Achatinella Survey and Monitoring Protocol

For TES Permit # 043638-8
May 24, 2012

A1. Summary

This survey and monitoring protocol is intended to be used as the initial monitoring protocol for a translocation or reintroduction. The level of monitoring required for each field population depends on its designation as a primary, secondary, or tertiary population. Primary populations are of highest priority for achieving stabilization, and thus require the most intensive monitoring (§A2). Accurate data on snail abundance must be obtained at regular intervals so that any changes in abundance can be addressed (e.g., extraction necessary). Secondary populations require an intermediate level of monitoring (§A3) to maintain their role as a safety net, should a primary population experience a catastrophic decline. Single-survey abundance estimates at less frequent (but regular) intervals will suffice for these purposes. Tertiary populations require a minimal amount of monitoring (§A4), limited to opportunistic surveys to determine presence/absence. This appendix will not address monitoring protocols for captive populations (which may be primary or secondary populations); those are discussed in Hadfield et al. (2004).

A2. Primary Populations

Field Site Delineation:

One must first delineate artificial boundaries for the population in the field. Defining the boundaries of a population will ensure consistency between surveys and at least some level of comparability among abundance estimates. This process is best done with a team of surveyors over two full days, as follows:

1) Several experienced field biologists survey a known snail locale for a half-day.
2) Each surveyor has a unique color of flagging tape with which to mark trees with snails in them, and a unique code is written on each flag.
3) Information is recorded for each flagged tree regarding snail abundance, and each surveyor records relative tree locations with a basic illustration.
4) Surveyors combine information to determine areas of high density, and then spend the second half-day searching un-flagged trees in the vicinity of high-density areas.
5) On the 2nd day, site boundaries are agreed upon which include a majority of trees with recorded snail presence, and a buffer zone to include some emigration on subsequent surveys. Site dimensions should not be narrow at any point to reduce the chance of emigration bias between surveys. Sites must also be small enough to be sufficiently searched (see next section) by 4 people in one day
6) Perpendicular rows of transects are then delineated with field markings to create a grid of discernable 5m x 5m squares within the site's boundaries.
7) Lastly, the grid is mapped using a GPS if possible to show the layout of flagged trees, which are given permanent unique markings in the field and corresponding codes on the map.

Capture-Mark-Recapture:

The type of markings given to a snail depends on the survey type (see next section). The most informative markings will come from populations where individuals are assigned a unique and permanent alphanumeric code upon first capture, and individuals can be identified on subsequent surveys. This method is called the "glue method" and is superior to the "paint method", both of which are described below and summarized in Table A1.

Table A1. Comparisons of different marking types

Type of marking	Advantages	Disadvantages
Glue method	Durability, additional data available	Time Consuming, training required, more snail handling
Paint method	No training required, limited handling, fast	Mark deterioration, precludes long-term analyses

With the glue method, the type of shell marking a snail receives at first capture is determined by its size. Snails with shells over 7 mm in length are marked using letters and numbers printed on 2 paper punch-outs. Six-point font characters are printed onto waterproof paper (Rite-In-The-Rain), and cut out with a leather punch. It is preferable to use 2 separate punch-outs rather than a single punch-out with both characters on it, for 3 reasons: 1) many tags are often lost via wind gusts prior to application, which leads to erratic numbering, 2) a larger size punch-out is needed to fit 2 characters on it -that is less flush with the shell- leaving more of the rigid tag's underside exposed to deterioration, and 3) with 2 separate tags, loss of one tag allows for an estimate of tag-loss rate rather than mistaking all unmarked snails as "new".

In the field, the snail's shell is gently dried and cleaned, and then a small drop of cyanoacrylic glue (Satellite City 'Super T') is applied to it. The 2 punch-outs are then placed onto the glue, followed by another drop of the transparent glue to protect the marks from deterioration. When the glue dries, the snail is returned to its capture location. Snails less than 7 mm in length receive small circular dots using paint pens. These different colored paint dots must uniquely identify individuals in the field until they grow large enough to receive the paper marks, which can take up to one year. For a further description of marking techniques, see Hall et al (*in press*).

With the "paint method", all snails captured on a single day are marked with a single color of paint pen. These marks wear down substantially over the course of a year, and new marks of a different color can be applied on a subsequent survey. This generic coding scheme precludes the use of more sophisticated analyses to estimate survival rates and other demographic parameters, which can be obtained using the glue method. Abundance measures can still be obtained with both methods.

Survey Type:

The methods outlined in this section will be strictly aimed at determining abundance. Some methods will have the potential to estimate additional parameters such as survival, growth rate, and emigration, with some additional effort. These modifications are described in the next section. For all survey types, the location of all snails will be recorded on every survey. Unmarked snails will be marked, measured (shell length and width), and aged (lipped or not lipped). Previously marked snails will be recorded, length taken unless growth rates are known (see next section), and aged (lipped or not lipped).

To determine abundance, the following site-specific methods must be completed in the exact same manner on at least 2 consecutive occasions in order to estimate abundance using the Lincoln-Peterson estimator (Chao and Huggins 2005). This method assumes that all marked individuals have a chance to randomly re-mix into the population prior to being surveyed again. In areas with low vegetation, this can happen in as little as 1 day. However, in areas with taller vegetation, up to 1 month may be necessary to be confident that the population has re-mixed. In no circumstances should this interval be longer than 1 month, because the method also assumes no birth, death, emigration or immigration occur between intervals.

The type of surveying method will depend on the habitat; some methods have been designed here exclusively to minimize impact to certain tree-snail habitat types. Sites are characterized as to whether they have an understory that is sensitive to trampling, and by the difficulty of the terrain to survey (e.g., steepness). This results in 4 permutations with which to classify a site. These permutations are outlined in Table A2, along with corresponding management recommendations that are described below.

Table A2. Snail site classification scheme and associated management recommendations

Understory	Terrain	Surveying Recommendations	Marking Method
Absent	Easy	2 survey days, full site survey	Glue
Absent	Difficult	3 survey days, full site survey	Glue
Present	Easy	2 survey days, random quadrat surveys	Paint
Present	Difficult	3 survey days, random quadrat surveys	Paint

For a site where trampling of the understory is not a concern, roughly 10-15 minutes of search time should be devoted to each quadrat. This should take a half-day to complete for sites between 500 and 1000m^2 in area, and is best done with 4 observers dividing a site into 4 transects of quadrats (and at least 1 other person marking and releasing snails). The number of observers can be adjusted for sites with larger or smaller areas. For the second half of the day, the surveyor with the most recorded snails will then survey the transect of the observer with the fewest number of snails, and vice versa. The other two surveyors will also switch transects. This is to homogenize recapture rates at the site for a given day, so as not to violate a key Lincoln-Peterson estimator assumption. All unique captures for that day are compiled, and the same process is repeated the next day with the

same surveyors doing the same transects. The "glue method" of marking should be used so that the maximum amount of data can be extracted from surveying efforts.

At sites with a substantial understory presence, fully surveying a site would cause excessive impact. For these sites, a random quadrat is selected and surveyed by all surveyors for 10-15 minutes total. The number of unique individuals is tallied, and then another random quadrat is surveyed. This iterative process continues until an adequate number of snails has been surveyed. To decide an adequate number of snails, a rough estimate of the population size is needed first (based on single day surveys, §A3). Generally, an adequate sample size is between ¼ and ½ of the rough estimate and can be determined using tables in Robson and Reiger (1964). A few examples using 4 typical *Achatinella* species population sizes are provided in Table A3. Surveying ceases when an adequate sample size has been reached, and the exact same quadrats are surveyed on the following sampling occasion.

The "paint method" of marking is used with understory-sensitive sites, since not surveying the whole site precludes analyses of variables other than abundance. A single color of paint pen is used by all surveyors to tally the number of unique snails on the first survey, and the process is repeated on the following survey with a different color. This 2nd marking is only to prevent a snail from the 1st day from being counted as recaptured more than once. Abundance is estimated as normal with the Lincoln-Peterson estimator, and then divided by the combined area of the quadrats surveyed. Finally, this density is multiplied by the area of the site to obtain an abundance estimate for that site.

Table A3. Minimum sample size needed using the random-quadrat method; examples are provided for range of population sizes that are typical of *Achatinella* species.

Rough Estimate of Population Size	Snails Needed Each Day
40	20
80	40
120	45
400	110

For sites with terrain that is easy to survey, only the minimum number (2) of survey days is required to achieve a reasonable rate of recapturing snails. Difficult terrain reduces this rate, and thus additional data are needed. Adding a 3rd consecutive survey occasion adequately accomplishes this goal (K. Hall, unpubl. data). Instead of using the Lincoln-Peterson estimator, multiple-recapture surveys require using the Schnabel method (Chao and Huggins 2005). Recommendations by site-type are summarized in Table A2.

Survey Intervals:

Primary sites need to be monitored at 6-month intervals to prevent emigration from influencing changes in abundance. If abundance drops are noted following analysis, an additional survey should be completed as soon as possible to determine the cause. This survey will be a combination of ground-shell surveys to rule out mortality, and surveys of

the area around the site to rule out emigration. Site delineations and management should be adjusted accordingly. Great care should be taken to conduct all surveys at the same time of day to keep recapture probabilities constant.

Sites where the "glue method" has been used can be further surveyed to obtain survival, emigration, and other important demographic estimates. This requires intensive monthly surveys of a high-density quadrat as in Hadfield et al (1993) to determine growth rates for 1 year. These data will be used to assign snails to different age-classes at every survey interval, even if not captured. Such data are necessary, as recapture probabilities and survival are known to vary with age (Hall et al *in press*). Program MARK (White and Burnham 1999) can then be used to obtain simultaneous estimates of all parameters of interest using likelihood-based model ranking.

A3. Secondary Populations

These populations require substantially less effort to monitor because of their lower priority level. For the initial survey, at least two surveyors search a known snail locale for several hours (day or night). The number of individual snails is recorded, along with shell measurements and any other pertinent data (e.g., weather observations). All trees with snails in them are then flagged with a blue and orange flagging combination for ease of relocation, the standard color scheme used by U.S. Army Environmental on O'ahu.

Subsequent surveys must be done annually to ensure that such populations are still of sufficient size to fulfill their role as a safety net for primary populations. As with primary population surveys, effort should be made to keep search efforts consistent.

A4. Tertiary Populations

Tertiary populations need only have presence/absence data taken (although additional information is surely warranted when possible). These surveys should be done when time permits, and records should be available to all interested parties in an annual inventory of all populations. At a minimum, these populations should be surveyed every 5 years to determine their ability to serve as secondary populations if needed.

A5. Field equipment checklist (primary population surveys)

For each fieldworker	**For the survey team:**
Relevant site maps	Paper punch-outs (glue-method sites only)
Notebooks	Fine-point tweezers
Snail collection containers (petri dishes)	Satellite city "super T" glue
Calipers for measurements	Glue remover
Orange and blue flagging tape	Permanent tree tags
Watch or timer	Paint pens of various colors