

RESPONSES OF A DECLINING AMPHIBIAN AND OTHER WILDLIFE TO  
CHANGES IN FISHERIES MANAGEMENT IN A CALIFORNIA  
WILDERNESS

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## Table of Contents

Project Summary .....	3
Introduction .....	5
Methods .....	6
<i>Study area</i> .....	6
<i>Experimental design</i> .....	6
<i>Field sampling</i> .....	7
<i>Biotic variables</i> .....	7
<i>Environmental variables</i> .....	9
<i>Analysis</i> .....	10
Results .....	12
<i>Trout</i> .....	12
<i>Cascades frogs</i> .....	13
<i>Insects</i> .....	14
<i>Garter snakes</i> .....	14
<i>Birds</i> .....	15
<i>Bats</i> .....	15
Conclusions .....	15
References .....	17
Table 1. Physical parameters, maximum number of <i>Rana cascadae</i> seen per year during visual encounter surveys, and annual catch per net-hour of trout for the 16 individual study lakes .....	20
Table 2. Summary of faunal sampling techniques, timing of sampling and purpose of sampling during the four-year study .....	21
Table 3. Bird species recorded > 100 times during 2003-2005 point count surveys and number of times recorded at any of the 16 study basins .....	22

## List of Figures

Figure 1. Map of the Trinity Alps study area .....	23
Figure 2. Total number of trout gill-netted from the fish removal lakes .....	24
Figure 3. Mean number of trout caught per hour of gill net set for each treatment .....	25
Figure 4. Mean total length and body condition of trout caught during 4-hr gill-net sets .....	26
Figure 5. Box plots showing the total lengths of trout caught at the stocked and stocking suspension lakes .....	27
Figure 6. Box plots showing the relative mass ( $W_r$ ) of trout caught at the stocked and stocking suspension lakes .....	28
Figure 7. Annual densities of <i>Rana cascadae</i> frogs and larvae .....	29
Figure 8. Relative change from 2003 in the number of untagged <i>Rana cascadae</i> caught for each treatment category .....	30
Figure 9. Mean number of large-bodied insects per lake collected in 2006 benthic sweep transects .....	31
Figure 10. Mean number of large-bodied insects caught in emergence traps .....	32
Figure 11. Percent of <i>Thamnophis atratus</i> and <i>T. sirtalis</i> stomach samples containing fishes or amphibians .....	33
Figure 12. Individual-based bird species accumulation curves .....	34
Figure 13. The nightly number of bat calls recorded during the summer of 2005 .....	35

Figure 14. Ratio of large- to small-bodied bat calls in relation to the mean annual number of large-bodied insects caught in emergence traps and to the number of trout caught per hour of gill net set each year.....	36
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Appendix A. Trinity Alps Basin Study 2005 field protocol. ....	37
Appendix B. Histograms of trout lengths of gill-netted fish .....	50

## Project Summary

Management of wildlife resources in wilderness areas is a challenge to State and Federal agencies because of mandates to protect native species and ecosystems, while providing recreational and economic opportunities for the public. Sport fish have been introduced to many formerly fishless waters in public lands throughout the western USA. The resulting fisheries foster recreation use of wilderness, however, widespread fish introductions have been implicated in the decline of several amphibian species including sensitive species such as the mountain yellow-legged frog and the Cascades frog (a Species of Special Concern in California). Our goal was to better understand the consequences of agency fish stocking policies regarding impacts to the native fauna that live in the lakes or feed on the insects and amphibians that emerge from the lakes. 2006 was the final summer of data collection for our 4-year research project assessing the effects of wilderness fisheries management options on distribution and abundance of fish, amphibians, emerging aquatic insects, birds, reptiles and bats. Our experimental design compared 12 wilderness lake basins subject to three different fisheries management techniques (continued fish stocking, cessation of stocking, and fish removal) to four historically fishless reference basins. Our 16 study basins were between 1920 to 2210 m in elevation, with water depths ranging from 2.7 - 11.2 m. The lake basins were grouped in four geographical blocks within which sites were randomly assigned for stocking suspension, fish removal, or continued stocking. There was one historically fishless basin in each block.

We collected pre-treatment data in 2003 at all basins and began trout removal from the four fish removal basins in the fall. In the following three summers we conducted repeat sampling of the 16 study basins. In each survey, amphibians and snakes were monitored via mark-recapture and visual surveys. We collected aquatic insect samples using benthic sweeps, odonate exuvia surveys, emergence trap samples and sticky trap transects. Bats were monitored via acoustic bat detectors, and we used point-count surveys and bird mapping to quantify bird abundances.

In 2003, large-bodied insects and frogs were less abundant in the 12 stocked lakes compared with historically fishless control basins. Over the following summers, Cascades frog numbers increased in the fish removal basins so that, by 2006, densities were not significantly different from the control lakes. Cascades frog numbers remained low at the stocked and stocking suspension lakes. Large-bodied emerging insect (such as caddis flies, mayflies and dragonflies) numbers increased in the fish removal lakes in the years following fish removals. We also found evidence that the introduction of trout in wilderness lakes expanded the range of the aquatic garter snake, *Thamnophis atratus*, based on our analyses of the diet, distribution and density of *T. atratus* relative to the common garter snake, *T. sirtalis*. The distribution and density of *T. atratus* matched the distribution and density of introduced trout instead of native amphibian prey, which they also opportunistically eat. Because populations of *T. atratus* are not reliant on native prey populations, they can reach high densities in the absence of high densities of amphibians (unlike *T. sirtalis*, which are dependent on amphibian prey). When *T. atratus* opportunistically prey upon native amphibians whose numbers may already be directly impacted by trout, they can cause significant additional declines. We observed 116 species of birds during our surveys over the four years and collected 793,652 bat call files. Our study shows that restoration of selected lake basins within wilderness areas of

northern California benefits a diversity of native fauna that use these lake basins and protects and enhance populations of sensitive amphibians.

### Introduction

In the western USA, thousands of historically fishless mountain lakes have been stocked with trout (primarily *Oncorhynchus*, *Salmo*, and *Salvelinus* spp.) since the late 1800s to increase recreational fishing opportunities (Pister 2001). Since 1945, the California Department of Fish and Game (CDFG) has managed the stocking of California's aquatic resources (Hopelain 2003). Using trucks, airplanes and packstock, CDFG has stocked the vast majority of California's lakes, including in remote wilderness areas. However, due to recent findings that fish introductions harm native aquatic species, CDFG is under increasing pressure to manage California's fisheries to improve conditions for these native species. For example, a recent California Superior Court ruling found that the CDFG must consider the impacts of fish stocking on sensitive aquatic species when making future fish stocking management decisions (California Superior Court of Sacramento County, 2007).

Although stocking non-native trout in wilderness lakes continues today, it already has been greatly reduced in some wilderness area lakes where sensitive amphibians occur, including the mountain yellow-legged frog (*Rana muscosa*) and Cascades frog (*R. cascadae*). The National Park Service and CDFG have also begun local restoration programs to remove introduced fish from specific mountain lakes (Milliron 2000, Knapp et al. 2007). Such actions are often contentious because the stocking program is popular with anglers and the public is aware that a multiplicity of reasons have been advanced for amphibian decline (e.g. UV radiation, pesticides, disease). Few experiments to date have shown that fish removals definitively promote amphibian population recovery. However, based on the increased occurrence and size of amphibian populations following stocking cessation in two National Parks (Knapp et al. 2001, Knapp et al. 2005) and following fish removals (Vredenburg 2004, Knapp et al. 2007, Walston and Mullin 2007), there is evidence that decreasing non-native fish populations could benefit sensitive amphibians. The relative benefits of stocking cessation versus fish removals have not been compared.

The consequences of introducing a top predator into mountain lakes and streams are just beginning to be understood. Introduced trout have been shown to affect nutrient cycling (e.g., Schindler et al. 2001), aquatic invertebrate composition and density (e.g., Knapp et al. 2001), and amphibian composition and density (e.g., Vredenburg 2004, Knapp 2005). Because the majority of aquatic insects and amphibians metamorphose from aquatic to terrestrial stages, these taxa become available as prey for terrestrial animals. The presence of trout in wilderness lakes reduces the emergence of these taxa and, therefore, may indirectly affect terrestrial predators by reducing the availability of prey. For example, Matthews et al. (2001) and Knapp (2005) found reduced occurrence and abundance of garter snakes in areas with trout, likely due to the reduced abundance of the snake's amphibian prey in these areas.

We addressed the impacts of introduced trout on the relationship between aquatic and terrestrial fauna by conducting a large-scale replicated experiment comparing the abundance and composition of several aquatic and terrestrial taxa in lake basins with and without introduced fish. Our overall goal was to test the response of aquatic and terrestrial wildlife communities to the different wilderness fisheries management options

currently being considered in California. To that end, we assessed differences among lake basins that were stocked, temporarily suspended from stocking, and had fish removed. These three fish treatments, which are currently being used by CDFG for maintaining both a recreational fishery and sensitive amphibian populations, were also compared with fish-free “control” basins. We focused on the quantity of fauna with both aquatic and terrestrial stages (insects and amphibians), and on some upland taxa that are known to feed on these (birds, bats and snakes).

## Methods

### *Study area*

The study was conducted within the Trinity Alps Wilderness in the Klamath-Siskiyou Mountains of northern California. The wilderness has approximately 100 named lakes occurring between 1,675 and 2,300 m elevation. No native fishes historically occurred in the lentic habitats of the Trinity Alps Wilderness. Recent surveys by CDFG and the US Forest Service (USFS) found that fish now occur in about 90% of the lakes greater than 2 m deep in the Trinity Alps, Marble Mountains, and Russian wildernesses. Analysis of the survey data from 730 water bodies in these wilderness areas found a negative relationship between the occurrence of fish and the presence and abundance of the Cascades frog (a sensitive species), long-toed salamander (*Ambystoma macrodactylum*), and Pacific treefrog (*Pseudacris regilla*) (Welsh et al. 2006). These landscape-scale correlations suggested the need to better understand the effects that introduced fish are having on the native fauna in these wilderness areas.

We surveyed 16 headwater lake basins distributed throughout the eastern half of the Trinity Alps Wilderness, where the vast majority of lakes in the wilderness are found (Fig. 1). Basins were chosen based on practical, physical and biological parameters such as having low recreation use, moderate elevations, and being small enough to facilitate fish removal. All lakes had inflows that did not support fish and outflows with fish barriers, making complete fish removal feasible. Also, the lakes supported or were within 1 km of habitat that supported *R. cascadae*, ensuring that amphibian population recovery was possible within the project period. Lakes were between 1,896 – 2,210 m in elevation, ranged from 0.3 ha – 1.98 ha, and were between 2.4 - 11.3 m deep (Table 1). Lakes occurred within mixed conifer to sub-alpine habitat zones with common trees being red and white fir, mountain hemlock, lodgepole pine and western white pine. Although we tried to match the physical parameters of the lakes, for historical reasons on average the fish-free sites were smaller ( $0.64 \text{ ha} \pm 0.2$  (mean  $\pm$  SE)) and shallower ( $2.67 \text{ m} \pm 0.15$ ) than the fish treatment lakes ( $1.16 \text{ ha} \pm 0.15$  and  $4.98 \text{ m} \pm 0.31$ , respectively). Two of the fish-free lakes had historically been stocked but did not support fish populations prior to the start of the study. The 12 fish-containing lakes had been stocked by CDFG with brook and/or rainbow trout for over 30 years prior to the start of the study.

### *Experimental design*

The 16 study basins were blocked into four groups of four lakes based on geographic location. The 12 fish-containing lakes were then randomly chosen as continue to stock lakes, stocking suspension lakes, or fish removal lakes. We sampled all lakes in 2003 prior to the implementation of the fish treatments. In the fall of 2003 we removed fish from the four removal lakes in fall and winter of 2003 and spring of 2004 using multiple

repeated gill net sets as described by Knapp and Matthews (1998). Post-treatment sampling was conducted in the summers of 2004-2006. During this period, CDFG maintained the fish treatments by stocking the stock lakes yearly with rainbow trout and withholding stocking from the stocking suspension and removal lakes. After the 2003 surveys, the initial lake randomization was adjusted slightly because a stocked lake (Shimmy) was found to be both fish-free and more comparable in size to other lakes than one of the existing control lakes that nearly dried up by the end of the sampling season. Shimmy Lake was reassigned as a control, a stocking suspension lake (Upper Stoddard) was reassigned to be stocked, and another lake (Luella) was brought into the study as a stocking suspension lake (Table 1). In 2006, brook trout (*S. fontinalis*) fry were found in Echo Lake, a removal lake, during visual surveys. Because trout removal failed at this site, we removed the Block 4 lakes from the Cascades frog analyses to maintain the balanced design.

#### *Field sampling*

Over the course of a field season (June – September), four crews of three people surveyed the 16 study basins in a nine-day period. Given five days off between trips, a total of six sampling trips were conducted during the summers of 2004 and 2005. In 2003, we conducted only five sampling trips to allow time for fish removals, and in 2006 heavy spring snow precluded us from conducting the first sampling trip. Field crews rotated randomly among blocks to reduce surveyor bias. Sampling methods are described below and timing and effort for the different faunal groups and techniques are summarized in Table 2. The 2005 field protocol is also provided in Appendix A.

#### *Biotic variables*

*Trout-* In mid-summer of all four study years, we sampled trout presence and density at each fish removal, continue stocking, and suspend stocking lake. A single 36 m long, variable mesh, monofilament gill-net was set perpendicular to the shoreline for approximately 4 hrs in each lake. Captured trout were identified and counted. Trout densities were estimated as catch per unit effort (CPUE: number of fish captured per hour of net set). A linear regression comparing CPUE to actual density of fish in the four fish removal lakes showed that CPUE and density are highly correlated ( $r^2 = 0.95$ ,  $P$ -value < 0.01).

*Amphibians and reptiles-* To determine presence and relative numbers of amphibians and garter snakes, we used a shoreline visual encounter survey (VES, Crump & Scott, 1994) in which we searched the shoreline and littoral zone habitats looking under banks and logs and in the substrates. When amphibians or snakes were found, we documented which species were present and counted the number of animals by life stage. In addition, adult Cascades frogs and the two species of garter snakes (*Thamnophis atratus* and *T. sirtalis*) were sampled via mark-recapture. This technique provided additional information about habitat use, movement patterns, and survival in habitats with and without fish. During mark-recapture surveys, lake, pond, wet meadow and riparian margins up to 50 m upstream and downstream of lakes were systematically searched for Cascades frogs and garter snakes. Animals were captured by hand or net. Frogs > 40 mm snout-vent length (SVL) and snakes > 340 mm SVL were individually marked using passive integrated transponders (PIT-tags)(see Pope and Matthews 2001 for PIT-tagging methods). To differentiate prey preferences of the two garter snake species, all garter

snakes caught in 2005 and 2006 were palpated to force regurgitation of any food in their digestive tracts. We recorded the species and life stage of stomach contents and measured the approximate length of fresh prey items.

*Benthic insects* – Littoral benthic macroinvertebrates were sampled by collecting six standard 1 m sweeps with a D-net at each lake each year from the dominant substrates at depths from 0.1 to 1.1 m. Three sweeps were taken during the second sampling period and three from the fourth sampling period. Sweeps followed bottom contours and sampled epibenthic and surficial sediment habitats. Large rocks and debris were carefully removed from the samples in the field then the samples were preserved in 70% ethanol for later processing in the lab. If a sample was too large to fit in a 8 oz Whirl-Pac®, it was halved until the amount of material fit in the bag. For samples that were split ( $n = 37$ ), the percentage of the sample processed ranged from 12.5% to 75% (median = 50%). In the lab, benthic insects were identified to order, except Odonates which were identified to family (Anisoptera or Zygoptera) and counted.

In addition to the yearly sweeps, in 2006 we collected 12 benthic sweep samples at each lake during all five sampling trips (one fish removal lake was not sampled during the first trip due to a nearby forest fire). At four equally spaced transects around the lake shore, three sweeps were taken at approximately 0.1 m, 0.5 m and 1.0 m deep. In the field, insects  $\geq 4$  mm were sorted and identified to order except Odonates which were identified to family. Within each taxon, individuals were grouped by size classes and counted. Lengths were estimated for each size class by measuring (from head tip to end of abdomen) up to three individuals per group.

*Emerging insects*- To quantify relative aquatic insect emergence from the littoral zone over the course of the summer, we set three 0.6 m diameter floating emergence traps for approximately 40 hrs at each lake every 2 wks. The lightweight, collapsible emergence traps were composed of polyester 'No-See-um' fabric secured to a tension frame dome made from fiberglass rods and floated by an inflated bicycle tube. One trap was set adjacent to the shoreline, one was set at approximately 40-50 cm over the dominant substrate in the lake and the last trap was set at approximately 1 m. Traps were checked twice during each sampling period by inserting an aspirator into a sleeve in the trap and collecting all insects found. Samples were preserved in 70% ethanol. Insects were identified to family, counted and measured.

In addition to the emergence traps, dragonfly and damselfly emergence was measured by collecting exoskeletons or exuviae that are often left clinging to near-shore emergent vegetation after the animal metamorphoses into a terrestrial adult. Each survey we collected exuviae from four 1 m x 20 cm plots of emergent vegetation along the edge of each of the main lakes. Plots were located at the cardinal directions around the lake.

*Terrestrial insects*- We estimated the distribution and relative abundance of emerged insects versus terrestrial insects in the terrestrial landscape using 4 transects of 40 cm<sup>2</sup> sticky traps set every 10 meters up to 40 m away from each lake (16 traps total per lake). Transects were set along lines going north, east, south and west from the lake. We collected and reset traps every survey trip.

*Birds*- To sample birds in the basins, we conducted double observer point-counts on two consecutive mornings at each study basin every two weeks. We used a double-observer approach to increase bird detection probability and reduce variation due to



differences in surveyor ability (Nichols et al. 2000). We sampled at up to six survey points in each basin spaced at 100 m intervals to increase the power to detect annual population trends in the basins (Thompson et al. 2002). Starting in 2005, we incorporated an area- and time-constrained bird mapping technique to gain more detailed information about resident and migrant species, territories, breeding and feeding. We set up one 300 X 50 m plot paralleling the shoreline of each study lake. During the survey, one person spent one hour in the plot locating and mapping individual birds, bird interactions, breeding behavior, feeding behavior, and nest sites.

*Bats-* Bat activity was measured in the fish removal and continue stocking lakes using Anabat II ultrasonic acoustic detectors and zero-crossing analyzers (storage zcaims). Starting in 2004, we set up one permanent acoustic monitoring station at each of the eight lakes on the first trip of each season. The detectors were set up within 1 m of the shoreline of the primary lake in the basin with the transducer (microphone) directed over the water. We attached the detectors and recording devices to solar chargers to maintain power throughout the summer. Bat activity was recorded every night all night (unless we had mechanical failure) on data storage cards. Cards were switched out and downloaded every two weeks. Although one cannot estimate density using bat detectors, their use to investigate differential use of habitats by bats has proven effective in identifying differences in activity in relation to habitat (e.g., Gehrt and Chelsvig 2004) and invertebrate activity (e.g., O'Donnell 2000).

#### *Environmental variables*

Elevation and surface area were obtained for each lake from GIS topographic coverage. We deployed two temperature loggers at each lake from the first to last survey of the season. Loggers were set at 0.5 m and 1.25 m deep and were maintained at these depths throughout the summer. Littoral zone characteristics were measured in July 2005 by sampling from approximately 25 evenly spaced transects around the perimeter of each lake. At each transect, depth, substrate, and presence of aquatic vegetation and woody debris were recorded at three specific distances from shore (0.1 m, 0.5 m, and 1.0 m). Estimated littoral zone slope was calculated by obtaining the slope of a least-squares line through the three depths at each specific distance from shore. The slopes for each transect were averaged to obtain a mean littoral zone slope for each lake. Littoral zone substrate categories were defined by dominant particle size and included silt (< 0.06 mm diameter), sand (0.06-2 mm), gravel (2-32 mm), pebbles (32-64 mm), cobble (64-256 mm), boulder (>256 mm), and bedrock (large, solid piece of embedded rock). We also recorded the % cover of terrestrial vegetation and dominant vegetation type (meadow, shrubs, willow, alder, conifer, forb) from the shoreline to 1.0 m from shore at each transect.

In addition, terrestrial vegetation and basin slope, aspect, and substrate were measured within 50 m of the lakeshore at each study basin. Along 50 m intervals, 6 m diameter plots were set up at random distances between 1 and 50 m from shore. In each plot, we used a clinometer to estimate slope and a compass to estimate the direction of the slope (aspect). We counted trees by species and measured diameter-at-breast height, measured shrub cover and height, and estimated cover of forbs and grasses. We also estimated the proportion of the substrate within each plot containing organics, sand, gravel, boulders, and bedrock.

### *Analysis*

Our analyses focused on differences and changes over time for trout, aquatic insects and Cascades frogs across treatments and in comparison to control lakes. We have not completed analyses on the indirect effects of trout on birds and bats so only initial results and summary information will be presented in this report. In addition, we are still processing the sticky trap samples, so we have not run any analyses from this sampling technique. For the amphibian and reptile surveys (both VES and mark-recapture), we have complete results from the four years. We also provide a fifth year of results for trout density (2007) since we have already collected and processed this data.

*Trout-* We compared trout densities in the 12 treatment lakes in 2003 to ensure that pretreatment densities were not significantly different among treatments. We used repeated measures ANOVA to compare catch per net-hour in stocked lakes versus stocking suspension lakes from 2004-2006 to see if differences occurred post-treatment. Removal lakes were not included in this analysis because, after 2003, we did not catch any trout in the removal lakes during the 4-hr gill-net samples. We also compared mean length and body condition of trout in stocked lakes versus stocking suspension lakes from 2004-2006. We used the relative mass ( $W_r$ ) condition index (Wege and Anderson 1978) to assess the relative body condition of brook and rainbow trout across treatments (see Pope and Matthews 2002 for  $W_r$  calculation). We also summarized the yearly composition of trout species and lengths of trout found in the individual lakes.

*Amphibians-* We first compared 2003 pre-treatment *R. cascadae* densities (number of frogs per 100 m of shoreline) in the 12 fish-containing lakes to ensure that pre-treatment values were not significantly different among treatment categories using one-way ANOVAs. We then ran a MANOVA to test for an overall treatment effect on VES-estimated densities of both frogs (subadult and adult combined) and larvae. Both densities of frogs and larvae were log-transformed (mean +1) to make variances more uniform. Separate repeated measures ANOVAs were then run to test for specific treatment and treatment by year effects on the yearly mean densities of frogs and larvae. In the repeated measures analysis, treatment was the between-subjects fixed effect and lake was the within-subjects effect. All analyses used Type III sums of squares. We used Tukey-Kramer multiple-comparisons to test for all pair-wise differences between means for treatments and years.

To compare survival, population growth rate, and recruitment among treatments, we used the mark-recapture data to parameterize Cormack Jolly-Seber and Pradel's reverse time population models using the software MARK (White and Burnham 1999). Given that we saw no differences between stock and suspend stocking lakes in the ANOVAs on densities of fish and frogs (see results), we combined the two fish treatments into a general "fish-containing" category for population analyses.

*Insects-* For aquatic insect analyses, benthic and emerged insects were grouped into four categories: predators (Odonata, Megaloptera or Coleoptera), trichopterans, ephemeropterans, and dipterans. Separate MANOVAs were run on the emergence and two benthic data sets with the insect groups as the response variables to test for differences in relation to the four treatments and blocks. We applied either log or power ( $Y^{0.05}$ ) transformations to normalize the response variables. We followed the MANOVAs with a series of repeated measures ANOVAs on each response variable to evaluate which

variables contributed significantly to the multivariate responses (Scheiner 2001) and test whether the insect groups were affected by a treatment by time interaction.

*Garter snakes*- We found two species of garter snakes in our 16 study basins: the common aquatic garter snake (*Thamnophis atratus*), a known fish specialist that also preys upon amphibians and the common garter snake (*T. sirtalis*), a local amphibian specialist in the Trinity Alps. We compared the diet and distribution of these two garter snake species in relation to introduced trout and amphibians to assess whether introduced trout may act as a supplemental prey source that facilitates the population increase and spread of *T. atratus* at the possible detriment of *T. sirtalis*. The presence of a common, consistent introduced prey source (fish) in sub-alpine mountain lakes of northern California may have allowed *T. atratus* to move upstream from their more typical natural trout stream habitats (Lind and Welsh 1994) into these historically fishless habitats. With the presence of introduced trout, *T. atratus* may be able to reach high densities independent of native amphibian prey. Even moderate predation by *T. atratus* on native amphibians could cause significant declines, especially if the amphibian population numbers are already depressed by other causes such as introduced fish.

We summarized the diet of the two species by comparing the proportion of trout or amphibian prey in stomach contents, obtained by palpating snakes encountered in the field. For the distribution analysis, we used the northern California high lakes dataset (Welsh et al. 2006) collected from the Klamath Mountains between 1999 and 2002. We used logistic regression to compare the occurrence of the two garter snake species with amphibians and trout while accounting for spatial autocorrelation and habitat variables. To estimate *T. atratus* and *T. sirtalis* densities, we used 2005-2006 mark-recapture data from Ward Lake and Hidden Lake, respectively. We estimated capture probabilities and population sizes using Jolly-Seber capture-recapture models in the program JOLLY ("robust design"; Pollock et al., 1990). This design includes several secondary sampling occasions within each primary sampling period (year) and allows for time-specific changes in parameters such as population size and survival.

*Birds*-We determined the relative detection frequency of bird species from the point count surveys. To see if species accumulation rates and richness varied between removal and stocked lakes, we also calculated individual-based species accumulation curves (Gotelli and Colwell 2001) using the software EstimateS (Colwell 2000).

*Bats*-We filtered noise from recorded echolocation passes using a modified filter in the program AnaLook (Titley Electronics) that separates individual call files by a combination of quantitative (minimum and mean call note frequency) and qualitative (call curvature and slope) metrics. We then tabulated and categorized the actual bat calls into call frequency groups using addition filters. Bat groups included 50 kHz bats, 40 kHz bats, and 25 kHz bats. In general, the higher call frequencies are made by smaller-bodied bats while the low call frequencies are made by large-bodied bats. We calculated the annual average ratio of 25 kHz bats to 50kHz bats per lake per year and compared the ratios between removal lakes and stocked lakes using ANOVA. We then related the mean annual ratio of calling activity of large-to-small bats to the mean annual emergence of large-bodied insects and density of trout at each lake using linear regression.

## Results

We completed 22 two-day sampling periods at each of the experimental lake basins over the four-year study (five sampling periods in 2003 and 2006 and six in 2004 and 2005). Insect sampling resulted in approximately 1,900 emergence trap samples, 384 benthic sweep samples, 1,042 exuvia samples and 3,570 sticky trap samples. During VES surveys, five species of amphibians were encountered fairly regularly including Cascades frog (*Rana cascadae*), Pacific chorus frog (*Pseudacris regilla*), western toad (*Bufo boreas*), long-toed salamander (*Ambystoma macrodactylum*) and rough-skinned newt (*Taricha granulosa*). An additional species, Pacific giant salamander (*Dicamptodon tenebrosus*) was found at Adams Lake. The aquatic garter snake (*Thamnophis atratus*) and common garter snake (*T. sirtalis*) were the only reptiles consistently found in association with lentic habitats. We identified 116 species of birds during the 44 point-count surveys per lake with 22 species observed over 100 times during the four survey years (Table 3). A total of 20,264 birds were identified during all the point count surveys. Eight bat detectors were set at the fish removal and stock lakes in summers 2004-2006. A total of 793,652 bat call files were recorded from 874 survey nights. These files represent calls from at least nine species of bats.

### *Trout*

In 2003, pretreatment densities of trout were similar in the three fish treatment categories ( $df = 2$ ,  $F = 0.25$ ,  $P = 0.784$ ) with an average catch of 3.57 trout per net-hour (95% confidence intervals 1.31-5.83). We removed 672 trout from the four fish removal lakes in the fall and winter of 2003 and did not catch trout again in the 2004-2006 four-hour gill net sets (Fig. 2). In 2006, however, we observed fingerling brook trout in the Block 4 fish removal lake (Echo Lake), which meant that we missed at least two trout in that lake during fish removals.

We used repeated measures ANOVA to compare 2003-2007 CPUE data from the stock and suspend lakes to see if a difference between treatments was observable (Fig. 3). We did not find any significant differences by treatment ( $df = 1$ ,  $F = 2.03$ ,  $P = 0.23$ ) or treatment by year ( $df = 4$ ,  $F = 0.63$ ,  $P = 0.13$ ). In one of the stocking suspension lakes (Hidden Lake), however, catch per net-hour dropped from 5.88 trout/hr in 2003 to 0 trout/hr in 2006 and 2007. We also compared total length and body condition of trout between treatments and across years using repeated measures ANOVAs. For total fish length, we saw no significant treatment ( $df = 1$ ,  $F = 1.28$ ,  $P = 0.3$ ) or treatment by year effect ( $df = 4$ ,  $F = 1.43$ ,  $P = 0.28$ , Fig. 4a), but there was again a significant lake effect ( $df = 6$ ,  $F = 14.57$ ,  $P < 0.001$ ). On average, Salmon Lake (stocking suspension lake) had the largest fish with a mean total length of 209 mm and Upper Stoddard (stocked lake) had the smallest fish with a mean total length of 136 mm (Fig. 5). Total length histograms of netted trout for each year at the stock and stocking suspension lakes are provided in Appendix B. For body condition, there was not a significant treatment ( $df = 1$ ,  $F = 0.24$ ,  $P = 0.64$ ) or treatment by year effect ( $df = 4$ ,  $F = 0.97$ ,  $P = 0.45$ , Fig. 4b) but there was a significant lake effect ( $df = 6$ ,  $F = 20.10$ ,  $P < 0.001$ ). Salmon Lake had the highest average  $Wr$  value (119) and Hidden Lake had the lowest  $Wr$  value (90.6, Fig. 6). A value of 100 equals the average body condition for all fish caught during the project.

In 2003, we caught three rainbow trout at Ward Lake and none at any other of the stocked or stocking suspension lakes but caught brook trout at all eight lakes. By 2005, we caught only rainbow trout at Upper Stoddard and Mavis Lake: the brook trout seem to have disappeared from these two stocked lakes. We continued to catch both brook and rainbow at Ward Lake and only brook at Deer Lake. Brook trout remained at three of the four stocking suspension lakes through 2007.

### *Cascades frogs*

2003 pre-treatment Cascades frog densities were similar among the treatment lakes ( $F_{2,9} = 0.82$ ,  $P = 0.47$ ). The total number of frogs and density of frogs and larvae increased dramatically at the removal lakes following fish removals but remained relatively low at the stock and suspend stocking lakes across all years of the project (Table 1, Fig. 7). The MANOVA comparing the density of frogs and larvae across treatments showed a significant treatment effect. In the repeated measures ANOVA for frog density there was a strong year ( $P = 0.005$ ) and treatment by year ( $P = 0.02$ ) effect and a strong trend in treatment effect ( $P = 0.07$ ). Pair-wise comparisons showed that the pre-treatment frog densities at the removal lakes were less than all control year densities and 2006 densities at the removal lakes. Post-treatment (2004-2006) densities at the removal lakes were greater than all years' densities at both the stock and suspend stocking treatment lakes. In the ANOVA comparing the density of larvae, there was a significant treatment ( $P = 0.005$ ) effect but not a year ( $P = 0.17$ ) or treatment by year ( $P = 0.3$ ) effect. Pair-wise comparisons showed that the stock and suspend stocking lakes had lower densities than the control lakes but that the removal lakes did not significantly differ from either the control or fish-containing treatments, although a trend toward an increase in density could be seen at the removal lakes (Fig. 7).

Between 2003 and 2006, 546 frogs were individually PIT-tagged at the fish-free control lakes, 321 were tagged at the fish removal lakes, 110 at the stock lakes and 79 were tagged at the stocking suspension lakes. The number of untagged adult *R. cascadae* (> 42 mm SUL) caught at the fish removal lakes increased yearly reaching a 10-fold increase by 2006 compared to the 2003 pre-treatment year, while the number of untagged adults remained relatively constant at the control, stock and suspend stocking lakes across pre- and post-treatment years (Fig. 8). In 2006, there was an increase of new frogs tagged (from a mean of 1 to 7) at Lion Lake, one of suspend stocking lakes, concurrent with a decrease in fish density at that lake (Table 1).

Fish removal improved frog recaptures. Using MARK to model Cascades frog population dynamics at removal, control and fish-containing lakes; we found survival to 2004 of frogs tagged during the 2003 pre-treatment year was 34% lower at the fish removal lakes (0.59; 95% CL 0.35-0.80) than the control lakes (0.79; 95% CL 0.69-0.87). Survival rates for frogs tagged in 2004 and recaptured in 2005 were similar at the fish removal lakes (0.76; 95% CL 0.56-0.89) and control lakes (0.79; 95% CL 0.68-0.87). By 2006, survival estimates of frogs tagged in 2005 increased to 0.94 (95% CL 0.63-0.99) at the removal lakes and were about 47% higher than survival estimates of frogs tagged during the same period at the control lakes (0.64; 95% CL 0.51-0.75) and 31% higher than the overall estimated survival rate of frogs at the fish-containing lakes 0.72 (95% CL 0.63-0.80). The estimated recapture probability for all years and all treatments was 0.73 (95% CL 0.67-0.78). Estimates of realized population growth rate ( $\lambda$ , where  $\lambda = 1$  means a stable population,  $\lambda > 1$  means a growing population, and  $\lambda < 1$  means a declining

population) ranged annually from 1.2 (95% CL 1.01-1.39) to 1.35 (95% CL 1.15-1.54) at the fish-free control lakes; 1.66 (95% CL 1.40-1.84) to 3.03 (95% CL 1.66-4.39) at the fish removal lakes and 0.91 (95% CL 0.59-0.99) to 1.21 (95% CL 0.88-1.54) at the fish-containing lakes. Recruitment estimates ranged from 0.45 (95% CL 0.35-0.55) to 0.60 (95% CL 0.42-0.76) at the fish-free control lakes; 0.77 (95% CL 0.51-0.92) to 1.75 (95% CL 1.17-2.32) at the fish removal lakes and 0.16 (95% CL 0.06-0.35) to 0.46 (95% CL 0.21-0.74) at the fish-containing lakes. Overall, the population growth rate of adult frogs was highly correlated with recruitment rate ( $r_p = 0.99$ ,  $n = 9$ ,  $P < 0.001$ ) but not with adult survival ( $r_p = -0.34$ ,  $n = 9$ ,  $P = 0.36$ ).

### *Insects*

We found a significant treatment effect ( $P = 0.04$ ) on the four insect groups (predators, trichopterans, ephemeropterans, and dipterans) using a MANOVA on the 2006 benthic transect data. Separate univariate ANOVAs found significant treatment effects on ephemeropterans (mayflies,  $P = 0.01$ ) and trichopterans (caddisflies,  $P = 0.05$ ) with less of both groups occurring in the stocked and stocking suspension lakes (Fig. 9). We found only a trend for dipterans (flies and midges,  $P = 0.1$ ) with greater numbers occurring in fish-containing lakes, and did not find a significant treatment effect for aquatic invertebrate predators ( $P = 0.3$ ). However, using repeated measures ANOVA, we found that large predators peaked significantly later in the 2006 season at stocked lakes compared to the fish removal lakes (treatment by trip effect:  $F = 4.48$ ,  $P = 0.05$ ).

We tested whether fish treatments affected the emergence of large-bodied insects (predators, trichopterans, and ephemeropterans combined) using repeated measures ANOVA. We found a significant treatment ( $F = 4.43$ ,  $P = 0.03$ ) and treatment by year ( $F = 2.64$ ,  $P = 0.02$ ) effect with the removal lakes having significantly more emergence of large-bodied insects than at fish-containing lakes in all post-treatment years but not in the 2003 pre-treatment year (Fig. 10).

### *Garter snakes*

We sampled stomach contents from 458 snake captures: 155 *T. atratus* and 303 *T. sirtalis* (J. Garwood's data included in totals). A total of 152 (33%) of the stomachs contained prey (57 *T. atratus* and 95 *T. sirtalis*), which produced 405 individual prey items. About half of the *T. atratus* with stomach contents had trout in their stomachs and the other half had amphibians with *R. cascadae* in 33% of the *T. atratus* with prey (Fig. 11). We found only amphibians in the stomachs of *T. sirtalis*. Five amphibian species were represented including all life stages except eggs. *Rana cascadae* was found in 66% of the *T. sirtalis* with prey in their stomachs.

Details on the results of the logistic regression models using the high lakes dataset to assess the distribution of *T. atratus* and *T. sirtalis* in relation to amphibians and fish are beyond the scope of this report but a manuscript on the garter snake data is currently in review at Biological Conservation. We will provide a copy of the paper after it is accepted for publication. In summary, we found that *T. atratus* presence at a water body is positively associated with presence of trout but not amphibians. In contrast, *T. sirtalis* is only positively associated with the presence of amphibians.

We found that *T. atratus* attained approximately twice the density of *T. sirtalis*, using the garter snake mark-recapture data from two basins where snakes were sufficiently abundant for population estimates. *T. atratus* had an average adult density of  $\sim 7$

snakes/ha over two sampling years (mean population = 29, range:  $15 \pm 4.8$  to  $44 \pm 17.6$ , capture probability:  $0.13 \pm 0.04$ ) while *T. sirtalis* had an average density of  $\sim 3$  adults/ha (mean population = 13, range:  $8 \pm 3.1$  to  $28 \pm 7.7$ , capture probability:  $0.23 \pm 0.06$ ).

### *Birds*

We are just beginning to look at the bird data so we cannot present any robust analyses. Our individual-based species accumulation curves for stocked and removal lakes showed remarkably similar species accumulation rates (Fig. 12). After four years of surveys and the identification of 116 bird species, an asymptote for species richness was not reached for individual stocked or fish removal lakes (Fig. 12).

### *Bats*

We have also just begun to analyze the bat data so only provide some preliminary results in this report. From 2004 to 2006, a total of 793,652 bat call files were recorded from 874 survey nights at the stocked and fish removal lakes. There was high variability in bat activity among lakes and from night to night (Fig. 13). Number of calls recorded per night varied from 5 to 3500. Instead of focusing on total numbers of bat calls per lake, we compared the proportion of calls made by large- (25 kHz) versus small-bodied (50kHz) bats at the lakes. We did not find a significant treatment ( $F = 1.64$ ,  $P = 0.25$ ) or treatment by year ( $F = 1.01$ ,  $P = 0.4$ ) difference in the proportion of large-bodied bats using repeated measures ANOVA. We did find a positive trend when we related the proportion of large-bodied bat calls at a lake with the mean annual emergence of large-bodied insects and a negative trend when we related large-bodied bats to yearly catch per net-hour of trout (Figure 14).

## Conclusions

This study has yielded several significant findings to date, with more expected as data analysis continues. Most importantly, this carefully controlled, manipulative experiment provides conclusive proof that fish removals are effective in restoring local populations of *Rana cascadae*, a state and federal species of special concern. In three years following fish removals densities of adult frogs in restoration basins were equivalent to fishless control basins. In 2007, there were several egg masses at all restoration basins and high recruitment levels appeared to be continuing.

We did not find any significant differences in density, body condition, or length of trout in stocked versus stocking suspension lakes, even though one of the stocking suspension lakes (Hidden Lake) appears to have gone fishless. The other three stocking suspension lakes maintained relatively consistent densities of trout for the duration of the study. There appeared to be a trend where densities are decreasing and body condition is increasing in stocking suspension lakes relative to the stocked lakes but more years of monitoring would be needed to verify this trend.

Large-bodied aquatic insects also increased in abundance following fish removals. These insects are important in both aquatic and terrestrial life stages; for example dragonflies and damselflies provide natural mosquito control (e.g., Finke et al. 1997). The terrestrial stages of aquatic insects also provide food for adult frogs so by removing fish from a lake, not only is predation pressure on larval amphibians reduced but competition pressure for food resources is also reduced (Finlay and Vredenburg 2007). In

addition, it appears that the composition of bats using Trinity Alps lakes is related to the abundance of large-bodied insects emerging from the lakes. There appears to be higher proportion of large-bodied bats at lakes with strong large-bodied insect emergence. Fish, which preferentially eat large-bodied aquatic insects (Progar and Moldenke 2002), may indirectly affect the composition of bats using Trinity Alps lakes by reducing the prey for large-bodied bats such as Big brown bat (*Eptesicus fuscus*) and Hoary bat (*Lasiurus cinereus*). We note that our largest data set for insects is still being constructed via lab work (sticky traps) but observations thus far suggest that these data will serve to strengthen our findings that trout decrease the emergence of large-bodied insects from Trinity Alps Wilderness lakes.

The research also showed that the presence of fish is strongly correlated with the distribution of garter snakes. In lakes with fish, *T. sirtalis* is largely replaced by the aquatic garter snake (*T. atratus*). Diet analysis strongly suggests that the lack of amphibian prey in lakes with fish causes the corresponding dearth of *T. sirtalis*. Approximately 100% of meals recovered from *T. sirtalis* were identified as amphibians. The diet of *T. atratus* was mixed; it contained an equal percentage of fish and amphibians. The presence of abundant alternative prey (fish) means that the density of *T. atratus* does not depend on amphibians, so it can drive amphibians to low levels without suffering a corresponding reduction in its population size. This food web configuration is known as apparent competition (Holt et al. 1994) or sometimes hyperpredation (Courchamp et al. 2000). Hyperpredation occurs when non-native prey (such as introduced trout) facilitate invasive predators (such as *T. atratus*), which then suppress native prey (such as Cascades frog).

In addition to the results from our main experiment, the data we collected is some of the best natural history data available for Trinity Alps Wilderness lake basins. We have documented sightings of Pine Martens at several lakes, we have generated a list of 116 bird species complete with relative abundances and breeding activity data, and our extensive bat surveys are the first completed for the area. We will continue to analyze the bat data to establish whether fisheries management of water resources indirectly affects the local abundances or taxonomic composition of the bat community. Bat activity over the water was often quite intense, as indicated by the number of call files logged.

Fisheries managers are almost always required to balance the needs of various stakeholders, such as anglers and packers, with the mission to manage California's diverse native fauna for its ecological value or intrinsic value to other stakeholders, such as hikers and birdwatchers. The idea that sport fish might cause amphibian population declines has been controversial, especially among anglers and fisheries managers, because most studies to date have been retrospective and correlational (but see Vredenburg 2004 and Knapp et al. 2007), and because amphibian declines have a variety of causes. This project demonstrated conclusively that fisheries management decisions can have large effects on amphibians, on large-bodied insects, and possibly on terrestrial predators that feed on emerging aquatic animals.

Our study shows that if managers restore some carefully selected lake basins within wilderness areas of northern California, this would preserve a diversity of native fauna that use these lake basins and protect and enhance populations of sensitive amphibians. In particular we show that removal of fish from some lakes can reverse local amphibian declines. This is of increasing urgency in high mountain lakes, because the invasive



fungal disease, chytridiomycosis, is currently decimating mountain yellow-legged frog populations in the Sierra Nevada and was recently found in Cascades frogs in both the Trinity Alps and Lassen area, where there are already very few populations remaining (Fellers et al. 2007). In most diseases, a small percentage of individuals have some natural disease resistance, however if the population becomes too small, not enough resistant individuals will occur and the population will be extirpated. We hope the information provided by this study will help fisheries and wildlife managers to balance angling opportunities with the need to maintain sizable amphibian populations.

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Table 1. Physical parameters, maximum number of *Rana cascadae* seen per year during visual encounter surveys, and annual catch per net-hour of trout for the 16 individual study lakes.

Lake	Block	Treatment	Elevation (m)	Max depth (m)	Area (ha)	Max. no. of frogs				Catch per net-hour of trout			
						2003	2004	2005	2006	2003	2004	2005	2006
Eagle Creek	1	fish-free control	1922	2.4	0.33	14	23	14	8	-	-	-	-
Section Line	1	fish removal	2182	4.1	0.99	17	151	102	264	1.40	0.00	0.00	0.00
Mavis	1	stocked	2042	4.2	1.52	29	26	24	10	0.18	4.65	0.92	8.64
Hidden	1	stocking suspension	2050	4.6	1.51	0	1	0	0	5.88	1.50	0.88	0.00
Found	2	fish-free control	2088	2.7	1.14	192	293	476	530	-	-	-	-
Adams	2	fish removal	1896	4.9	0.67	4	36	33	47	7.40	0.00	0.00	0.00
Upper Stoddard	2	stocked	1951	3.5	0.24	4	6	10	17	3.57	0.25	4.75	2.96
Lion	2	stocking suspension	2135	7.3	1.44	4	5	2	9	7.80	6.50	6.28	3.75
Shimmy	3	fish-free control	1958	3.0	0.78	32	69	40	52	-	-	-	-
Little Caribou	3	fish removal	2191	5.3	1.32	11	20	22	52	1.89	0.00	0.00	0.00
Ward	3	stocked	2172	7.0	1.98	0	2	1	0	8.25	6.50	5.15	8.08
Salmon	3	stocking suspension	2179	4.0	0.66	17	16	25	24	1.04	2.75	1.16	1.68
C26062	4	fish-free control	2056	2.4	0.31	8	5	5	5	-	-	-	-
Echo*	4	fish removal	2213	5.2	1.30	31	25	22	25	3.30	0.00	0.00	0.00
Deer	4	stocked	2179	5.8	1.31	9	3	4	3	7.20	6.25	10.87	9.03
Luella	4	stocking suspension	2117	3.8	0.94	-	1	3	2	-	3.75	0.93	9.51

\*In 2006 crews found brook trout fry in Echo Lake. Because trout were not completely removed from the Block 4 removal treatment, the block was removed from the analyses.

Table 2. Summary of faunal sampling techniques, timing of sampling and purpose of sampling during the four-year study.

<b>Taxon</b>	<b>Technique</b>	<b>Years Implemented</b>	<b>Frequency</b>	<b>Purpose</b>
<b>Insects</b>	Emergence traps	2003-2006	3 traps, 2 nights, every trip	Estimate of composition, number and size emerging from lakes
	Benthic sweeps	2003-2006	3 sweeps, 2 trips per year	Estimate of composition, number and size in benthos
	Odonate exuvia sampling	2003-2006	4 plots every trip	Identification and size at metamorphosis
	Sticky traps	2003-2005	16 traps set for 2 wks every trip	Abundance and proportion of aquatic vs. terrestrial insects up to 40 m from shore
<b>Trout</b>	Gill-netting	2003-2006	1 4-hr set per year	Density, size and species of trout
<b>Amphibians</b>	Visual encounter surveys	2003-2006	1 every trip	Presence and abundance of species by lifestage
	Mark-recapture (adult <i>Rana cascadae</i> )	2003-2006	1 every trip	Recruitment, movement, population size and growth
<b>Reptiles</b>	Visual encounter surveys	2003-2006	1 every trip	Presence and abundance of species by lifestage
	Mark-recapture (garter snakes)	2004-2006	whenever encountered	Recruitment, movement and growth
<b>Birds</b>	stomach palpation	2005-2006	whenever encountered	Diet
	Point count surveys	2003-2006	2 mornings every trip	Composition and relative abundance
	Bird mapping	2005-2006	2 mornings every trip	Composition and behavior
<b>Bats</b>	call recording	2004-2006	1 detector recording nightly all summer	Species group identification and index of activity

Table 3. Bird species recorded > 100 times during 2003-2006 point count surveys and number of times recorded at any of the 16 study basins.

Species	Count	Species	Count
Dark-eyed Junco	4909	Townsend's Solitaire	318
Mountain Chickadee	3317	Fox Sparrow	312
Red-breasted Nuthatch	2846	Red crossbill	295
Yellow-rumped Warbler	1869	American Dipper	194
Steller's Jay	1248	Rufous Hummingbird	185
American Robin	714	Olive-sided Flycatcher	184
Golden-crowned Kinglet	514	Green-tailed Towhee	178
Pine Siskin	460	Hermit Thrush	160
Brown Creeper	409	Lincoln's Sparrow	156
Clarck's Nutcracker	406	Hermit Warbler	116
Northern Flicker	347	Nashville Warbler	112

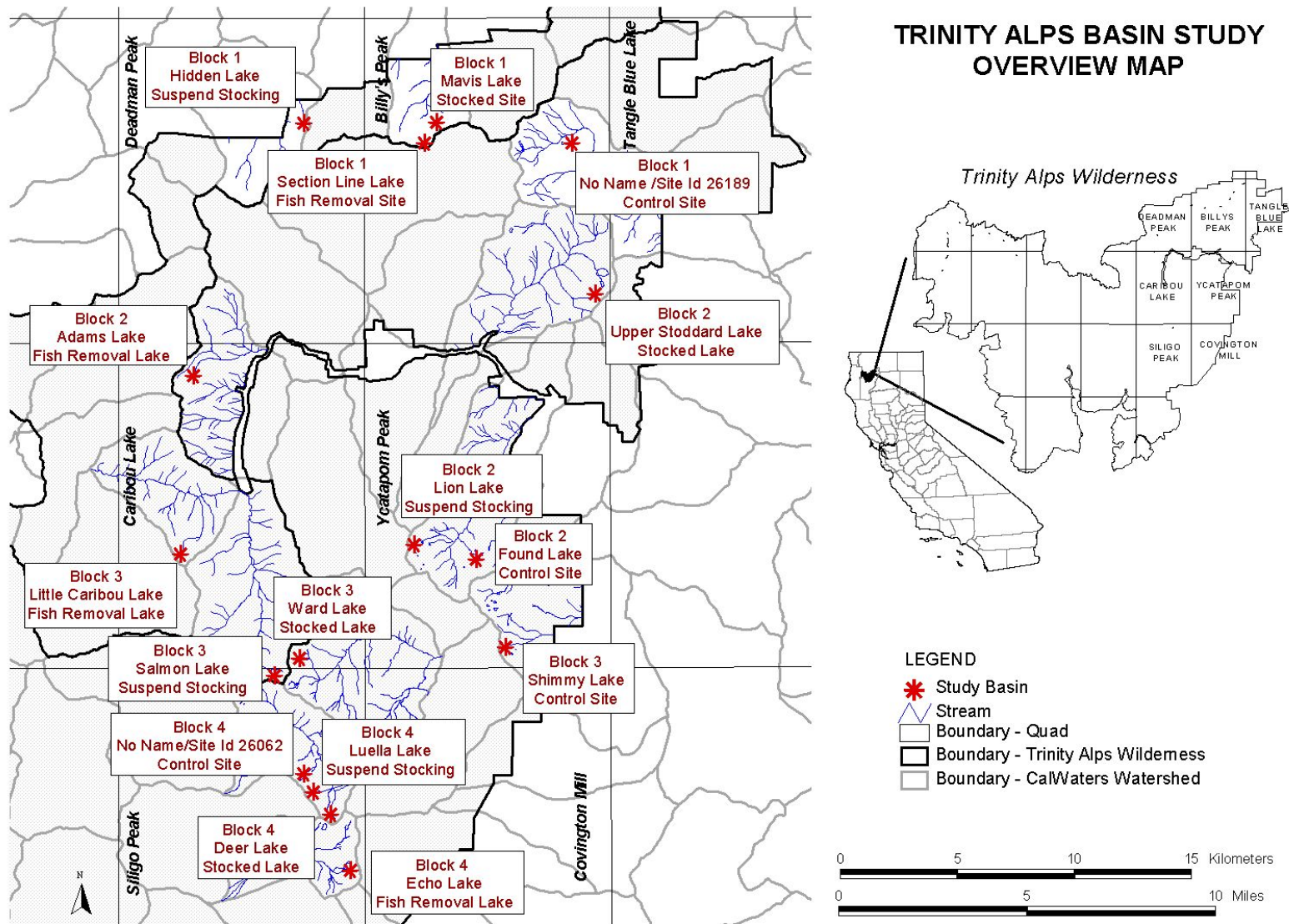


Figure 1. Map of the Trinity Alps study area.

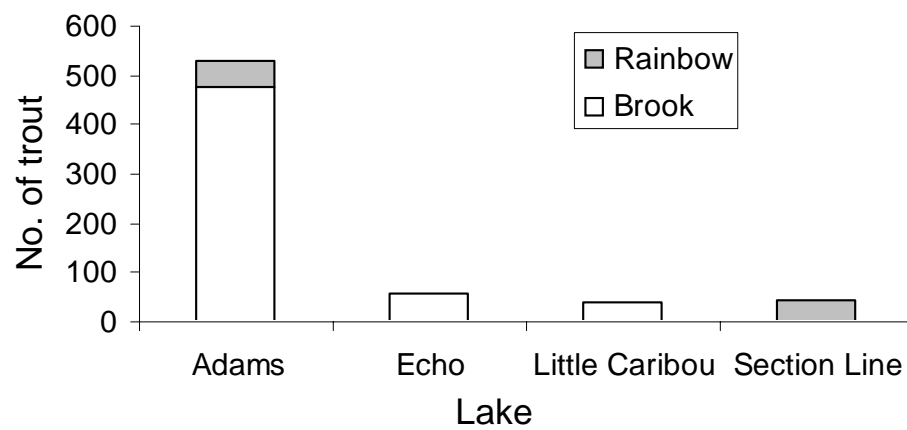


Figure 2. Total number of trout gill-netted from the fish removal lakes in fall 2003 and spring 2004. Fish caught in nets set over-winter are not included.



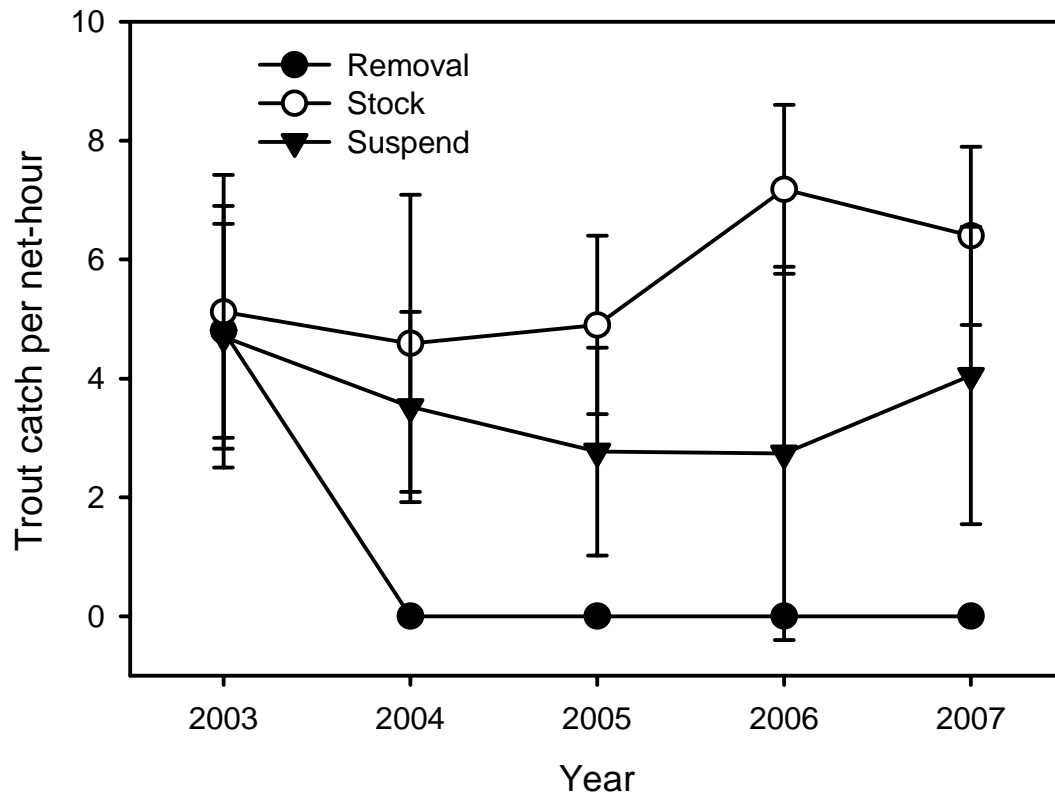


Figure 3. Mean number of trout caught per hour of gill net set for each treatment category over five years. 2003 was pre-treatment (before fish removals) and 2004-2007 were post-treatment. Error bars represent  $\pm 1$  SE.

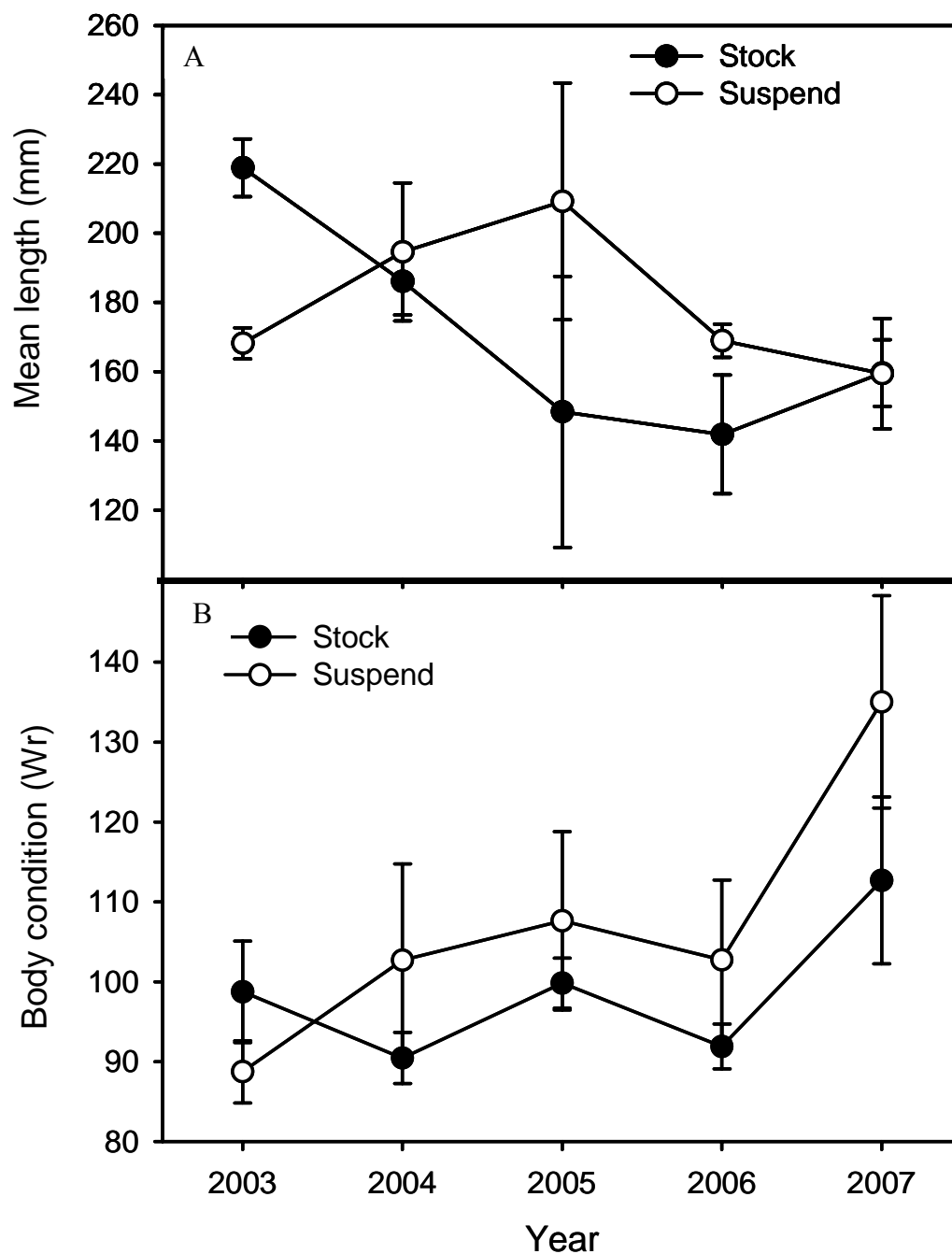


Figure 4. Mean total length (A) and body condition (B) of all trout caught during 4-hr gill-net sets at each stocked and stocking suspension lake  $\pm 1$  SE. SEs are for differences among lakes within each treatment. The mean body condition of all trout caught at the eight lakes over all years equals 100.

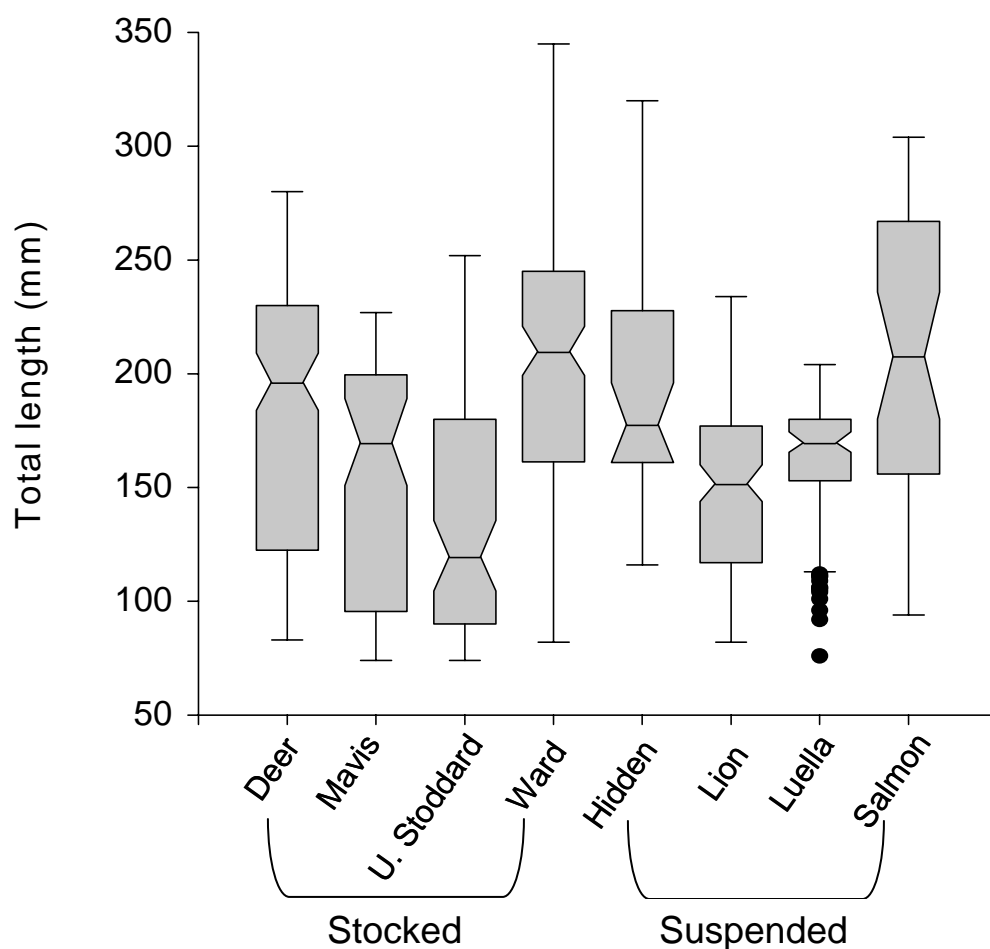


Figure 5. Box plots showing the total lengths of trout caught at the stocked and stocking suspension lakes between 2003 and 2007. The solid line within each box represents the median, the bottom and top borders indicate the 25th and 75th percentiles, the notches represent the 95% confidence intervals, the whiskers below and above each box mark the 10<sup>th</sup> and 90<sup>th</sup> percentiles, and dots indicate points outside the 10<sup>th</sup> and 90<sup>th</sup> percentiles.

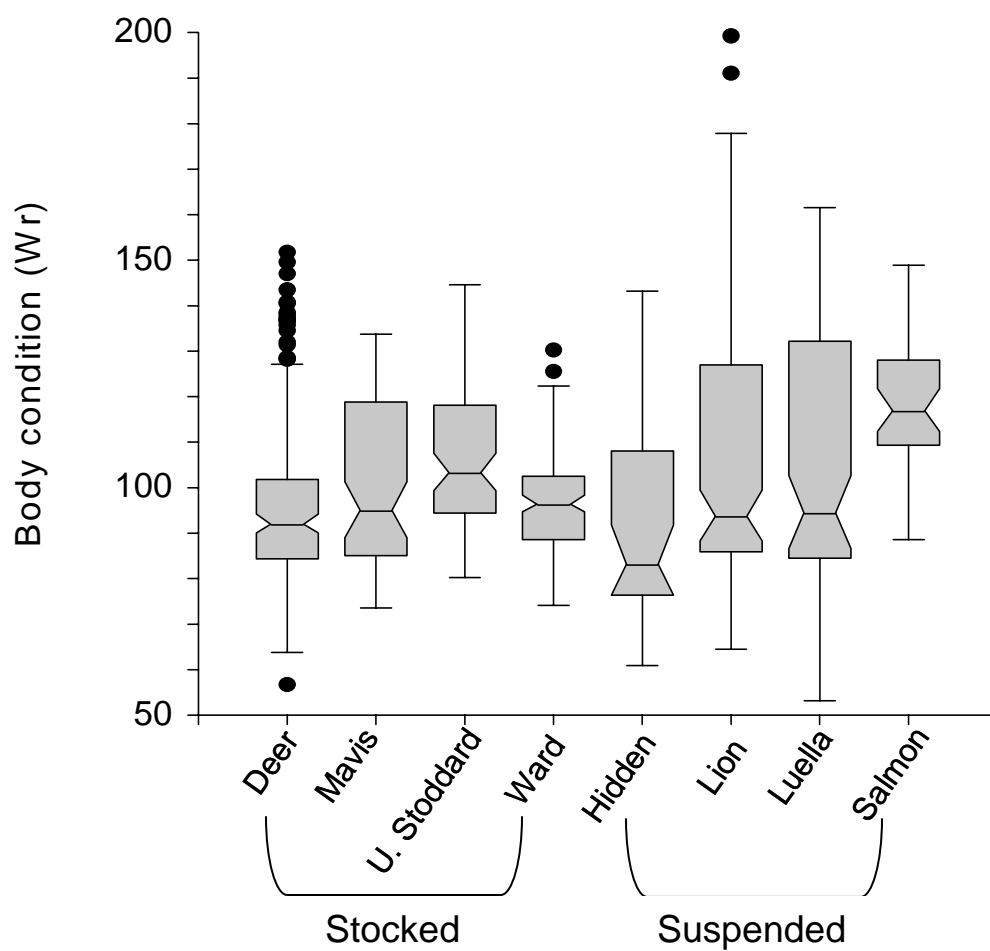


Figure 6. Box plots showing the relative mass (Wr) of trout caught at the stocked and stocking suspension lakes between 2003 and 2007.

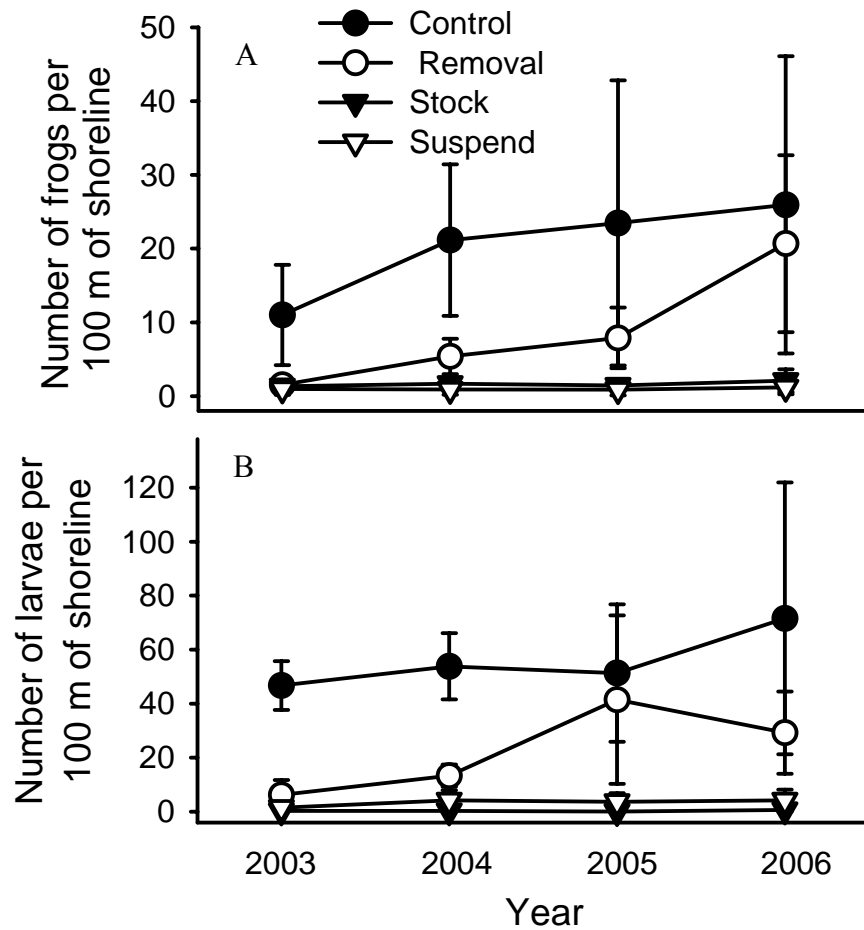


Figure 7. Annual density (mean  $\pm$  SE) of *Rana cascadae* frogs (A), and larvae (B) in fishless control lakes, fish removal lakes, stocked lakes, and lakes where stocking was suspended throughout the study. Three study lakes are included in each treatment and control category since Block 4 lakes were removed from amphibian analyses due to the presence of trout in the fish removal lake, Echo. Fish removal in the removal lakes began in the fall of 2003 following the 2003 surveys.

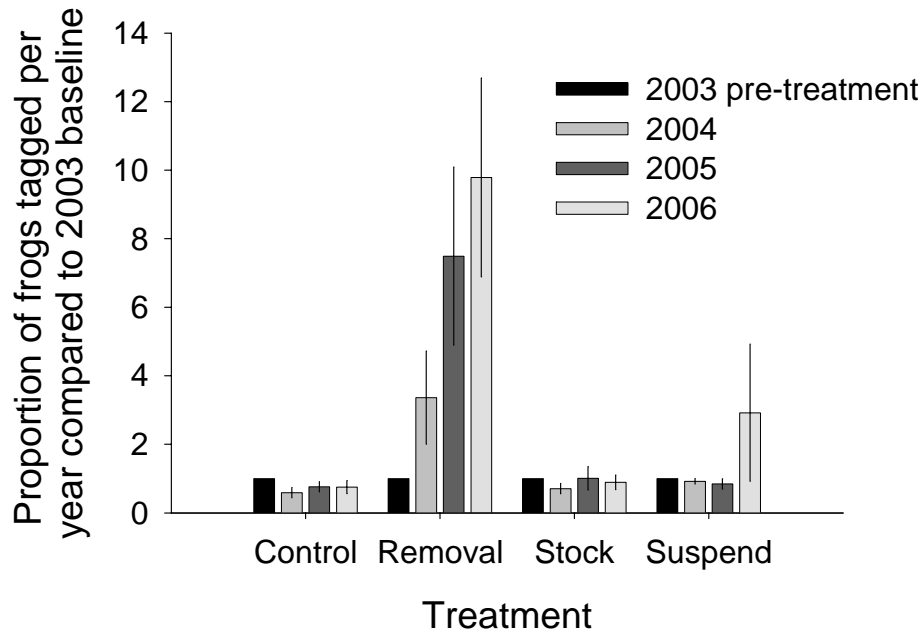


Figure 8. The average yearly change from 2003 in the number of untagged *Rana cascadae* frogs > 42 mm caught for each treatment category. For each lake, the total number of untagged frogs per year was divided by the total number of untagged frogs caught in 2003. Bars represent the average proportion of untagged frogs for lakes in each treatment category and lines indicate  $\pm$  SE. For all treatments, 2003 values equal 1 (no. untagged frogs in 2003/ no. untagged frogs in 2003).

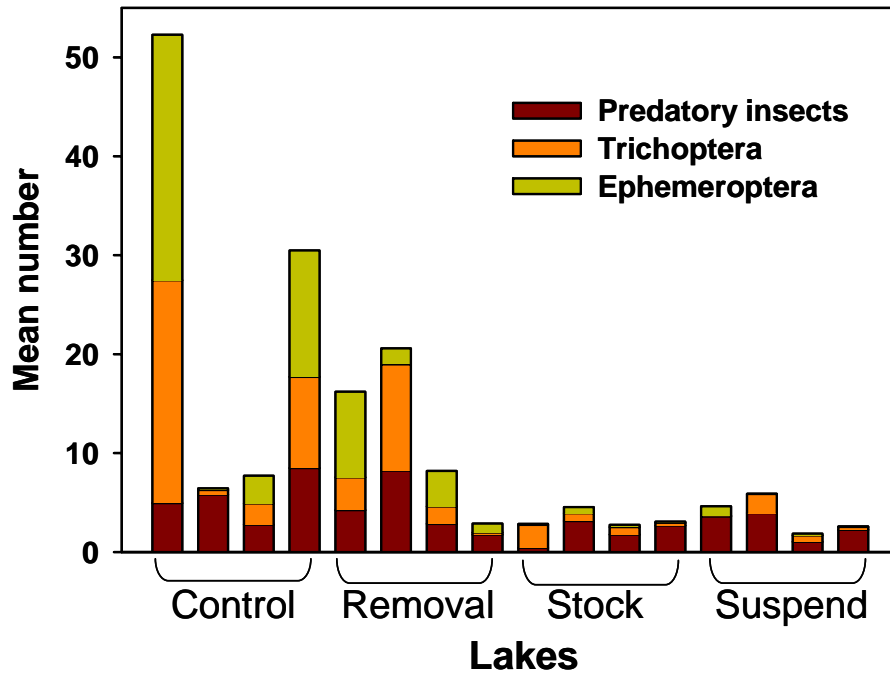


Figure 9. Mean number of large-bodied insects per lake collected in 2006 from benthic sweep transects. Bars are stacked with the mean number of predatory insects (e.g., odonates, megalopterans), trichopterans, and ephemeropterans differentiated by color. Individual lakes are grouped by treatment category.

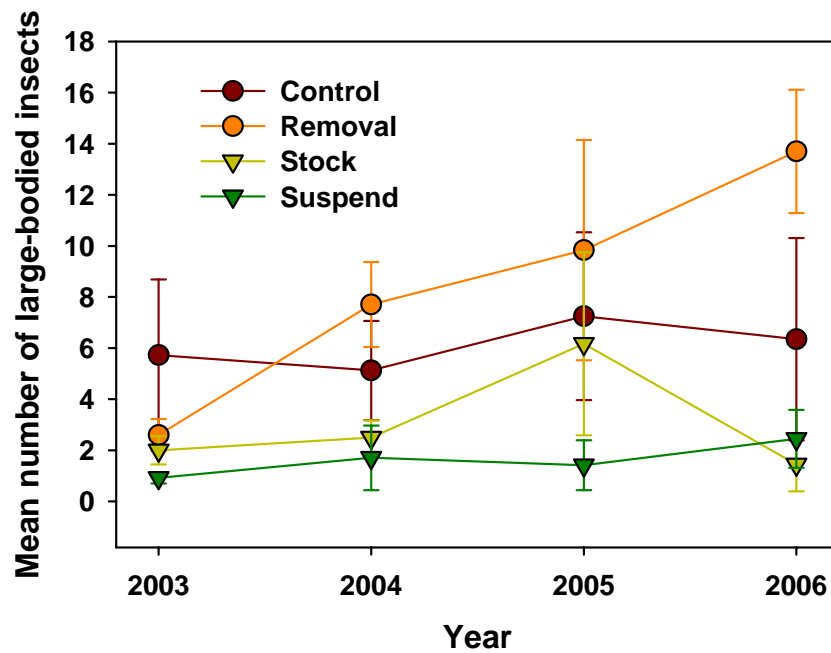


Figure 10. Mean number of large-bodied insects caught in emergence traps per year for each treatment category. 2003 was pre-treatment (before fish removals) and 2004-2006 were post-treatment. Error bars represent  $\pm 1$  SE.



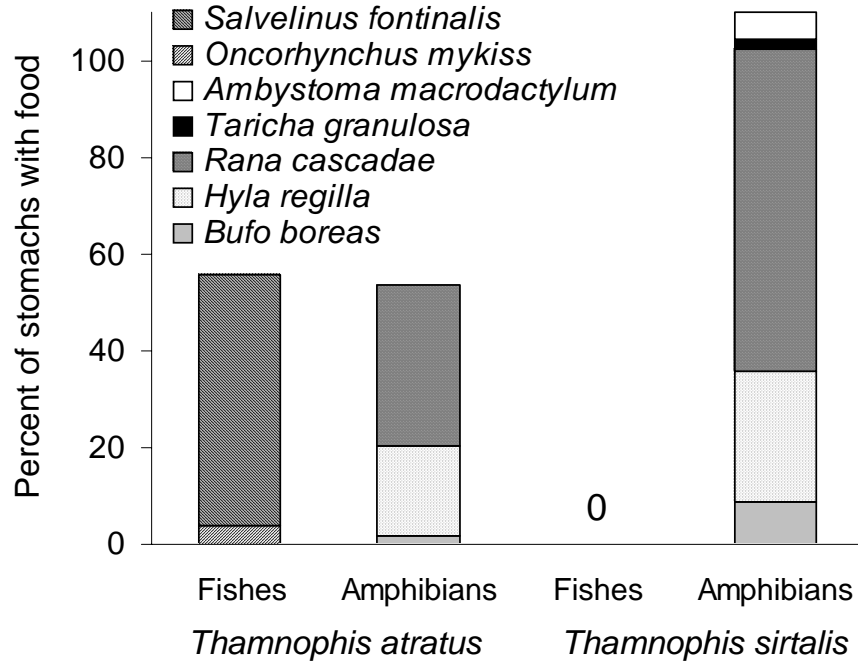


Figure 11. Percent of *Thamnophis atratus* and *T. sirtalis* stomach samples containing fishes or amphibians. Prey types are distinguished by species. For *T. atratus*, the combined percent of fishes and amphibians is greater than 100 because some snakes had both fishes and amphibians in their stomachs.

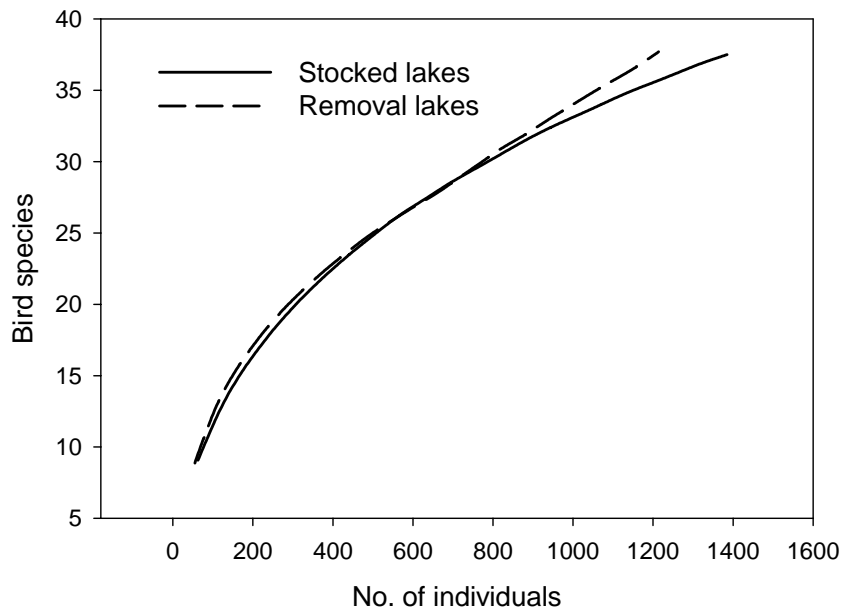


Figure 12. Individual-based bird species accumulation curves based on the point count data collected from 2003 through 2006 for lakes stocked with trout annually and lakes where trout were removed in 2003.

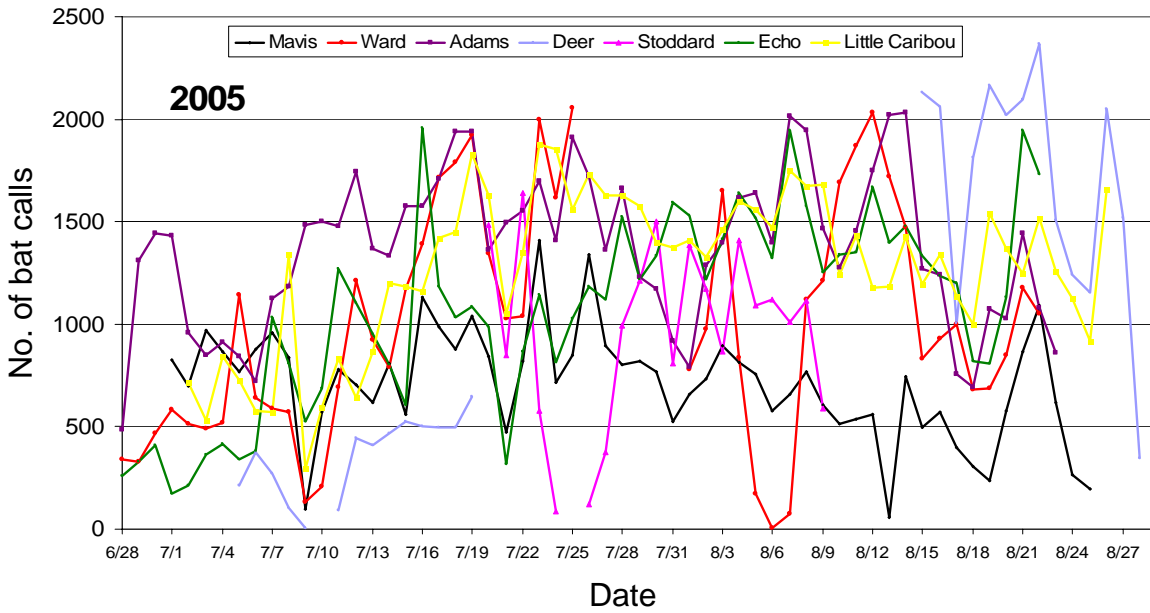


Figure 13. The nightly number of bat calls recorded at each study lake during the summer of 2005. Note the variability at a single lake from night to night and among lakes on the same night.

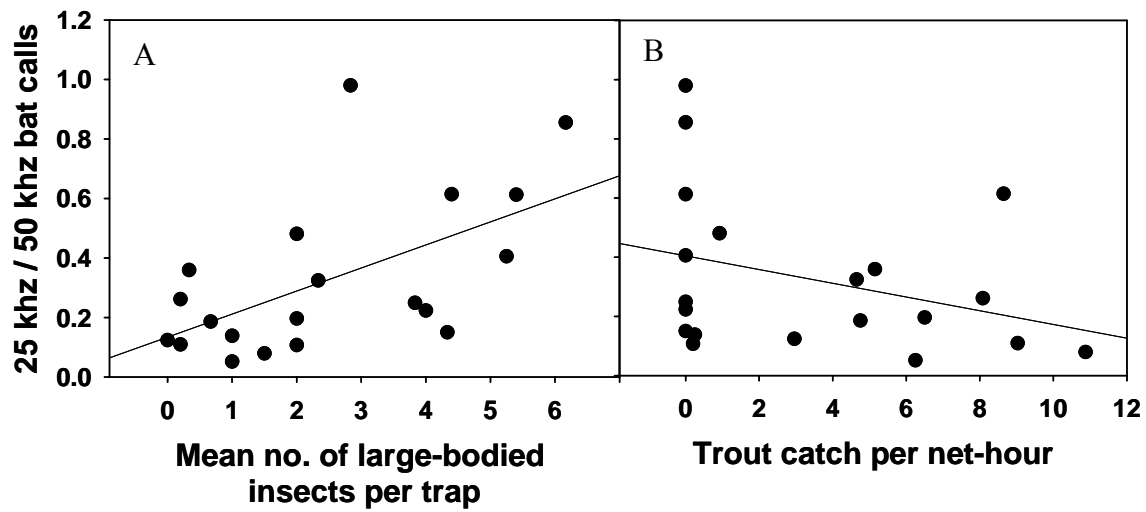


Figure 14. Ratio of large- to small-bodied bat calls recorded per lake per year in relation to the mean annual number of large-bodied insects caught in emergence traps (A) and to the number of trout caught per hour of gill net set each year (B). The estimated least-squares regression line is included for each graph.

Appendix A. Trinity Alps Basin Study 2005 field protocol.

**2005 Trinity Alps Basin Study Protocol**

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June 15, 2005

**Overview**

Our overall goal is to test the response of aquatic and terrestrial wildlife communities to the different wilderness fisheries management options currently being considered in California. To that end we will quantify differences among lake basins that are stocked, temporarily suspended from stocking, have fish removed, and are fish-free 'control' basins. We will focus on the composition and quantity of fauna with both aquatic and terrestrial stages (insects and amphibians), and on upland taxa that are known to feed on these (birds, bats and snakes). Specific hypotheses, stated as null hypotheses, include the following list:

1. There is no difference in amphibian abundances and habitat use between lakes with different quantities of introduced fish.
2. There is no difference in the abundance of garter snakes between basins with different quantities of introduced fish.
3. There is no difference in the biomass of emerging aquatic insects among lakes with different quantities of introduced fish.
4. There is no difference in the abundance of flying aquatic insects along transects up to 40 m away from lakes with different quantities of introduced fish in the lakes.
5. There is no difference in the abundance of birds in the vicinities of lakes with different quantities of introduced fish
6. There is no difference in bat activity in the vicinities of lakes with different quantities of introduced fish.

The project is an ecosystem-scale, replicated manipulative experiment. Whole-lake experiments are challenging and demanding in terms of personnel, resources and time, but they are extremely valuable in yielding realistic predictions of the dynamics of large, complex systems. In this study there are four replicate study basins in each of the four fisheries management categories for a total of 16 study basins. The control basins were not randomly assigned because there are only a handful of lakes without fish. The remaining 12 basins were blocked into four groups based on geographic location, then lakes in each block were randomly chosen as stocking suspension lakes, removal lakes, or continue to stock lakes. We used gill nets in September and October 2003 and summer 2004 to remove the fish from the chosen 'removal' basins.

<b>Block</b>	<b>Remove Fish</b>	<b>Suspend Stocking</b>	<b>Stock</b>	<b>Control</b>
1	Section Line Lake	Hidden Lake	Mavis Lake	Eagle Creek Benches
2	Adam's Lake	Lion Lake	Upper Stoddard	Found Lake
3	Little Caribou Lake	Salmon Lake	Ward Lake	Shimmy Lake
4	Echo Lake	Luella Lake	Deer Lake	SiteID 26062

**Surveys**

IMPORTANT: Recent studies indicate that chytrid fungus is the likely proximate cause of amphibian die offs in several parts of the world. Little is known about this fungus but it is believed that it can be transferred from site to site on gear. Disinfect all gear used in a lake (dip nets, waders, booties, gill nets, emergence traps) before moving from one site to another. To disinfect your gear, use a 0.5% solution of Quat 128. Fill your collapsible decontamination bucket about half full of water and add half of a small

## Pope et al., Trinity Alps Basin Study Final Report

nalgene of Quat to the water. After removing mud and debris from your gear, soak all items for 5 minutes. Dispose of the disinfection solution >100 m from water on bare soil or rock. Do not rinse the disinfected equipment with lake water as this will negate the disinfection process.

All crews will be on the same sampling schedule with each trip being 9 days long and each crew visiting one Block of 4 sites per trip. Crews will rotate among all blocks. We will have 5 days off in between trips. If trip delays are incurred for any reason (weather, injury, car trouble), do not try to make up the time. Skip whatever was missed and continue the survey at the scheduled study basin. Do not extend the trip duration for any reason. The other crews waiting will send in a search and rescue party if you are not out on the scheduled day. Because we are coming from different directions, we will all meet at the Weaverville Forest Service office on Highway 299 at 10:00 am on the first day of each trip. On the last day of the trip we will meet at the Weaverville FS office by 3 pm. Crew leaders must stay in Weaverville until all crews have returned from a trip.

**Block Notebooks:** There will be a specific notebook for each block that will go with the crew leader who is on the block. Read the entries from previous crews before heading out on the trip. At the top of the first clean page record the trip #, crew members, date and first lake visited. Record information for each of the 4 lakes in the order you visit them. This notebook is for inputting information that crews experience that might be helpful for the following crews or to Karen when she is processing data sheets and samples. For example, if a crew got lost on the way to a lake because a tree fell across the trail intersection, write this down so the next crew doesn't also get lost. Also record if a hike was especially difficult and crews should plan for more time or if you found a great new route to the site. Record where bat detectors were located including GPS coordinates and if there were any survey problems or if you set any traps in an odd fashion. Record water quality data and GPS coordinates of temperature probes. Also write any unique sightings including uncommon birds seen, any nests, dead animals, or warnings of sick animals. It is not a notebook for recording raw data. You may also take note of peaks to climb for good views or great fishing opportunities. Any crew member can write in the book but it is the crew leader's responsibility to make sure it is appropriate and that all important information is clearly recorded.

**Data Sheets:** Fill out a data sheet for every survey (point counts, Anabats, emergence traps, sticky traps, visual encounter, mark-recapture, and littoral zone habitat) making sure all required information is recorded. The header on all field forms includes **Block #, Trip #, Crew, and Basin**. **Block #** is the number for the group of study lakes you are visiting, **Trip #** is the number for the 9-day trip you are on (e.g., first trip is Trip # 1). For **Crew**, record the initials of all crew members. This is important to ensure that we can contact a particular individual if there are questions about data collected for a particular site. Put the name of the main lake of the particular basin you are surveying in **Basin**. The header information must always be filled out completely because it creates a unique identifier for the data you collect. In addition, some data sheets require a SiteID number. This is a number pre-assigned by CDFG to uniquely identify every mapped lentic water body in California. This is a critical number, as it will be used to link the data sheet to the particular body of water in the basin. You can find the site IDs on the site lists for each block and on the color GIS maps you will be taking into the field. Check the Site ID carefully before recording it on the data sheet.

**Date:** Write as day-month year (10-Jun-01) and always use the three-letter abbreviation for month.

**GPS Coordinates:** On some forms UTM coordinates are required. This is a pair of numbers that are basically x and y coordinates. In our area, they are North and East. Use the GPS unit to obtain the UTM coordinates. Make sure your GPS is set up to NAD 27. Record the 7-digit UTM North coordinate in the "UTMn" section of the data sheet. Record the 6-digit UTM East coordinate in the "UTMe" section of the data sheet. These coordinates are critical as they will be used to locate the feature on Geographic Information System and future surveys.

**Birds – Point counts:** We will be using a double-observer point-count approach to survey birds at each basin. Point count surveys will be conducted on two consecutive mornings at each basin. Begin surveys within 15 minutes after dawn (the actual start time will vary among trips). Before heading to the first point, decide on the primary and secondary observer for the first point then switch roles for consecutive points.

Fill out the header on the data sheet including Block #, Trip #, Basin name, and the initials of the primary and secondary observer for each point. Be sure you both have binoculars, field guides, and notebooks. The secondary observer should also have a watch with seconds, 2 pens, data forms with the header section already filled out, and list of birds with 4-letter codes. Set the first point ~10 m from the outlet of the main lake approximately 25-40 m from the lake shore. In some basins that have steep outlet streams the point will be closer to the water's edge. After the 5 minute survey walk around the lake approximately 100m in either direction and start another survey ~ 25-40 m from shore. Continue with point surveys until there are a total of 6 in the basin or you have run out of space to set any more with at least 100 m between them. On the following morning, return to the same locations for the points but visit them in the opposite order so that the last point surveyed on the prior day becomes the first point surveyed. Also alternate observers so that for any given point an observer is both primary and secondary observer. Pick points in the range of habitats in the basin and with good vantage points for viewing birds.

Approach the point with as little disturbance as possible, and begin the count as soon as you are both oriented, and confident you can estimate a 50 m radius accurately. Point counts are 5 minutes duration at each point. The secondary observer should use a watch timer to start and stop the survey. Record the time the survey begins at each point at the top of the data sheet. When the survey begins, the primary observer identifies all birds seen and heard and communicates (via speech and gesture) to the secondary observer the species detected, approximate distance of the detection, and method of detection (visual, song, call, drumming). Also for each species, identify any indication of breeding status in the Breeding Observation column. The secondary observer records all information given by the primary observer and includes a P in the **P or S** column for birds observed by the primary observer. The secondary observer is also surveying the area for birds. Birds detected by the secondary observer but not by the primary observer are also recorded by the secondary observer and an "S" is written in the P or S column. Make every effort to avoid double counting individuals detected at a single point. Any birds observed between or after surveys but not during a survey should be recorded on the data sheet. In the Point # column, record an "I" for incidental and record the rest of the information in the same way as during the survey. If the bird was observed while moving between points, state the location in the comment section (e.g., "seen between pts 2 and 3"). If something interferes with your ability to detect birds during the 5-minute count, stop the count until the disturbance has passed and start the entire 5 minute survey over. Put a single line through the interrupted data and note what happened on your form.

No attracting devices or "pishing" should be used. Use the standardized 4-letter abbreviation for **Species Code** (e.g., DEJU for dark-eyed junco, CLNU for Clark's nutcracker). For unknown species, record "XXXX." For unknown members of various families, use "XX" plus two letters to signify the family – for example, "XXHU" for unidentified hummingbird. You can follow birds after the completion of a point in order to verify or discover identification. For birds not on listed on the key or rarely seen birds, give the 4-letter code in the Species code column and also write the common name in the comments column. If no birds are detected at a point write "No birds detected" on your form

Record every species within 100m of the point. In the **Distance** column, record the approximate detection distance to the bird or if it was a 'fly over'. The estimated distance recorded is the distance from the point to the first location an individual was observed, regardless of its behavior. If the bird subsequently moves, do not change the original distance recorded. If a bird is flying (but not "flying over" – see below), or perched high in a tree, the distance recorded is to the point at which a plumb line would hit the ground if hung from the point at which the bird was first observed. This distance should be estimated as though a tape were laid across the ground, including any intervening topographic features.

Birds that are flying over but not using the habitat on the study area are recorded as "fly over" in the Distance column. Birds flying below the canopy level, flying from one perch to another, or actively foraging on or above the study area are recorded as described in the previous paragraphs.

A bird flushed from within 10 m of the point when you arrive should be included in the count. Birds that are flushed from farther away should be recorded as an incidental if they are species that did not occur during the count.

In the **Method of ID** column, record the behavioral cue that alerted the observer to the presence of the individual – S for song, V for visual, C for call, and D for the drumming of a woodpecker. Always fill out

this column – it is not optional. If a bird uses a different cue after it has been detected by the first cue (e.g., visual after heard sing), add the additional cue to the column and circle the original detection cue.

**Breeding Obs.:** Record any potential indications of breeding if noted for species at each point as follows. CO=copulation, DI= territorial display, DD=distracton display, FC= food carry, FL= fledglings, MC= material carry, NF= nest found, PA= pair (male and female together).

After each point count is completed, both observers must take one minute to review the data sheet to ensure that all columns are properly filled out and that the 4-letter codes are correct for the birds seen. These are early morning surveys (pre-coffee) so be extra diligent to review for mistakes. It is much better to catch mistakes when there is time to correct them.

### **Area-Constrained Search**

Each morning following the point counts, one crew member will conduct one hour-long area-constrained searches for birds. These surveys will focus on assessing breeding and foraging activity within 50 m of the lake shore.

### **Bats- Anabat II detectors:**

We will be using acoustic bat detecting equipment that allows us to record bats remotely in a wilderness setting for two weeks at a time. The key to this innovation is a zero-crossing analyser (storage zcaim), which saves compressed zero-crossing data from acoustic events on compact flash memory cards, such as are used in digital cameras. Daily recording periods are programmed on the 256 MB data storage cards by software prior to taking them into the field, and cards will be downloaded to a computer between data collection trips. The low-power system demand (i.e., for storage zcaim and ultrasound detector) means a small solar panel and storage battery are adequate for this study. A total of 8 bat detectors will be used in this study. All blocks will have 2 detectors that will be set in the fish removal and stocked lakes for the entire season. After the first trip when detectors are installed, all crews have to do is ensure that the system is working well, switch out the 256 MB compact flash card, and clean the solar panel and microphone. Switch out the card on Day 2 at the basin and leave the system running until the next crew comes to the basin on the following trip.

### **Station setup -**

The detectors will all be set up within 1 m of the shoreline of the primary lake in the basin with the transducer (microphone) directed over the water. Make sure to pick a location that is inconspicuous and as far as possible from the trail and any established campsites. The entire detector assembly will be attached to a 2 meter length of 3/4" steel EMT mast hammered into the ground. The transducer assembly ("bat hat") will be attached near the top of the mast using hose clamps so that the transducer is angled down at 45°. The four-watt solar panel will be attached below the bat hat and directed toward the most open aspect (south if possible) to maximize sun exposure. The panel will provide daily power to a 1.3 Ahr gel cell that will be placed inside a waterproof box with the detector and zcaim. The box will also be secured to the pole at ~ 1/2 m height. All boxes will be locked with a combination lock set at "151".

### **Detector setup-**

Detector/battery charger/battery/zcaim units will already be in position in the boxes with most connections in place for the first trip so that all that needs to be done is to attach the transducer, solar charger, and box to the pole; plug in the transducer and solar charger to the system; make sure the settings are correct; and turn on the system. First attach the brown Anabat cable from the transducer to the Anabat through the hole on the right side of the box. Put the double wire cable from the solar charger through the 3-piece gland nut in the left hole in the box being careful not to lose any pieces. Make sure the protected part of the cable is in the gland nut. Using the small screwdriver, attach the wires to the green solar panel terminal (from the battery charger unit), black to black and red to red. Make sure the settings on the Anabat are correct (see **Anabat Settings** below) and turn on the power on the detector and zcaim. While the box is still open, rub fingers together in front of the microphone and make sure sound is heard through the detector. Check the LEDs on the zcaim (see **Zcaim Settings** below).

For trouble shooting issues, we will now go through the entire set up. Open the empty box with a single pink foam pad in the bottom. Mount the small battery charger unit to the Velcro on the top wall of the box with cables facing down. Plug one of the barrel connectors from the charger to the "12 volt" labeled socket



on the side of the Anabat.

Attach one end of the 8 pin DIN cable to the “recorder timer” labeled socket on the Anabat. Screw the brown Anabat cable from the transducer through the right hole on the bottom of the box and plug into the front of the Anabat. Set the Anabat in the box and place a strip of foam pad at the top and right side of the box between the box and detector. Insert the battery into the bottom right of the box and attach the battery cables from the charger unit to the black and red terminals on the battery (black to black, red to red). Outside of the box, put the microphone into the PVC shroud then attach to the brown Anabat cable. Hook the strain relief at the base of the aluminum bracket.

Turn on the Anabat and rub fingers together in front of the microphone to make sure it is working. Test with the Timer switch in the “Off” position. Once checked, turn down the volume, set the timer back to “On”, and set the sensitivity and division ratio (see **Anabat Settings** below). Put the two layers of foam with the holes for the dials over the Anabat.

Make sure a new 256 MB flash card is installed in the back of the zcaim. Put the other end of the 8 pin DIN cable from the Anabat into the zcaim socket labeled “bat detector”. Plug the other barrel connector from the charger unit into the zcaim. Power up the zcaim to make sure it is working correctly (see below). Set the zcaim on top of the Anabat. Put the double wire cable from the solar charger through the 3-piece gland nut in the left hole in the box being careful not to lose any pieces. Make sure the protected part of the cable is in the gland nut. Using the small screwdriver, attach the wires to the green solar panel terminal (from the battery charger unit), black to black and red to red. Make sure all cables are neatly tucked in the box. Lay the roll of foam and screwdriver over the cables and carefully shut and seal the box.

#### **Anabat Settings-**

Move the 'TIMER OFF / ON' switch on the side of the Bat Detector to the 'TIMER ON' position.

Set the 'DIVISION RATIO' to 8 and 'SENSITIVITY' to the penned mark which will likely be near 7. We will calibrate all detectors to each other so it is very important to set the sensitivity exactly to the mark.

Put the 'TAPE' switch on the top of the Bat Detector to the 'OFF' position

Rotate the 'VOLUME / OFF' control on the top of the Bat Detector from the 'OFF' position to position 2 (or minimum volume level in ON position). You can turn it up when listening to make sure the detector is working.

#### **Zcaim Settings –**

If a new CF Memory card is not already inserted into the CF ZCAIM, then you must insert an initialized CF card. Make sure the unit is OFF at this stage. To insert a CF card into the CF ZCAIM, remove the rear panel CF CARD access plate marked ‘COMPACT FLASH CARD ACCESS - REMOVE SCREWS’ by rotating the two cover plate screws counterclockwise. Once the plate is removed, insert a CF card into the rear panel card socket, and push in firmly to ensure the card is seated properly. Replace the rear panel CF CARD access plate, tightening the screws in a clockwise direction until they are hand tight, and the cover is seated securely on the CF ZCAIM. The rear card access plate can only go on one way, with the bulge in the plate lining up to fit over the ejector button on the CF socket. Ensure the rear access plate is fitted to prevent dirt and moisture from entering the CF ZCAIM CF socket and damaging the electronics inside.

The CF card will be programmed so that it is in Standby Mode during the day and Record Mode during the night. Whenever the ZCAIM is powered up without a CF card being installed, it will enter Serial mode, as indicated by the STATUS and ERROR LEDs being lit. The ERROR LED in this situation is not indicating an error condition, other than that there is no CF card installed.

To turn on the power, press the CF ZCAIM front panel ‘POWER ON / OFF’ push button. The ‘STATUS’ LED should light while the ZCAIM boots up. This process could take as little as one second, but could take a lot longer if the installed CF card already contains a lot of data. After bootup, the ZCAIM will enter Standby Mode unless it is nighttime and it will enter Record Mode.

If the CF ZCAIM is in Record mode, only the RECORD LED will be lit. If it is in Standby mode, only the STANDBY LED will be lit. In either of these modes, you can always switch between modes by pressing the ‘RECORD / STANDBY’ push button. Check the setup at least once during the day and once during the night to ensure the system is recording properly.

NOTE - If none of the expected LED display conditions occur or the ‘ERROR’ LED is lit, consult the LED STATUS DISPLAY table below to ascertain the status of the CF ZCAIM for any fault conditions.

While the ZCAIM is in Record mode, any detected signals will be shown by the ‘DATA’ LED on the front panel of the CF ZCAIM flashing in sympathy with the call.

While in 'RECORD' mode, the CF ZCAIM will continue to store analysed Bat calls sounds onto the CF card as long as the CF card has space available to store the data. Once the CF card is full, then recording of bat calls will stop and the CF ZCAIM will indicate the 'CF CARD FULL' status by having the 'RECORD' 'STANDBY' STATUS' and 'ERROR' LEDS all lit. The information written to the CF card will not be overwritten with any new data following a 'CF CARD FULL' condition.

When collecting a CF card, first turn off the ZCAIM by pressing the front panel 'POWER ON / OFF' push button. Shortly after, all LEDS should extinguish and the CF card is safe to remove. Place the full CF card in a ziplock with and place safely in the crew leader's backpack. Install the new empty card while the back is still open. Make sure to fill out the AnaBat data sheet with the # of the CF card removed (written on card with sharpie) and the # of the card installed.

**CF zcaim status table (as displayed on the front panel of the storage zcaim)**

Note - The DATA LED does not indicate any status of the CF card in the CF ZCAIM, the DATA LED only flashes when Bat call sounds are detected, and the CF ZCAIM is in the 'RECORD' mode

CONDITION	RECORD	STANDBY	STATUS	ERROR
Boot Up Processing	OFF	OFF	ON	OFF
Record Mode	ON	OFF	OFF	OFF
Standby Mode	OFF	ON	OFF	OFF
No CF Card Detected (= Serial Mode)	OFF	OFF	ON	ON
Bad CF Card File	ON	OFF	ON	ON
CF Card Error	OFF	ON	ON	ON
CF Card Full	ON	ON	ON	ON
Sleep Mode	ON	ON	ON	OFF

**Bat visual surveys:** Every evening at dusk (~15 min after sunset), one crew member should take 10 minutes to walk near the lake shore and record in the visual survey section of the Anabat data sheet the number of bats seen flying in the basin. Also record if you hear any of the bat vocalizations and what the bats are doing (foraging over the water, flying in the trees, etc.). Note if you see one or several birds flying out of a particular tree and if they appear to stay or leave the basin. Record the start and end of the survey period and the crew member doing the survey.

**Insects-**

**Emergence traps:** We will be using light weight tension frame collapsible aquatic insect emergence traps modified from a design by Bill Rainey of Stillwater Sciences. Emerging insects are retained in semitransparent fabric dome (with an access sleeve) sewn from white polyester 'No-See-um' mesh. The dome is draped over a tensioned frame composed of four 3 ft. lengths of white 1/8" fiberglass rod. The base perimeter ring is assembled by connecting two 3' long fiberglass rods together using 2 metal ferrules. The dome is supported by the two other lengths of rod which are formed into tensioned arches by inserting both their ends into the four T-shaped connectors distributed at equal distances around the perimeter ring. The float for the trap will be a lightly inflated bicycle inner tube. Lay the netting over the frame and inner tube and use cable ties through the holes in the Dacron fabric to secure the fiberglass frame and inner tube into the Dacron sleeve at the base of the netting. For anchoring the trap, tie three lengths of nylon cord to the holes in the Dacron sleeve. Fill three small Tyvek bags with rocks and tie to the other end of the cords. Shorten the cord as necessary so that there is minimal slack in the lines.

The fiberglass rods splinter and break easily. Be extra careful when handling them. The tips are especially fragile and care must be taken to keep them from splintering. When setting up the traps always use silicone or grease from your chapstick to lube the tips before inserting them into the ferrules. Do not set up in the dirt because the ferrules easily fill with dirt and the fiberglass tips break when forced into a ferrule with dirt. Either keep the rods above ground while constructing traps or use a tarp for setup. If splintering starts, use the piece of sandpaper and sand it down. When transporting the rods, store them in a protected place such as a fishing rod case.

Install three emergence traps in the main lake in the study basin on the first evening you reach the basin. Traps will be checked the following morning and then left in place until the second morning when they will be checked again and packed up. Set one trap in silt/emergent veg habitat with the shallow side of the trap situated at the shoreline ("Shoreline" trap), another trap in shallow (~30 cm) water within 3 m of the shoreline over the dominant substrate in the lake ("Dominant" trap). The third trap should be set directly out from the second trap in about 3/4 to 1 m deep water ("Deep" trap). Try to disturb the substrate as little as possible by setting the traps gently over it without trampling the area under the trap. In the Set section of the data sheet, record the set time, water temperature collected at the location of each trap, depth collected from the deep side of each trap, substrate, % emergent vegetation, and any additional location information or comments.

The following morning at approximately 9:00-10:00 (after bird surveys and breakfast), take the same data sheet, aspirator, 3 plastic containers with 3 lids, 3 emergence trap labels, thermometer, the container of ETOH, and plastic dropper to the lake edge. Record the collection date and air temperature. In the Insect Collection part of the data sheet, record the collection time and water temperature taken at each trap before removing insects.

To collect the insects, unknot the sleeve in the netting, insert the aspirator and put the tubing end in your mouth (make sure it is clean first) to suck the insects from the netting into the container. Be extremely thorough making sure all insects are collected, even off emergent vegetation and the seams of the netting. Once all insects are collected, re-knot the sleeve. Carefully remove the rubber stopper and quickly slide on a white container lid. Pull open the lid slightly and drop several drops of alcohol into the container using a plastic dropper so that all insects are completely coated. Once insects are dead, add enough ethanol so that insects are completely submerged. Fill out the label, insert it into the container and put the lid back on. Seal the lid with parafilm. Insert the sample into a ziplock with the Block and Trip # clearly written on the outside with a Sharpie. The crew leader is responsible for keeping all samples stored safely and upright in their pack. Make sure the data form is completely filled out. For each trap, record the general insect types and approximate number in the comment section (e.g., "~25 midges and a damselfly"). Clearly note if no insects were found in a trap and there was no sample vial. Be sure to note the locations of the traps in the lake, if the traps moved overnight due to wind, and if any insects escaped from the trap or container.

**Sticky traps:** At each basin on Day 2, collect 16 sticky traps set two weeks prior by the previous crew and set 16 new traps. After the first trip, you can collect and set sticky traps at the same time. Bring with you a sticky trap data sheet and 2 pens/pencils, 16 new traps and twist ties, the nylon cord with meter measurements, cloth measuring tape, plenty of cellophane, pocket knife/scissors, Sharpie, gallon-sized ziplock, and a compass. Label the ziplock with the block #, trip #, date, crew, and basin name. Before setting each trap, label the outside of the sticky trap along the white strip using the Sharpie with the basin name, date set, transect and trap number (e.g., **Echo, 21-Jul-05, N3**). We've found it is easiest to do this task before leaving camp.

The traps will be set in four transects of 4 sticky traps around the main lake in the basin. Use a compass and start the transects from the lakeshore as close to north, east, south and west as is feasible. At the north end of the lake, use the measuring cord to walk 10 m north and prepare to set the first trap (trap #1). Walk 10 more meters north and set trap #2 and 10 more meters to set trap #3. Trap #4 will be set 40 m from shore. Measure the 4<sup>th</sup> trap distance directly to the lake to ensure that it is 40 m. Do not assume that the traps set by the previous crew are at the correct distances. Always use your measuring cord and if distances from the previous crew are off, note it on your data sheet and reposition the traps more accurately if possible.

If there is nothing at the correct distance along the transect to set the trap to, move to the nearest usable vegetation to the east or west that still maintains the same distance from the lakeshore and set the trap. Note the distance moved from the transect in the comment section of the data sheet. Follow the same methodology for the east, south, and west transects.

Peel the trap apart and reverse the fold so the sticky side is out. **With the Sharpie, write the letter "F" on the side of the trap that will be facing the lake.** Set the trap so the "F" side is directly facing the lake. Attach to edge of vegetation or stick with a twist tie. As best as possible, set the trap so both sides are exposed and not touching vegetation. Hang the traps at a height of 1/2-1 m off the ground.

For each directional transect record the **trap number** as 1 for the trap set closest to the lake, 2 for the second trap, 3 for the third, and 4 for the trap furthest from the lake. Record the time the trap was set and the height of the trap measured from the ground to the base of the trap. For **Attachment**, record specifically what the trap is hung from (e.g., pine branch). In **Dominant Cover**, record the 2 primary cover types within a 2 m radius of the trap. Categories are conifer, shrub, forb, bare rock, willow, aspen. Record the distance from the trap to the lake and any additional comments. If there is other water (e.g., pond or stream) closer to the trap than the distance of the trap to the lake, measure the distance from the trap to the water and record in the comments section. Try to avoid this situation if at all possible.

To collect the traps that were set 2 weeks prior, untie the twist tie and carefully spread a sheet of cellophane so that it completely covers each side of the sticky trap with as few wrinkles as possible. Fold the trap with the sticky side in. Trim any excess cellophane from the edges of the closed trap. Make sure that the trap was properly labeled by the previous crew. If not, label it with the basin name, transect and trap number, and date collected (label "Col." after date). In the comments section of the Retrieval portion of the data sheet make note that the trap was not labeled and the set date is unknown. When collecting the traps, record the trap number and time each trap was collected in the Retrieval portion of the data sheet. Also record any comments about the insects collected on the trap or disturbances to the trap that may have modified the number of bugs collected (e.g., found stuck to vegetation or laying on the ground). After all traps are collected from a site, wrap the entire bundle (16 traps) in a piece of cellophane and store the ziplock.

**Odonate Exuvia Surveys:** Every trip collect dragonfly and damselfly exuviae from four 1 m x 20 cm plots of emergent vegetation along the edge of each of the main lakes. Locate the four plots by standing at each of the cardinal directions around the lake (use the sticky trap transects as reference) and walk to the left to the first patch of emergent vegetation >2m long. Start the transect ½ meter into the vegetation patch from both the side and shoreline. Use your measuring tape to get the measurements exact and **do not** collect any exuvia seen outside the sampling plot. Bend down and carefully collect all larval exoskeletons observed in the quadrant and put them in a whirl bag labeled with the basin name, date, crew members and trip #. Also add a label to the bag with the same information. You may combine all 4 quadrants at one lake in one bag. If the samples are wet, add a little ethanol to the bag. Seal the bag with air so the samples do not get crushed. Store the samples in a Rubbermaid-type container. If a quadrant lacks vegetation, locate a second plot randomly in the nearest quadrant with vegetation. You may conduct this survey at any time during the day and on either day of the trip.

**Benthic Sweeps:** Benthic insects will be sampled from the littoral zone of each study lake on the second and fourth trip by collecting three 1 m<sup>2</sup> sweeps with a D-net. Collect the three samples from the littoral zone in the following substrate categories: emergent vegetation, organic/silt, and rock. To collect the samples, take the D-net and sweep it across the top of the substrate for 1 meter then quickly rotate the net and sweep again over the same area. Complete a total of 4 passes (forward and back twice) in one constant motion. Take the sample to the shore and empty the net into a rectangular Rubbermaid container. Use water to remove all debris from the net. Remove any large rocks or sticks and sharp pine needles from the sample. If the sample is too large to fit into a whirl bag, half or quarter the sample as necessary. Remove extra water from the sample before putting it into the bag. Add ethanol to the sample so that there is at least a 1:1 ratio of ethanol to the sample (2:1 is preferable) and swirl. Fill out a label being sure to note the substrate the sample was collected from and if the sample was reduced at all. Place the label in the bag but near the top so it does not fall into the sample. Also, use a sharpie to label the outside of the bag. Seal the sample tightly and store in the Rubbermaid container.

#### **Amphibians and Reptiles-**

Unlike the bird, bat, and invertebrate surveys, visual encounter and mark-recapture surveys will be conducted at every water body in the basin including temporary ponds and stream reaches. Each lake and pond already has a unique Site ID that is labeled on the basin map. This is a **critical** number, as it will be used to link the data sheet to the particular body of water in the basin. This ID will be on color GIS maps available for crews to take into the field. Check the Site ID carefully before recording it on the data sheet.

**Visual encounter surveys:** To conduct an amphibian/reptile visual encounter survey (VES), one crew member alone will walk slowly around the perimeter of the site, visually scouting for amphibians and reptiles near the shoreline and in the littoral zone. Count the number of adults, sub-adults, larvae, and egg masses you find of each species. Use the sterilized hand net or aquarium net to catch amphibians for

identification if necessary. You can also use it to stir areas of silt in an attempt to see any hiding salamander larvae. During the first couple trips, check carefully for egg masses in submerged vegetation, under rock ledges and on the underside of submerged wood. Begin VES surveys after 10:00 and finish before 14:00 since most amphibians in high elevations of the Trinity Alps are diurnally active and you still have to conduct mark-recapture surveys.

Record detections on the VES field form. Each species/life stage/survey method/location combination detected should be recorded in a separate row (e.g., HYPE adults detected **visually** during the timed survey in one row, and HYPE adults detected **aurally** during the timed survey in another row). If animals were seen in an associated water body such as in a meadow or small pool near the pond being surveyed or in the inlet or outlet stream, then circle either P/I/O/M for pool, inlet, outlet, and meadow. In addition, each species/life stage/survey method combination detected incidentally (e.g., detected before or after the timed survey) also should be recorded at the bottom of the sheet in the **Incidentals** section.

**Wind:** Enter the appropriate letter to represent current wind conditions at the site in the wind section of the data sheet. If there is no breeze present, write “C” for calm. If there is an intermittent or steady light breeze present, write “L” for light. If there is an intermittent or steady moderate wind present, write “M” for moderate. If there is an intermittent or steady heavy wind present, as evidenced by white-capped waves on the surface of a water body, write “S” for strong.

**Weather:** Enter the appropriate letter to represent the current weather condition at the site. If the sky has less than 5 percent cloud cover, write “C” for clear. If the sky has 5-50 percent cloud cover, write “P” for partly cloudy. If the sky has 51-95 percent cloud cover, write “M” for mostly cloudy. If the sky has 100 percent cloud cover, write “O” for overcast. If it is raining, write “R” and if it is snowing, write “S.”

**Water Color:** Circle **clear** if the water is clear and **stained** if the water is colored (e.g., tannin stained brown, algal green). Do not circle both clear and stained, choose one.

**Water Turbidity:** Circle **clear** if you can see clearly to the bottom or **cloudy** if suspended particles prevent you from seeing clearly to the bottom.

**Air temperature:** Measure air temperature from the lakeshore and in the shade at 1 m above the lake surface. Record air temperature in Fahrenheit (°F). Temperature should be measured immediately before starting the VES at each site.

**Air temperature time:** Record the time the air temperature sample was taken in 24-hour time (Example: 1500 for 3pm).

**Water temperature:** Measure water temperature approximately 0.5 m out from shore and 10 cm under the water surface. Record water temperature in Fahrenheit (°F). Take the water temperature immediately following the air temperature.

**Water temperature time:** Record the time the water temperature sample was taken using 24-hour time.

**Survey start time and end time:** Record the time at which the amphibian/reptile survey began and ended using 24 hr time. The start time is the time the amphibian/reptile survey began, not the time you arrived at the site.

**Total survey duration:** Record the total time spent searching for amphibians/reptiles. Do not include time spent surmounting lake-side obstacles (e.g., cliffs), identifying specimens, or recording notes. If two people survey the same site by walking in opposite directions around the lake perimeter, the total survey duration should include the time spent surveying by each person (20 minutes each = 40 minutes total). Record time in minutes, and round off to the nearest whole minute (ex: 42).

**Amphibian observers:** Record the first initial and last name of all people involved in the amphibian/reptile survey of that particular site. This will usually be a single observer except for large lakes where 2 observers can walk in opposite directions and end the survey when they meet up again on the opposite bank. If two observers are used, make sure to correctly consolidate all data collected onto one field form.

**Fairy shrimp:** During the amphibian survey, be on the look out for schools of fairy shrimp. The distribution of these crustaceans is poorly known for Northern California, so we are interested in describing localities. Circle Y if you see fairy shrimp at the site or N if you do not.

**Species:** Use the 4 letter abbreviation for amphibians and reptiles. Species abbreviations are provided as a footnote at the bottom of the field form. If you are unable to ID a garter snake to species, use THSP for *Thamnophis* species.

**Life stage:** Life stage abbreviations are “A” for adult, “S” for sub-adult, “M” for metamorph, “L” for larvae, and “E” for egg masses. Be sure to differentiate metamorphs (metamorphosed during the current season) from subadults (metamorphosed last year or earlier) if at all possible.

**Tally:** Record detections using your favorite tally method.

**Total:** Record the total for each row.

**Survey method:** Circle the method used. Most detections will be “visual” and some will be “aural” (e.g., Pacific tree frog adults heard calling but not visually detected would be recorded as an aural detection). In addition, circle “Dip Net” if you use a dip net to catch an individual of a species/life stage combination for identification. Consult the field guide provided for adult, larval, and egg mass identification. Further, circle “Tissue” if you collected tissue samples from individuals in that species/life stage combination.

**Comments:** Record any interesting observations made during the survey (e.g., Cascades frog larvae found only in shallow lagoon on NW side of lake).

**Chytrid surveys:** Periodically capture larvae with the dip net and inspect their mouthparts for deformities using a 10x hand lens. Handle the tadpoles out of water as little as possible. If you find any larvae with deformed mouthparts, carefully write down what you saw in your notebook and on the datasheet. Clean all gear with Quat extra carefully before going to any new water body even in the same basin. Conduct these surveys after completing the VES survey at a site but before moving to the next site. When the fungus attacks the larvae, it deforms their mouthparts. Record your findings in the Block notebook and after the trip, immediately report them to Karen.

### **Frog Mark-Recapture**

This survey technique will only be used for Cascades frog adults and large subadults (> 42 mm SVL). Mark-recapture surveys provide information on the seasonal movements, habitat use, population size, growth and condition of the study organism. Following completion of the VES surveys, conduct mark-recapture surveys for frogs in all water bodies in the study basin. The goal is to conduct these surveys in the afternoon sun when frogs are out basking and feeding. Before heading out, make sure you have mark-recapture forms, basin map, 2 pencils/pens, calipers, Pesola scales, several clean ziplock baggies, thermometer, Pocket reader, PIT tags in ethanol, and tagging scissors or injector and blades. Wash your hands carefully before starting survey. There should not be any sunscreen, mosquito repellent or lotion residues on your hands when handling amphibians. All crew members will work together on this survey. If chytrid symptoms have been observed in the basin you must wash your hands or use separate latex gloves for each individual handled. Store all frogs in their own clean ziplock. Sterilize all equipment in ethanol after contact with each frog. Note any malformations or sloughing skin on the data sheet. For small water bodies in basins without disease symptoms, catch all frogs possible and put in ziplocks or the cleaned collapsible bucket filled part way with water (if there are lots of frogs in a small area). Once all frogs are captured, process them one at a time and release them back into the pond. Only use this technique if no chytrid symptoms have been found in the basin. For large lakes and ponds, survey along the shoreline and catch frogs as you come across them. Put captured frogs individually in a ziplock and process them immediately. Release at the site of capture.

### **Data Sheet**

**Site ID:** Always include the site ID where the animal was captured.

**Length:** Measure the snout-vent length of the animal in mm using the calipers and record on data sheet.

Hold the animal flat and measure from the tip of the snout to the vent.

**Weight:** Put the animal in a ziplock and weigh using a Pesola spring scale. Make sure the scale is set to zero before weighing. Hold the scale from the metal loop at the top and block it from the wind using your body. Once an accurate weight is obtained in grams, record it in the tot. weight column then take the animal out of the bag and weigh the bag. Record this value in the bag weight column.

**Sex:** For adult frogs, sex can be determined by looking for secondary sex characteristics. Males have enlarged thumb pads and muscular forearms. In the sex column, write M for male, F for female or U for unknown.

**Recapture:** Use the PIT tag reader to swipe over the animal to check if it already has a PIT tag. Scan both the dorsal and ventral surfaces to check for tags. If it is a recapture, write Y in the Recapture column and record the PIT tag number in the **Tag #** column. Weigh and measure the animal before releasing it. If it is not a recapture, record N in the Recapture column. If the animal is less than 43 mm and, therefore, is too

small to be tagged, write “too small” in the recapture column since you do not know if it has been captured before or not.

**Tag #:** If the frog is greater than 43 mm SVL, it can receive a PIT tag (see below for PIT-tagging methodology). Record the PIT tag number of the tag inserted into the animal. All frogs > 43 mm (Recaptures Y or N) should have a tag number recorded in this column unless the animal is injured or sickly.

**Condition:** Record the overall condition of the animal as G for good, F for fair, P for poor, or D for dead.

**Comments:** Include any unique identifiers, comments on the condition, or problems with the tagging.

**PIT-Tag insertion:** Pull a tag from the alcohol and set on a clean surface such as the Nalgene lid of the PIT tag container. Scan the tag and record the new number on the data sheet. Place the tag within easy reach. An assistant holds the frog at the forearms with the top of the head in the palm of their hand. They stabilize their arm on a rock or knee. The tagger dries the skin on the back of the frog with a clean cloth then pinches the skin to the right or left of the sacral hump and uses clean surgical scissors to make a small v-shaped incision in the pinched skin with the point of the V toward the hind end of the animal. Insert the tag into the v and slide it to the other side of the back. Release the frog after making sure weight, length, sex and PIT number have been recorded without error. Clean scissors between each use by rinsing in ethanol.

### **Snake Diet Study and Mark-Recapture**

Any time a snake is encountered in the study basin, catch it if at all possible. The only exception is if you are in the middle of a VES survey and the capture will disrupt habitat yet to be surveyed. When caught, identify the snake to species and put it in a snake bag. If you cannot process the snake immediately, make sure the bag is not kept in direct sun. Process all snakes within 30 minutes of capture. First, run the PIT reader over the length of the body checking to see if it was already tagged or if it recently ate one of the tagged frogs. If a tag number is found, check to see if it is on the snake list. If not, record the number in comments and we will check it later to see if it was one of the tagged frogs. Weigh the snake in the bag then weigh the bag separately; minus the bag weight from the total weight to get the snake weight. Measure the length of the snake from the tip of the nose to tail using the cloth measuring tape. Also measure the tip of the nose to vent and record in S-vent length. Record all measurements on the snake mark-recapture data sheet. Once measurements are complete, check to see if the snake is gravid by gently rubbing the sides of the body cavity along the posterior half of the body feeling for ova (feels like a series of lumps). If the snake is not a gravid female, palpate it to remove any stomach contents. Hold the snake with one hand directly below the vent. Using the thumb of your free hand put mild pressure at the base of the snake's stomach (about 2 cm anterior to the vent) and slowly move your thumb down the length of the body while maintaining the mild pressure. As you approach the head, the snake will open its mouth and release any stomach contents. Early in the season, snakes often do not have food in their stomachs so don't worry if nothing comes out; just record that the stomach was empty. If stomach contents are released, record what was found to species and lifestage if possible and measure the approximate length of fresh prey items. Palpate all snakes captured including adults and subadults of both *Thamnophis atratus* and *T. sirtalis* (unless it is a gravid female).

**Tag insertion:** PIT-tag garter snakes that are >340 mm snout-vent length. Use the PIT tags specifically labeled for snakes. Pull a tag from the alcohol and insert into the injector blade. Remove any excess alcohol. Scan the tag and record the new number on the data sheet. Place the tag within easy reach. An assistant holds the snake as still as possible at the head and just above the vent. The tagger uses the injector and inserts the tag into the muscle on the right side of the body about  $\frac{3}{4}$  the distance from the head to the vent. This is to avoid damaging the reproductive or lung areas. Insert the tag under a scale, not through it. Angle the tag up toward the head making sure to enter muscle but not the body cavity. Fill out the data sheet (see above).

### **Gill netting**

On trip 3 we will set a gill net in the primary lake in the Suspend stocking, Continue stocking, and Fish Removal lakes to assess fish density, size and condition.

Our fish survey methods are designed to provide an accurate representation of fish species composition and size structure in lakes and ponds, as well as provide an estimate of catch per unit effort (CPUE) at each location. In order to get reasonably accurate measures of CPUE, all net must be set for a similar amount of time. We will set one net in each lake for a minimum of 4 hours and maximum of 8 hours. Try to set the nets around noon so they can be checked after amphibian surveys but before dinner. Then you will be able to supplement your dinner with fresh trout. Gill nets should be set in an area frequented by fish but where there are minimal snags along the bottom. If possible, choose an area that is 3-8 m deep and free of woody debris. Before setting a gill net, submerge the entire net (still contained on the handle); dry nets are much more susceptible to tangling. To set the net, one person holds the fine mesh end of the net on the shore. The other person gets in the float tube with a bag filled with a few small rocks, the float, and the net lying across the float tube (lead line on your left and net handle in your right hand or vice versa). Paddle backwards slowly while feeding out the net. The net should be set perpendicular to the shore. If you encounter a tangle while feeding out the net, shake the net. Do not pull on the net as this will often tighten the tangle. Shaking will nearly always rid the net of the tangle. When you get to the end of the net, attach a float to the handle and then clip the bag with small rocks to the bottom of the net. Paddle backwards until the net is taught, and then drop the bag. Record the time and depth of the end of the net when you finish setting the net. On the data sheet circle the appropriate location and provide a brief description of the area in which the net was set in the Comments section.

After 4-8 hours, retrieve the net by getting in the float tube and pulling the mid-lake end of the net up by the float. Detach the float and the bag. Pull the net toward you, placing the float line on one side of the float tube and the lead line on the other. If you pull any live fish, stun them by whacking their head with the bag of rocks or the back of your hand. Continue pulling in the net until you reach the shore. To carry the net to an area for fish removal, cradle the net over your arms keeping the lead line on one side and the float line on the other. Lay the net down in a meadow, granite slab, or on a sandy flat (stay away from areas with lots of woody vegetation, pine needles, pine cones, and sharp rocks since they will get snagged in the net). Spread out an arms length of net and remove any fish. Spread the next length of net over the first so the net is folded like a fan and remove fish. Continue until you have removed all fish from the net. Restraining the net onto the handle, rinse the net in the lake, sanitize the net using Quat-128, dry in the shade, tie the net in a knot to prevent tangling, and stuff it into a sack. Nets should be stored and transported in stuff sacks to keep them from getting tangled and to keep them out of the sun.

If no fish were captured, write "no fish" across the fish portion of the data sheet. If fish were captured, record the species, length, and weight of all fish. Species abbreviations are given as a footnote. Measure fish using the vinyl tape laid out on the ground. Measure fish total lengths to the nearest mm. Weigh fish using a Pesola spring scale. Before weighing fish, ensure that all debris (small rocks, etc.) are removed from the fish. Use the 100 g scale for all fish <100 g, and the 1 kg scale for larger fish. Cut open stomach of fish and record identifiable contents in the comments section of the data sheet.

**Net set time and date:** Record the time when you completed the net setting process, not the time when you started setting the net. Record the time as 24 hr time. Record the date on which the net was set.

**Net pull time and date:** Record the time when you began pulling the net and the date on which the net was pulled.

**Carcass disposal-** This is very important as we don't want the carcasses attracting the attention of backpackers or bears. When more than 5 trout are to be disposed, carcasses should either be buried at least 100 feet from water or deposited in an untreated burlap sack and sunk into the nearest deep water body downstream of the study basin. Be sure to pop the swim bladder of all fish that will be sunk. When disposing of less than 5 fish, carcasses may be sunk into the deepest part of the lake from which they were removed.

### **Littoral Zone Habitat Surveys**

Depending on snow conditions at individual lakes, littoral zone habitats will be surveyed either the first, second or third trip. Our goal is to conduct the surveys as close to amphibian breeding times as possible to record conditions available to breeding adults and eggs. One crew member should be selected to conduct all littoral surveys for that crew. Start the survey at the lake outlet and walk the specified distance for the specific lake to the first transect. Approximate distances to use between each transect are listed in the block notebooks. Distances were calculated in an attempt to obtain ~25 transects per lake. At each transect, you will collect information at 3 points in the littoral zone perpendicular to the shoreline, and the first 1.5 m of terrestrial habitat. Littoral zone points are measured 1) 0.1 m from shore, 2) 0.5 m from shore, and 3) 1.0 m



## Pope et al., Trinity Alps Basin Study Final Report

from shore. At each point, record 1) water temperature at 5 cm deep (°F), 2) depth in cm, 3) substrate, and 4) presence of living or dead aquatic vegetation (i.e., last year's emergent veg) within a 10 cm radius of the point. Collect temperatures first, trying not to stir the water before collecting them. If depth drops off quickly so that it can not easily be measured, you can record it as deeper than 1.5 m and give your best guess. The dimension associated with each substrate category is the particle diameter so that silt particles are < 0.06 mm in diameter; sand particles range from 0.06-2 mm in diameter; gravels range from 2-32 mm in diameter; pebbles range from 32-64 mm in diameter; cobbles range from 64-256 mm in diameter; boulders are > 256 mm in diameter; and bedrock is a large solid piece of rock embedded into the shoreline. Additionally, if the point lands on downed wood, record either submerged or emerged wood as the substrate. In this case, the depth should still be taken to the real bottom of the lake, not the top of a log. Note any amphibian eggs encountered along transects, including species, attachment substrate, and depth. Record the % cover of terrestrial vegetation and dominant vegetation type (meadow, shrubs, willow, alder, conifer, forb) along an imaginary line from the shoreline to 1.0 m from shore at each transect.

### **Field review of datasheets**

Following each survey type, the crew leader will review all datasheets for completeness and clarity. Once review of a datasheet is completed, the crew leader initializes the field review box at the bottom of the datasheet.

### **Public outreach**

During our surveys, we will undoubtedly be asked many questions by the public. Be polite but keep your responses brief and simple. For example, if someone asks what you are doing, inform them that you are conducting a study of the fauna of several lake basins throughout the Trinity Alps. Try not to get into discussions about potential changes in fish stocking, amphibian declines, etc. These are hot button issues, and should be avoided.

Appendix B. Histograms of trout lengths of gill-netted fish during density surveys from 2003 to 2007 for the individual stocked and stocking suspension lakes.

