, a tributary of Wilder Creek with several perennial pools that persist during the dry season when connecting stream beds do not contain surface water. An ephemeral stream drains directly into Wilder Creek from the southwest corner of the plot (see Appendix C.3).

1.3. Coverboards

Plywood coverboards are used to sample herpetofauna by providing artificial refuge, and are less disruptive to the surrounding habitat compared to other sampling methods. Time-constrained and transect searches require disturbing existing coarse woody debris and leaf litter, and drift fences with pitfall traps pose a threat for the animals, which may die upon landing at the bottom or consume one another in the pit (Tietje and Vreeland 1997, Hesed 2012). There are 115 coverboards set on a systematic grid throughout the FERP (see Section 2.1).
1.4. Aquatic Sampling

Aquatic sampling is an integral part of long term amphibian monitoring. Salamanders are known to play key roles in the ecology of many headwaters streams, and monitoring larval development can provide important information about salamander population growth (Heyer et al. 1994; Davic and Welsh 2004; Becker et al. 2013). Three species of salamanders found on campus breed aquatically: the California giant salamander (*Dicamptodon ensatus*), rough-skinned newt (*Taricha granulosa*) and California newt (*T. torosa*; Haff et al. 2008). Both species of newt mate and lay eggs between late December and early June, while California giant salamanders most likely mate in spring (Blaustein et al. 1995, Petranka 1998). We observed rough-skinned newts in amplexus and California giant salamander larvae on the FERP while establishing coverboards and during the pilot study completed June 2015. Periodic sampling to track larval development and metamorphosis in these pools will determine if breeding efforts of these salamanders are successful.
2. Coverboard Establishment and Maintenance

2.1. Coverboard Establishment

• There are 115 plywood coverboards that are 1.27cm (1/2in) thick squares measuring 0.61x0.61m (2x2ft), with a surface area of 0.37m² (4ft²).

• They are placed on a 40x40 m grid across the plot.
  - 79 coverboards were placed systematically “ongrid” every 40 m, centered at 1m W of corner posts.
  - 36 (half) of the ongrid coverboards are paired with a second board “offgrid” at a randomized location at a cardinal direction (N, S, E or W) and offset distance of 1m, 4m or 6m from the corner post.
  - When it was not possible to use the preselected spots due to obstacles such as trees, trails and streams, boards were placed at the selected distances in different cardinal directions.

• Vegetation and duff under the coverboards were removed to establish them on bare mineral soil for standardized sampling and reduction of potentially confounding factors such as mold growth.

• Stakes made from PVC reinforced with rebar were pounded to keep coverboards on steep slopes and in ephemeral streams from moving.

• Coverboards were established between Sept 2014 and Feb 2015, then given at least five weeks to weather in place before beginning sampling (Hesed 2012).

• Monitoring began on 15 March 2015 and will continue every two weeks throughout the year.

2.2. Clearing Vegetation

• Coverboards should be regularly monitored and maintained to keep coverboards from being overtaken by vegetation so they can be easily moved during sampling.

• When possible, simply redirect or move vegetation. Keep in mind that woody plants, including trees, shrubs and vines, are being studied on the FERP and should be left as undisturbed as possible. Never cut tagged plants.

• Remove vegetation growing beneath the coverboards, such as sprouts and runners, during each field monitoring day when sampling the coverboards. If a plant is too deeply rooted to be removed by hand, make a note of it in the notes section of the “Coverboard Sampling Data Sheet” (see Appendix C.2.1) and return to it the next field day with garden tools (see Appendix B.2) for removal. Ideally the coverboards should be placed directly on bare mineral soil, but major roots and root systems need to be left under the coverboard to minimize damage to the surrounding vegetation.

• At the end of every academic Quarter, perform maintenance on all coverboards. Use clippers to trim surrounding herbaceous vegetation to keep it from encroaching on the coverboards, as well as a hand cultivator and trowel to smooth the soil under the boards and clear disturbances from growing roots and tunneling animals (see Appendix B.2).
2.3. Replacing Old Coverboards

- The typical plywood coverboard needs to be replaced every 3-5 years based on level of degradation (Hesed 2012). They should be replaced when they break or warp and no longer provide effective cover for herpetofauna, when they cannot be lifted as one piece, or do not lay mostly flat on the soil.

- Place new coverboards with the dimensions described in Section 2.1 in exactly the same locations as the old coverboards.

- They must all be clearly marked with a black oil-based paint pen with:
  - Their specific coverboard number and location to allow for easy data collection and avoid confusion in the field.
  - Mark coverboards close to trails with “Do Not Disturb” and “Gilbert Lab”. Write the FERP website (http://ferp.ucsc.edu) on the coverboard so curious people can see what sorts of studies are happening in the area and be more inclined to respectfully leave the coverboards in place.
  - If a coverboard breaks or is greatly damaged during a field day, replace it that day or the following day if a new coverboard cannot be acquired immediately. This will allow them to weather for the two weeks before the next data collection. It is important to allow them to weather for as long as possible before they are sampled (Hesed 2012).
  - Always record what day a coverboard is replaced on the “Coverboard Sampling Data Sheet” (see Appendix C.2) and in the log on Google Drive as described in Section 5.3.

2.4. Special Considerations

- Coverboards placed at extreme angles need extra support from PVC pipe reinforced with rebar to keep them in place

- Coverboards placed in seasonally flooded areas will degrade faster than those placed in dry areas and should be evaluated yearly for potential replacement. These coverboards also must be reinforced with PVC and rebar to ensure they do not float away in the streams.
3. Permits, Animal Handling, Sanitizing Equipment and Field Safety

3.1. Permits

- To handle animals for any scientific purpose at UCSC, you must be added to the UCSC Natural Reserves’ Live Vertebrate Animal—Non-Biomedical protocol through the UCSC Institutional Animal Use and Care Committee (IACUC). The appropriate paperwork and the training can be acquired by contacting the UCSC Campus Natural Reserve Manager; as of 2015, this is Alex Jones.

- Email Alex Jones (asjones@ucsc.edu) or the UCSC Campus Natural Reserve Steward asking to be placed on the animal handling protocol through the American Association of Laboratory Science (AALS). In this message you will also need to send your resume or CV. They will then send you the appropriate links to the next steps.

- The UCSC Natural Reserves staff member will start the Occupational Health and Safety Service (OHSS) process and you will receive an email when the animal handling risk assessment is ready for your review. You will then be prompted to complete a medical questionnaire. In order to fill out the questionnaire to the best of your ability, please have information on vaccines, allergies, prescription medications and general health history on hand. The questionnaire will be reviewed by a doctor who may contact you with questions. After this, you will receive an email from OHSS when the risk assessment is ready for your final review.

- You will need to complete the online IACUC training for animal handling. Currently, the trainings consist of the module for Option B: Laboratory Animal Welfare. Be sure to complete and pass all portions of this training.

- Once these steps are completed, IACUC members will review your materials and add you onto the protocol once everything clears. Be sure to keep in contact with Natural Reserves staff to let them know your progress to keep everything on track.

- Complete this as soon possible. The process can take several weeks, and if delayed and will keep you from handling the animals in the field.

3.2. Animal Handling

- The following safe animal handling practices will reduce stress to animals during sampling. Gently pick up the animals by lifting them from the ground with two fingers grasping the sides of the animal. For larger herpetofauna like lizards, it is appropriate to hold them gently by their hips or shoulders. Handle snakes with two hands with one at the base of the head and the other towards the base of the tail.

- It is important that you keep the animal low to ground when handling and measuring. The animals will move and attempt to escape. If they fall, it is better for them to drop a smaller distance from the ground to reduce trauma.

- Minimize time spent handling the animals. Time spent handling the animal in and out of the bag stresses the animal.
• Take all measurements with the animal in a plastic bag. Gently place the animal at the bottom of the bag and roll the bag to keep the animal from moving. If the animal is a larger snake, a pillow case can be used.

• It is important that the amphibians encountered in the study are kept moist. After all measurements are taken, pour clean, non-chlorinated water onto the animal so it does not dry out from handling. If an amphibian begins to feel dry or “tacky” during handling, immediately pour clean, non-chlorinated water on the animal.

• After all animals have been measured, replace the coverboard, place the animals next to it and encourage them to take refuge back under the coverboard. Do not force them under the coverboard or place the coverboard on top of them as it may harm them.

• Any animal in the area of the coverboard, including under or on top of the coverboards, needs to be measured. This does not include animals next to the board. Make an attempt to capture snakes and lizards that dart away if it can be done without harming them. It is best to try and grab the animal at a point where it is thick, so grab snakes behind the head and grab lizards by their sides.

• Never hold a lizard or salamander by its tail, as they may lose it as a defense mechanism.

• To reduce possible disease transfer
  o Place each animal in a previously unused plastic bag. Discard the bag after a single use and do not use it again unless properly sanitized in a bleach solution (see Section 3.3). Please make an attempt to place these bags in an appropriate recycling bin.
  o Rinse off or change your gloves after handling every animal.
  o During aquatic sampling, change your gloves between pools.

• All of these techniques will be described and shown to new technicians in the field by a supervisor. Ask your supervisor for any clarifications needed.

3.3. Sanitizing Equipment

To prevent the spread of disease, it is important to clean dip nets and plastic containers before and/or after each trip to the field using the methods of Fellers and Freel (1995). Plastic bags may also be reused if properly sanitized.

• Fill a bucket with 10% bleach solution by combining 1:9 parts bleach to water.

• Submerge equipment in solution for 1-5mins.

• Remove and rinse thoroughly to remove all bleach, which can be harmful to animals and degrade equipment.

• Let the equipment dry overnight, allowing chlorine to evaporate before use or storage.

• Dispose of bleach solution properly by pouring the solution down a sink drain on campus (if sanitizing equipment off campus, never pour bleach in to a septic system).
3.4. Field Safety

Working on the FERP comes with some safety practices to keep in mind.

- Many of the coverboards are in or around poison oak (*Toxicodendron diversilobum*). You will be provided with a coverall suit to shield you from the plant’s toxic oils which can cause a rash if it comes in contact with your skin. You must also wear latex or nitrile gloves to protect your hands from the oils. Be aware of what poison oak looks like and know how to identify it in all seasons. Avoid it when practical and wash your hands and arms with Tecnu after every field day to remove any oils that may have gotten on you. Tecnu is available in the FERP lab. It is not recommended to participate in this study if you have severe reactions to poison oak as it is abundant within the study area.

- There are a lot of downed logs and sharp changes in topography. It is easy to fall or trip in these areas. Please use caution when traveling between coverboards to minimize the chance of falling. Use the safest route and follow the trails when available. Avoid walking on logs or on an unstable platform of any kind.

- Be aware of branches or other poking hazards in your surroundings to avoid eye injuries. Please cross streams and ravines as little as possible and find the path with the lowest gradient, as frequent crossings lead to increased erosion and can be dangerous. Working in steep ravines, especially during aquatic sampling, requires extra care and caution. To avoid falling, notice the slope, keep your balance, and never do anything outside your comfort level.

- Always travel with at least one other person and know where you are in relation to Chinquapin Road and Empire Grade at all times. Cell phone reception is poor on the FERP. In case of an emergency it is imperative you know where to go to contact emergency services. Cell phone reception tends to be best at the junction of the Seven Springs Trail and Chinquapin Road and at Empire Grade (see map in Appendix C.3), depending on your cell phone carrier. Find where your phone does and does not work on the first day in the area.

- If any injury/medical incident does occur, such as animal bites or possible transfer of zoonotic disease, please get immediate attention. These must also be reported to UCSC Natural Reserves staff. A supervisor will go over this information in more detail with you in person.
4. Field Methods

4.1. Coverboard Sampling Protocol

- **When to Sample**
  - Coverboard sampling occurs every two weeks, as close to the same weekday as possible, to retain consistency and allow for the coverboards to re-weather in place after the disturbance of sampling them (Hesed 2012).
  - To keep data consistent, check all coverboards in the same way, at roughly the same time, every field day. Begin sampling between the hours of 10:30 AM and 1:30 PM to stay within the range of consistent measurements while allowing for scheduling preferences.

- **Sampling**
  - Use the “Coverboard Grids with Locations Lists” (see Appendix C.2) to navigate and mark your progress and the “Coverboard Sampling Data Sheet” (see Appendix C.2) to record data. Also, see Section 4.3 for detailed instructions on measuring and recording data.
  - Record the coverboard number and time when you flip the coverboard on the “Coverboard Sampling Data Sheet” (see Appendix C.2).
  - All herpetofauna found under and on top of the coverboard need to be measured. Do not measure animals found next to the coverboard or in the surrounding area. You may need to go after animals that flee when you arrive.
  - The coverboards are assessed first by a visual scan to see if any animals are present on top of the coverboard and second by checking underneath the coverboard.
  - To check underneath the coverboards, you stay to one side of the board while lifting an edge, rather than standing in front of the opening edge. This way, if an animal is startled by the coverboard being lifted, they have an escape path away from you, which reduces risk of injury. However, you will need to keep track of the animal to measure it.
  - When multiple animals are found under a coverboard, number them on the “Coverboard Sampling Datasheet” in the order measured (see Figure 2).
    - Record all of the measurements for herp number 1 before placing subsequent animals in a bag. This will keep the animals from incurring unnecessary stress and keep their temperature measurement more accurate.
    - Keep track of the subsequent animal(s) to make sure they do not escape. If the animals do try to escape, place each of them gently and individually in wide open bag(s) where you can monitor each animal’s activity.
    - Reduce unnecessary stress when practical, but make sure all animals found in the space of the coverboard are measured.
After all the animals have been measured, gently pour clean non-chlorinated water on amphibians, replace the coverboard, place animals next to it and encourage them to take refuge back under the coverboard. Do not force them under the coverboard or place the coverboard on top of them as it may harm them.

4.2. Aquatic Sampling Protocol

- **When to Sample**
  - Begin sampling only after larvae hatch and grow to a less vulnerable size, be careful not to sample when eggs are in the water. Yearly breeding times vary depending on rainfall but tend to be from February through April (Propper, 1991)
  - Dip nets are disruptive to habitat so sampling should be less frequent than with coverboards. Sample pools once per academic quarter to track seasonal changes in populations.
  - On a regularly scheduled sampling day, conduct aquatic sampling with a third team at the same time as coverboard sampling.

- **Pre-Sampling**
  - Use stratified random sampling to select pools from each stream section (see Appendix C.3).
    - Streams are divided into four sections named: “Cave Gulch Creek”, “Upper Cave Gulch Creek”, “Rio Granulosa” and “Salamander Spring” (see Appendix C.3).
    - Choose a total of eight pools to sample by selecting two pools from each of these sections.
    - During randomization, keep in mind that pool numbers are non-sequential. During the pilot study, six pools (two from each of three stream sections) were sampled, but more sampling may be possible with more than one team working concurrently.
  - Make sure equipment is sanitized before leaving the lab (see Section 3.3).
  - Understand that pools are generally not closed systems. It is possible for animals to enter and leave the sampling area unnoticed, so work quickly and look for stragglers.
  - A team of five is most efficient, with two people retrieving animals with nets, two people taking measurements and one person recording data.
  - Start at the pool that is farthest downstream and work upstream to avoid contamination by disturbed sediment.
  - Collect water from the pool in a clean container before sampling because water will become clouded as sediment is disturbed. Adults require small amounts of water to stay moist, while larvae must have enough water to swim freely.
Dip net size is determined by pool dimensions (See Appendix B.2).

- The large dip net is required for sampling deeper pools.
- The medium aquarium fish net is most efficient in small shallow pools.
- Oddly shaped pools with deeply undercut banks and unmovable logs require a combination of both nets, where the large net is used to bring animals up from the bottom and the medium net is used to remove them from the water.
- Only use the small aquarium fish net for transferring larvae from plastic containers to bags during measurement.

- **Recording Pools**
  - Record information about each pool sampled on the “Pools Worksheet” (see Appendix C.2).
  - Before sampling begins, record the pool number, with initial turbidity and movement. These only need to be recorded once per pool.
    - During the pilot study, we performed a visual assessment to recorded values using the following scales:
      - Turbidity: 1=clear, 2=slightly cloudy, 3=very murky.
      - Movement: 1=stagnant, 2=little movement, 3=more movement, 4=obvious current.
      - However, it would be more accurate to measure turbidity, and movement using sensors. With the proper equipment, pH and temperature could also be recorded during this step (see Appendix B.6 for recommendations).
  - Write down the time when each sampling event begins and ends.
  - Record the number of individuals of all species combined found in each sampling event as described in Section 4.3 (see Figure 2).
  - To decide when sampling is complete for each pool, use the "Pools Worksheet" to track the number of samples when no additional animals are removed from the pool. Sampling is complete after four samples with zero new individuals.
  - Individual based accumulation curves can also be created using the "Pools Worksheet" (see Appendix C.2)

- **Sampling**
  - See Section 4.3 for detailed instructions on measuring and recording data.
  - Find the pool by looking for a blue flag with the number of the pool written on it. Record pool number on both the “Aquatic Sampling Data Sheet” (see Appendix C.2) and the “Pools Worksheet” (see Appendix C.2).
  - One sampling event is a sweep of the entire pool starting at the downstream end and moving all the way to the upstream end. This may involve multiple passes to cover every section of the pool.
- Write down the time when each sampling event begins and ends on the “Pools Worksheet” (see Appendix C.2).
- During the first sampling event, remove rocks from the pool and place them on the bank, then scoop up hidden animals.
- Write down the time when each animal is removed from the pool on the “Aquatic Sampling Data Sheet” (see Appendix C.2).
- Place each animal in a separate clean container with lid and release only after sampling is complete. Place large animals such as California giant salamanders in large containers.
- Line up containers in a shaded area in the order the animals are removed from the pool.
- Measure animals in that order.
- After measuring an animal, return it to the container with lid and place in a shaded area separate from the unmeasured animals.
- Continue sampling until zero individuals are found during sequential sampling events (three events for small pools, four for large pools).
- After sampling is complete, return all rocks and branches to the pool.
- Release animals back into the water before moving upstream to the next pool.
- **Handling Larval Salamanders**
  - Gently remove larva from dip net and place into container of water by tipping the net directly into the container.
  - Larval salamanders have gills and need water to breathe, so make sure there is enough water in the container to fully cover the animal and take steps to minimize their time out of the water.
  - Remove the larva from the plastic container using the small aquarium fish net to place inside of the plastic bag with minimal water transfer.
  - Measure using the same methods for measuring adults (see Section 4.3).
  - Take photos with the animal submerged in water in the plastic container whenever possible. However, for clarity some photos of the animal may need to be taken while it is inside the bag.
- **Post-Sampling**
  - When you return to the lab, sanitize all equipment before storing (see Section 3.3)
Figure 1. “Object 1” is a pool mapped from post 380,300 and “Points 1-4” represent points along the edge of the pool that can be used to create a polygon (Points 1-4 would be labelled 1A-1D)

- Mapping New Pools
  - Record mapping data using the “Mapping Pools Data Sheet” (see Appendix C.2)
  - Assign new pools the next integer in the sequence of pool numbers. To retain consistency in the data set, these numbers will not change as new pools are added. Keep in mind that pool numbers are non-sequential when you randomly select pools to sample (see Section 4.2: “Recording Pools”).
  - Pound a 0.3 m to 0.5 m long piece of PVC into the ground in a spot that obviously marks the location of the pool, but will not be easily moved by erosion or flooding. Use longer pieces for sandy soil.
  - Mark the number of the pool on the PVC and a blue flag inserted into the post, using a black oil-based paint pen.
  - Take distances and bearings from the nearest FERP corner post (see Section 1.2) to four points (or more for large or irregularly shaped pools) around the edge of the pool (Figure 1). Use a Sighting Compass (Suunto KB-14; see appendix B.2) and Hypsometer (Haglöf Vertex Laser, VL400; see Appendix B.3), which can be checked out from the Gilbert Lab.
  - Measurements will continue to be taken from corner posts to retain consistency with the way other pools were mapped.
  - On the “Mapping Pools Data Sheet” (see Appendix C.2), sequentially assign a letter to each point so that when placed in order they form a line around the outside edge of the pool (ex. pool 1, has points 1A, 1B, 1C and 1D). Fill out the entire record line for each point (see Figure 1).
  - These points will be used to create low resolution polygons in ArcGIS, a Geographical Information Systems (GIS) program. A suggestion for a future

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student project is to design a protocol for collecting more detailed information about pools and streams (see Section 7.2.)

- Enter Data in the “DataEntry_PoolsMapping” file in the “Herps on the FERP/Data Entry” folder in Google Drive.
- Update the map using ArcGIS.

- Hydroperiod
  - At the beginning of each month walk the length of the streams and record presence vs absence of water in each pool on the “Hydroperiod Data Sheet” (see Appendix C.2).

### 4.3. Measurements and Data Recording

<table>
<thead>
<tr>
<th>Trap</th>
<th>Herp No.</th>
<th>Species ID</th>
<th>SVL (cm)</th>
<th>Weight: raw (g)</th>
<th>Weight: bag (g)</th>
<th>Weight: herp (g) raw-bag</th>
<th>Animal Temp (°C)</th>
<th>Photos</th>
<th>Date</th>
<th>Time (24-hr)</th>
<th>Initials</th>
<th>Notes</th>
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</thead>
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<td>ENSAES</td>
<td>5</td>
<td>6.5</td>
<td>2</td>
<td>4.5</td>
<td>22.2</td>
<td>3/15/2015</td>
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<td>FG</td>
<td>BOARD NEEDS TO BE MARKED</td>
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<tr>
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<td>3/15/2015</td>
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<td>FG</td>
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<td>26.0</td>
<td>3/15/2015</td>
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<td>FG</td>
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<td>THAMAT</td>
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<td>31.5</td>
<td>25.0</td>
<td>3/15/2015</td>
<td>1318</td>
<td>FG</td>
<td>ON TOP OF BOARD</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Example of data collected during coverboard sampling.

- Board/Pool
  - The number of the coverboard or pool sampled.

- Herp Count
  - Record the number of herps found at each site in the order in which they are measured.
  - For Coverboards:
    - For boards with one or more animal, record the “Herp No.” from 1 to N, where N is the total number of animals found under the coverboard “Coverboard Sampling Data Sheet” (see Appendix C.2).
    - Record “0” in the “Herp No.” column for coverboards with no animals.
    - The count restarts at each new coverboard.
  - For Aquatic Sampling:
    - For pools with one or more animals, record “Herp No.” from 1 to n, where n is the total number of animals found in the pool on the “Aquatic Sampling Data Sheet” (see Appendix C.2).
    - Record “0” in the “Herp No.” column for pools with no herpetofauna.
    - The count restarts at each new pool, not at the beginning of a new sampling event.
• Sample/Stream
  o Aquatic sampling only:
    ▪ “Sample”: a sampling event is one sweep of the entire pool, which may involve multiple passes with a net.
    ▪ “Stream”: record the stream segment using the codes CG (Cave Gulch Creek), UCG (Upper Cave Gulch Creek), SS (Salamander Spring) and RG (Rio Granulosa).

• Time
  o Record the time to the minute using the 24 hour system (e.g., 1345 instead of 1:45pm).
  o For sampling using coverboards, record the time when you lift up the coverboard on the “Coverboard Sampling Data Sheet” for all animals present, not specific times of sampling for each animal (see Appendix C.2).
  o For aquatic sampling, write down the time when the animal is removed from the water on the “Aquatic Sampling Data Sheet” (see Appendix C.2).
  o On the “Pools Worksheet”, write down the times when sampling events begin and end (see Appendix C.2).

• Temperature
  o Measure animal body temperature first to avoid changes due to handling.
  o Place the animal in a bag with minimal exposure to your body and hold it in the shade so that the temperature is not raised by the sun.
  o Turn on the handheld thermometer (Omega HH-25TC) with a thermoprobe attachment, to the 200 setting, not the 400 setting (see Appendix B.2).
  o Touch the thermoprobe wire to the belly of the animal. Make sure that your hand is far enough away from the tip so that your body temperature does not affect the reading, and that the belly of the animal is pressed directly to the surface of the bag.
  o Record the temperature to 0.1°C when the number stops fluctuating.

• Weight
  o Attach the clip of the Pesola scale to the top of the bag and allow bag to hang freely, shielding it from the wind so that it does not affect the measurement (see Appendix B.2).
  o Round measurements to the nearest 0.5 g.
  o Record the weight of the empty bag in the “weight: bag (g)” column.
  o Place the animal inside the bag and record the total weight in the “weight: raw (g)” column.
  o Calculate the weight of the animal by subtracting the weight of the bag from the raw weight and record in the “weight: herp (g) raw-bag” column.

• Length
• Measure all animals from the tip of the snout to the back of the vent (Figure 3).

• For salamanders, lizards and frogs: gently place the animal at the bottom of the bag, roll the bag to keep the animal from moving, hold it as straight as possible and measure it with a clear ruler.

• For snakes: two people are required to hold the animal as straight as possible and measure it outside of the bag with a cloth measuring tape (McDiarmid et al. 2012).

• Record the length rounding to 0.1 cm.

<table>
<thead>
<tr>
<th>Total Length (TL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snout to Vent Length (SVL)</td>
</tr>
</tbody>
</table>

Figure 3. Snout to vent length (SVL) is measured from the tip of the snout to the back of the vent; total length (TL) is measured from the tip of the snout to the tip of the tail.

- Stage
  - Aquatic sampling only: record stage of development, E=egg, L=larva, A=adult (includes metamorphosed subadults).

- Notes
  - Record additional information in the "notes" column.
  - It is presumed that animals are found under coverboards unless “on top of board” is recorded in notes.
  - Record if gravid (pregnant with young or carrying eggs).
  - Record any other unusual observations.

4.4. Identification

- Species ID
  - Record the species of each animal using the “Species Code List” (see Appendix C.1). Codes are created by combining the first four letters of the genus followed by the first two letters of species.
Even if the taxonomic name of the species changes, the code will remain the same to retain consistency in the dataset.

- Photographs may aid in identification of difficult species.

4.5. Photography

- Like voucher specimens, photographs strengthen the power of observations and hold up over time as new information about animals is discovered (Heyer et al. 1994). Coloration and morphology can be used to identify information that may have been missed in the field such as species, gender, developmental stage, and other information not on the data sheet.

- Photos
  - For each animal, photograph the full body (both dorsal and ventral), head, eye, vent, tail and limbs.
  - Take photos of the animal on the ground if possible to make them presentable and to represent the natural habitat.
  - However, it is important to get a clear image with minimal stress to the animal, take photos inside of the bag if it is not possible to easily photograph on the ground.
  - Photos are numbered starting with “1” and listed in the order taken (ex. you take five photos of the first animal and label them “1-5”, then you take six photos of the next animal and label those “6-12”). Photos will be renamed this way when they are uploaded.
    - We found this method to be the most effective way of recording photo numbers during the pilot study after some experimentation with format. It is especially useful with cameras that do not show photo numbers such as camera phones and allows standardization of numbering.
  - Do not use the same numbering sequence for photos not taken for research purposes, such as photos of researchers at work and interesting non-herp organisms found in the study area (See Section 5.2: “Uploading Photos” for upload instructions).
5. Handling Data and Recording Progress

5.1. Google Drive

Upload all data and files related to the project to Google Drive. This includes, but is not limited to:

- “Log: Herps on the FERP progress” (see Section 5.3)
- “Data”
  - Enter all data collected in the field (from coverboard sampling, aquatic sampling, mapping pools, microhabitat data collection, etc.) to “DataEntry_xxx” Google Sheets in this folder.
  - Save any processed data, etc. to this folder (.csv, .xlsx, etc.)
- “Data Sheets, Maps, Etc.”: contains forms listed in Appendix C.
- “Herps Photos”: upload photos taken during sampling to this folder.
- “Literature”: store important papers and other relevant background information in this
- “Scans”: scan and upload data sheets to this folder before entering data.

5.2. Uploading Photos

- Open the folder “Herps on the FERP/Herps Photos”
- Create a new subfolder titled with the date and your name in the format “YYYY_MM_DD_FirstLast”.
- Upload photos to the new subfolder.
- Change the name of the file if necessary to make sure that each photo is labeled with the correct number so that it can be properly matched to the data (see Section 4.5 Photography).
  - Use leading zeros so that photos appear in order (e.g. “001” instead of “1”).
  - Change the name to match the number recorded in the field if it is different than the one from your camera (e.g. change “IMG5003” to “001”).
  - Add your initials and the date the photo was taken to the end of each name.
  - For example, the first photo recorded in the field would be labeled: 001_AMH_9May2015.jpg
- Upload photos not taken for research purposes to the “Non Research Photos” subfolder within “Herps Photos.”
- You must sign a “Photo Release Form” authorizing “Herps on the FERP” and the University of California, Santa Cruz to use your photos for research and publication (see Appendix C.4). Photos will be credited to the name that appears on the folder that contains them.
5.3. Keeping a Log

- It is important to keep a log to see project progress over time and to answer any questions or confusion from specific days. Use it to record what happens in the field, the lab or any important meetings.
- The log is a Google Doc labelled “Log: Herps on the FERP Progress” in the Google Drive folder “Herps on the FERP.”
- New Entries
  - Write a new log entry as soon as you return to from the field after each day in the field.
  - Enter new information at the bottom of the older logs to keep them in chronological order, in both the “Dates of Fieldwork” and “Work Log” sections.
  - Record the date and name(s) of people who were there that day in bold font for clarity.
  - Under the data and name(s) heading, use bullet points to record what was done that day, who was on each team, who took photos, and anything notable or unusual about the day.

5.4. Data Entry

- Scan data sheets before entering data in Google Drive. They can be scanned in the Gilbert FERP Lab using the scanner next to the computer or scanned at an outside location.
  - Then upload the scans to the “Herps on the FERP/Scans” folder in Google Drive.
  - Label the data sheets as scanned with the date and initials of the person who scanned them in the format “Scanned by Initials, Date” on both sides of the data sheet.
  - Then place the data sheets in the Herps on the FERP binder in the Campus Natural Reserve office (Natural Sciences Two Building in room 463).
  - After they have been scanned and uploaded, the data can be entered in the appropriate spreadsheet within the “Herps on the FERP/Data” folder in Google Drive.
- To Enter Data
  - We use a double-entry system for entering data to ensure accuracy. The file “Data Entry” has three tabs: “Instructions”, “Original”, and “Duplicate”. All data are entered twice; once on the “Original” sheet and once on the “Duplicate” sheet. The data entered on the “Original” and “Duplicate” should be entered by two different people. This should be done manually--do not copy and paste from one page to the other!
  - Get the binder with the data for the Herps on the FERP
  - Open "DataEntry_xxx" from the "Data" folder in the "Herps on the FERP" folder in Google Drive
  - Turn on caps lock on the keyboard
Enter the data from the data sheets in the appropriate columns on the tab "ORIGINAL" or "DUPLICATE" following these rules (DUPLICATE data entered only when ORIGINAL entries complete)

- Always enter data in the same order as they appear in the datasheets
- Always enter datasheets in the order they are found in the binder
- Include your initials in the column for "Data Entered By" and today's date in the column "Entered Date"
- When you finish a page, fill in your initials and the date on the physical datasheet in the "Data Entered By (Original) / Date" DATE INITIALS" or "Data Entered By (Duplicate) / Date" space as appropriate.
- Do not remove the datasheets from the Herps on the FERP binder so the datasheets can be reliably found.

5.5. Data Cleanup

- Make sure that all current data have been entered into both the "ORIGINAL" and "DUPLICATE" sheets.
- Find the last entry that has been cleaned up by looking at the "Checked_by" column.
- Select all rows that have not been checked (DO NOT compare entries that have already been cleaned up).
- Copy and paste these entries from the "ORIGINAL" and "DUPLICATE" sheets into separate Excel files.
- In Excel, sort the data by: 1st - “Date”; 2nd - “Board/Pool”; 3rd - “Herp No.”
- Use the "Compare Files" function on the "Inquire" tab (both files need to be open for this to work).
  - Check for any differences between the files.
  - Remember that there is no board number 9 (because 9 is a litter trap only location and is paired with 37 which is a coverboard only location).
  - If it is not clear which entry is correct, cross reference the physical data sheets
  - Make sure to check that there is an entry for every board and that there are no duplicate entries for the same herp
- Create a single merged sheet with the corrected data
- Remove all formatting (can be done by saving to .csv)
- Copy and paste it into the "CLEAN_DATA" sheet in this file (below the last existing entry)
- Enter your initials and the date in the "Checked_by" and "Checked_date" columns in all three: "ORIGINAL," DUPLICATE" and "CLEAN_DATA" sheets.
6. Microhabitats

6.1. Update this section each time a new protocol is created.

6.2. Soil Moisture Sampling Protocol

(by Eva Jason, Quinn Doud and Alex Shenton)

- Record data on the “Soil Sampling Data Sheet” (see Appendix C.2)
- In The Field
  - Remove any duff on top on the mineral soil and record the height (cm) of duff.
  - Take samples of the soil next to every coverboard, and randomly choose 20 coverboards to take cores from both the outside of the coverboard and underneath the coverboard.
  - Place corer (see Appendix B.2) into ground at the bottom right corner when facing towards the corner post (looking East) of the coverboard and remove a 10 cm section of mineral soil.
  - Place into bag and label each with a sharpie indicating which coverboard it came from and if it was collected from outside the coverboard or under the coverboard.
- In the Lab
  - Weigh using a scale that measures to 0.01 g (Scout SC2020) and record the weight (g) of the empty bag and the combined weight (g) of the bag with soil.
  - Place in drying oven at 100 °C for 48-72 hrs.
  - Reweigh the sample after it is dried and record the weight (g).
  - Determine grams of water in sample by subtracting the weight of the dried soil from the weight of the moist soil.
  - Calculate the percent water in solution with the equation:
    \[(100 - \text{dried sample}/\text{moist sample}) \times 100\]
7. Analyses

7.1. Pilot Study

Data for the Herps on the FERP pilot study were collected using the methods outlined in this manual between 15 March 2015 and 9 May 2015. Two scientific reports were prepared to address separate questions outlined below.

- Basal Area of Woody Vegetation Surrounding Coverboards
  - Question: Is there a relationship between basal area of woody vegetation and salamander abundance, and at what scale is there an effect, if any?
  - Hypothesis: We expect that salamander abundance will increase with an increase in basal area. An increase in basal area is correlated with higher cover and potentially cooler conditions, leading to less water loss from the forest floor. An increase in basal area may also lead to a higher amount of coarse woody debris, allowing for more available microhabitat for the salamanders. A variety of distances from the coverboard will encompass the home ranges of the salamander species.

- Salamander abundance and distribution by genera relative to streams
  - Question: Is there a relationship between the number of salamanders occupying coverboards and their distance from streams and pools? If so, is there a difference between genera of salamanders that breed aquatically and those that breed terrestrially?
  - Hypothesis: Herps that breed aquatically should be more common under coverboards close to water. The distribution of genera that breed terrestrially should not be correlated with distance to water.

![Figure 4](image.png)

**Figure 4.** Hypothesized relationships between salamander occupation of coverboards and distance from water, with differences between genera that breed aquatically versus terrestrially.
7.2. Recommendations for Future Studies

Here are some suggestions for future student projects using this monitoring system:

- Create a field guide for the various herpetofauna on campus.
- Preform correlative analysis of herpetofauna distribution data and meteorological data from the FERP.
- Collect and analyze microhabitat data for each coverboard
  - Temperature under and around coverboards
  - Soil moisture (see Section 6.2)
  - Coarse woody debris
  - Soil type
  - Surrounding vegetation
  - Substrate type under the coverboards
  - Canopy cover and height
  - Elevation and slope
- Collect hydrological data for pools and streams
  - Average depth, surface area and volume
  - Turbidity measurements using a sensor
  - Water temperature and pH
  - Streamflow
- Ground-truth the remaining potential ephemeral streams during the rainy season using the “Potential Streams Map” created from LiDAR data (see Appendix C.3)
- Expand aquatic sampling and hydroperiod records to include stream segments between pools.
- Create a comparative study using coverboards outside of the FERP to sample different habitat types.
- Analyze photographs for patterns in coloration and morphology.
- Design a mark and recapture study.
- Amend this protocol to include the methods used in these studies.
8. Conclusion

8.1. Summary

The Herps on the FERP monitoring system uses the techniques described above to accurately track spatial and temporal patterns in the populations of herpetofauna on the UCSC FERP over time. These techniques will minimize impacts on the surrounding forest and stress to the animals. The system is set in a forest comprised of mixed evergreen forest, second growth coastal redwood forest and the headwaters of Cave Gulch Creek, which allows for sampling in a variety of habitats. The coverboards are placed on a systematic grid with random offgrid coverboards in random cardinal directions at random specified distances. Aquatic sampling takes place once a quarter to monitor the water-breeding salamanders through a series of sweeps through the pools with a dip net. The FERP also has myriad other data that can be cross-analyzed with the herpetofauna data. If all aspects of the protocol are followed correctly, this long term monitoring system will be a safe and thorough way to collect data.

8.2. Recommended Reading


Haff, T.M., M.T. Brown, and W.B. Tyler (eds). 2008. The natural history of the UC Santa Cruz campus, 2nd Ed. Environmental Studies Department, University of California, Santa Cruz, CA, USA.


9. Acknowledgements

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GIS Group Project: Brennan Byrnes, Yasmine Shahbaz and Remy Vincent

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10. Literature Cited


Haff, T.M., M.T. Brown, and W.B. Tyler (eds). 2008. The natural history of the UC Santa Cruz campus, 2nd Ed. Environmental Studies Department, University of California, Santa Cruz, CA, USA.


Appendix A: Glossary

**Adpressed Limbs** - limbs pressed parallel to sides of body

**Amplexus/Amplex** - when "male clasps female with his hind limbs prior to mating" (Petranka 1998)

**Anal Plate** - ventral scales on snakes that indicate the location of the vent

**Ancillary** - supplementary; providing something additional to a main part or function

**Antecedent** - something that came before something else and may have influenced or caused it

**Anura (Order)** - frogs and toads

**Arboreal** - "living in trees" (Petranka 1998)

**Axilla** - (armpit) area directly under the joint where the arm connects to the shoulder.

**Biphasic Life Cycle** - (amphibians) having an aquatic larval stage and a terrestrial stage during development (Heyer et al. 1994)

**Caudata (Order)** - salamanders

**Cloaca** - see Vent

**Costal Grooves (Folds)** - "parallel, vertical grooves (folds) on the sides of a salamander's body" (Petranka 1998)

**Coverboard** - a board placed on the ground to acts as an artificial cover refuge and is used to attract animals for sampling.

**Crest** - (as in "tail crest") a ridge or prominence on a part of an animal body

**Direct Development** - (amphibians) no independent larval stage during development (Heyer et al. 1994)

**Dorsum/Dorsal** - The upper surface of an organism

**Ephemeral Stream** - a watercourse that only holds water for brief periods of time (Dodds & Whiles 2010)

**Estivation/Estivate** (or alternate spelling: Aestivation/Aestivate) - a state of dormancy or torpor during hot dry months

**Fossorial** - adapted to digging; living underground

**Granular Glands** - aka "poison glands" on the tail of Ensatina salamanders that secrete a sticky milky substance used to deter predators (Heyer et al. 1994)

**Gravid** - carrying eggs or young (pregnant)

**Ground-Truth** - to check maps created from remotely sensed data (such as LiDAR elevation data) against true physical conditions in the field.

**Gular Fold** - external flap/fold across lower throat region

**Herpetofauna/Herps** - the group comprised of reptiles and amphibians collectively
**Herpetology** - the scientific study of reptiles and amphibians

**Hydroperiod** - period of time during which surface water is present (Dodds & Whiles 2010)

**Intermittent Stream** - a stream that dries out during part of the year (Dodds & Whiles 2010)

**Inventory** - a study of a specific area to determine species richness; indicates only the presence vs absence of species (see also Monitoring) (Heyer et al 1994)

**Life History** - the way in which an organism partitions its time and energy among growth, maintenance and reproduction

**Mental Glands** - “secretory glands found on the chin of male salamanders that are used in courtship” (Petranka 1998)

**Metamorphosed** - fully developed

**Microhabitat** - The precise places where individual organisms occur within the general environment (Heyer et al 1994)

**Mixed Evergreen Forest** - California forest type characterized by stands of broadleaf evergreen trees and conifers; especially Douglas fir (*Pseudotsuga menziesii*), tanoak (*Notholithocarpus densiflorus*), live oaks (*Quercus spp.*), Pacific madrone (*Arbutus menziesii*) and bay laurel(*Umbellularia californica*) (Haff et al. 2008).

**Monitoring** - used to determine species composition and abundance through time (see also Inventory) (Heyer et al 1994)

**Mottled** - coloration pattern: blotchy, speckled or marbled

**Nasolabial Grooves** - hairline furrows running from the upper lip to the nostril openings; found on *Plethodontid* salamanders and helps them smell liquids from the ground. (Haff et al 2008)

**Newt** - salamander in the family *Salamandridae* with fully developed lungs and aquatic breeding strategy (Petranka 1998)

**Nuptial Pads** - developed by male newts during mating season so they can hold onto females in amplexus (Petranka 1998)

**Oviparous** - reproductive strategy where young hatch from eggs external to mother’s body

**Oviposition** - the act of laying eggs

**Ooviviparous** - reproductive strategy where eggs remain in mother until they hatch, followed by live birth

**Paedomorphism/Paedomorphosis** - the retention of juvenile characteristics in adults; in amphibians when larval metamorphosis is incomplete and they remain in the aquatic stage of a biphasic life cycle (Heyer et al 1994).

**Plethodontidae (Family)** - a family of lungless salamanders

**Reticulate** - netlike coloration pattern

**Riparian** - Land and associated habitat surrounding a stream
**Salamandridae (Family)** - family of salamanders known as newts

**Snout-to-Vent-Length (SVL)** - measurement from tip of snout to back end of vent (Petranka 1998)

**Spermatophore** - "packet of sperm perched atop a gelatinous pedicel" (Haff et al. 2008)

**Squamata (Order)** - all lizards and snakes

**Stratified Random Sampling** – Sampling units are divided into groups called “strata” and samples are randomized within strata (Heyer et al. 1994).

**Subadult** - an animal that is fully developed but has not reached sexual maturity

**Tail Autotomy** - when animals, commonly lizards and salamanders, lose their tail to distract predators

**Tail Crest** - (see Crest) crest developed by newts entering aquatic stage during mating season

**Torpor** - “a state of lowered physiological activity typically characterized by reduced metabolism, heart rate, respiration, and body temperature that occurs in varying degrees especially in hibernating and estivating animals” (Merriam-Webster Online Dictionary)

**Total Length (TL)** - "distance from the tip of the tail to the tip of the snout of a straightened animal" (Petranka 1998)

**Trogloidyic** - cave-dwelling

**Turbidity** - a measure of the clarity of a liquid

**Unken Posture** - defensive position displayed by Taricha newts with back depressed, head raised vertically, eyes closed, limbs extended outward, with tail curled over body (T. granulosa) or with tail raised outward from body (T. torosa) (Petranka 1998)

**Vent** - single opening on the underside of the body leading to genital, digestive and urinary systems (Cloaca)

**Venter/Ventral** - the lower surface of an organism

**Vernal Pool** - a pool that only holds water during part of the year

**Viviparous** - reproductive strategy; embryo develops inside mother with no egg and live birth

**Vomeronasal Organ** - very sensitive olfactory organ in the snout used primarily for smelling pheromones (Heyer et al 2008).

**Voucher Specimen** - a specimen (usually cadaver) retained for reference
Appendix B: Materials

B.1. General Field Equipment

- Rite-in-the-Rain Copier Paper
- Latex or nitrile gloves
- Suits
- Tecnu: outdoor skin cleanser
- Binder for storing data sheets

B.2. Herpetofauna Sampling Equipment

- Handheld thermometer: omega hh-25tc (3)
- Thermometer attachments: omega part # 5sc-tt-t-40-36
- Scale: Pesola light- line spring scale (50 gram) (3)
- Scale: Pesola medio-line spring scale (2500 gram) (3)
- Translucent ruler (3)
- Cloth measuring tape (3)
- Compass: Suunto A-10 (for navigation) (3)
- Mechanical pencils
- Paint pens: oil based, black
- Hammer to pound stakes
- Dip net: fine mesh large aquatic net (1)
- Dip net: medium aquarium fish net (1)
- Dip net: small aquarium fish net (1)
- All weather clipboard (3)
- Plastic containers: Sure Fresh reusable rectangle containers & lids
  - Small (3 cups/24 fl oz)
  - Large (10.35 cups/82.8 fl oz)
- Plastic bags: Ziploc
  - Small (sandwich)
  - Large (gallon)
- Plastic bags: tall kitchen
- Over the shoulder bags for carrying equipment in the field (3)
- Bottles filled with filtered water (3)
- Plywood: ½ inch (1.27cm) thick, cut into 2x2-ft (0.61x0.61-m) squares (115)
- PVC: 1’–1.5’ long pieces for staking boards
- Rebar: ½ in to reinforce stakes
- Garden tools: clippers, hand cultivator and trowel
B.3. Mapping Equipment
- Sighting compass: Suunto KB-14 (for mapping)
- Hypsometer: Haglöf Vertex Laser, VL400
- Blue flags for pools
- PVC: 1’–1.5’ long pieces for marking pools

B.4. Soil Sampling Equipment
- Soil core tools
- Bags: 4 oz Nasco whirl-pac bags, serial number 1843
- Sharpie
- Drying oven
- Scale: Scout SC2020

B.5. Software
- Google Drive
- R
- ArcGIS

B.6. Purchase Recommendations
- Cameras to standardize quality for analysis of coloration (3)
- Lizard catching devices (2)
- iButtons (20)
- Hand lenses (3)
- Turbidity sensor (1)
- Stream flow meter (1)
- pH sensor for sampling water (1)
- Water temperature thermometer (1)
Appendix C: Attached Forms

C.1. Checklists and Species Codes
    C.1.1. Field Materials Checklist
    C.1.2. Mapping Pools Checklist
    C.1.3. Species Codes List

C.2. Data Sheets
    C.2.1. Coverboard Sampling Data Sheet
    C.2.2. Coverboard Grids with Location Lists
    C.2.3. Aquatic Sampling Data Sheet
    C.2.4. Pools Worksheet
    C.2.5. Mapping Pools Data Sheet
    C.2.6. Hydroperiod Data Sheet
    C.2.7. Soil Sampling Data Sheet

C.3. Maps
    C.3.1. Study Area: FERP Map
    C.3.2. Pools and Streams Map
    C.3.3. Potential Streams: FERP Map
    C.3.4. Regional Streams Map
    C.3.5. Directions to FERP
    C.3.6. Map to the FERP

C.4. Other
    C.4.1. Photo Release Form