At our June 4 committee meeting, you suggested that I revise the design for this summer's mesocosm experiment to increase replication, and explore Roland Knapp's Sierra Nevada alpine lake data to find lakes with and without frogs which have otherwise comparable data sets. I provide summaries below.

2010 Mesocosm design:

I updated the design for the 2010 mesocosm experiment. The objectives of the experiment are still a) to assess the effect of *Rana sierrae* tadpoles and mayfly nymphs on epibenthic producers, and b) to assess the sublethal effects of infection with the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd) during the tadpole stage on growth and survival during the subadult stage.

I will use a factorial design rather than the previously proposed response surface design, crossing presence or absence of tadpoles with presence or absence of mayfly nymphs, with each treatment replicated four times (Fig. 1). Moderately high densities of each consumer will be used in the "presence" treatments. Two replicates of each of the four treatments will be infected with Bd (Fig. 2, in khaki). The treatments will be randomized within the 16 mesocosm tanks (Fig. 3).

The revision allows for replication of each treatment. The response surface design included two temporal replicates of the entire experiment, but no concurrent replicates of the specific treatments. This is no longer feasible given a short season due to heavy snow pack, meaning that tadpoles will not be available until mid-July.

Tanks have been colonized by algae obtained from Convict Creek in April 2010 (Figure 4). Tadpoles will be collected from Marmot Lake (ca. 3500m elevation) in Humphrey's Basin, John Muir Wilderness as soon as the ice melts. Mayfly nymphs will be obtained from a roadside pond (<2500m elevation) in western Yosemite National Park, where we expect the majority of nymphs to be small individuals which will metamorphose the following summer.

Consumer-Resource Experiment

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) causes local extinctions of yellow-legged frog (*Rana muscosa* and *R. sierrae*) populations in alpine lakes throughout the Sierra Nevada. Within these lakes, the two most abundant grazers on benthic material are yellow-legged frog tadpoles and mayfly nymphs (*Ameletus spp.* and *Callibaetis spp*). To evaluate the consumptive and competitive interactions of these abundant grazers, I will use a factorial design to cross presence/absence of tadpoles with presence/absence of mayfly nymphs (Figure 1). Treatments will be randomized among 16 concrete tanks at SNARL (Figure 3). Mesocosm tanks are all contiguous, and adjacent to Convict Creek, which is the water source. Tanks are 1.07 m x 1.22 m and are filled to a depth of .84 m; they contain 1.09 m^3 water.

I will measure the response of epiphyton growth in relation to consumer presence. Twenty-five arrays of 12 small porcelain tiles will be placed at the bottom of each mesocosm tank and five arrays will be placed on the shallow shelf where tadpoles bask in warm surface water (Figures 5 and 6). Five softball-ish sized rocks will be placed on the tank bottom, and will provide habitat for consumers and substrate for epiphyton. For tiles on the shelf, five arrays on the bottom, and the rocks, epiphyton sampling will occur every seven to ten days. Five more arrays on the bottom will be sampled at two weeks, then placed back in the tank to serve as substrate for further epiphyton growth. Five more tiles will be sampled similarly at three weeks, and five will remain unsampled until the end of the experiment. All tile arrays will be sampled again at the conclusion of the experiment, expected to be 6-8 weeks. This "step-wise" sampling with replacement schedule will provide average daily growth rate when epiphyton is just colonizing, average daily growth rate over intermediate time scales, and growth rate over the 6-8 week experimental period.

Each day, I will monitor tadpole survival, metamorphosis, and mayfly emergence. On each sampling day, I will collect the substrates. I will scrub epiphyton from the substrate and these samples will be combusted to calculate biomass of epiphyton growth (ash-free dry mass) since the last sample date. I will capture, stage, and weigh tadpoles. If individuals are to be marked, then I will also collect individual measurements of body size (length, asymmetry of hind limbs). I will collect a sample of mayfly nymphs, performing sweeps of the tank with a D-net until 10 sweeps yield no more mayfly nymphs. I will count mayfly nymphs, and collect a cumulative biomass by water-volume displacement of a subset of nymphs placed in a graduated cylinder. The measurement of the cumulative masses of each consumer will indicate the occurrence of competitive effects on consumer growth. After sampling, I will place the substrates, tadpoles, and mayfly nymphs back into the mesocosm.

Tadpoles will be removed from the experiment when they reach Gosner stage 42, at which point their rasping mouthparts atrophy and they stop feeding until the subadult stage. The experiment will end when the last tadpole reaches stage 42. All tiles will be sampled, mayflies will be collected, weighed, and measured.

Based on my 2009 field experiment, I expect epiphyton growth to be similar in the mayfly-present and mayfly-and-tadpole-present treatments. I expect epiphyton growth in these two treatments to be lower than epiphyton growth in the no-consumer treatment. I expect the tadpole-present treatment will not differ from the no-consumer treatment, as no effect of tadpoles on epiphyton growth was observed in the field experiment.

Sublethal Effects Experiment

Bd infects only keratin on amphibians. Keratin occurs in the mouthparts of tadpoles and in the skin of post-metamorphic frogs. Our previous work on Rana muscosa/R. sierrae has found no detectable effect of Bd infection on tadpole growth and survival, although it is lethal to post-metamorphic individuals. A recent paper by Garner et al. (2009) found, in a different amphibian species, that exposure to Bd during the tadpole stage, even in individuals that did not become infected, reduced survival after metamorphosis. Their explanation was that a metabolic cost was accrued in fighting off the infection, which reduced subsequent survival. However, this seems unlikely because no one has yet been able to detect an adaptive immune response against Bd in tadpoles. We will test for sublethal effects of infection with Bd during the tadpole stage on growth and survival both before and after metamorphosis. In the Bd infected treatments, prior to being placed in the tanks, the tadpoles will be exposed to a mix of Sierran Bd strains grown from culture. The infection status of each individuals will be assessed on regular intervals using a non-invasive "tadpole soak" protocol and real-time quantitative PCR.

When the tadpoles reach Gosner stage 42 they stop feeding on algae, as their rasping tadpole mouthparts morph into frog mouthparts. At this point the tadpoles will be removed from the mesocosms and half of the tadpoles from each tank will be treated with an antifungal compound (Itraconazole), which is effective at clearing individuals of Bd infection. This will give us four new treatments for the second part of the experiment:

(a) not infected during tadpole stage - No Itraconazole
(b) not infected during tadpole stage - Itraconazole treated
(c) infected during tadpole stage - No Itraconazole
(d) infected during tadpole stage - Itraconazole treated

Individual growth and survival will be followed for several weeks after removal from the mesocosm tanks. The main comparison in which we are interested is (b) versus (d). This comparison will allow us to address the question: does infection during the tadpole stage have negative effects that carry over to the subadult stage (even if the subadults are no longer infected)? Infected individuals in treatment (c) are expected to exhibit the symptoms of chytridiomycosis (lethargy, cessation of food intake, lack of righting reflex, and death if not treated). Comparing (a) and (b) will allow us to detect any negative effects of Itraconazole treatment.

Our expectation, based on prior work in this system, is that Bd infection will have no effect on tadpole growth or survival, or on growth or survival of individuals treated with Itraconazole prior to metamorphosis.

Lakes to be used for analysis comparing benthic macroinvertebrate communities in lakes with and without mountain yellow-legged frogs:

I searched Roland's database of lake surveys for lakes that fit the following criteria:

- No fish observed during the initial survey
- At least one survey of benthic invertebrates performed
- At least one amphibian survey performed

A set of 125 lakes fit these criteria (Table 1). These are predominantly medium to large lakes, >1 ha in surface area (as indicated by a 0 as the second digit in the LakeID). All are above 2000 m (6500 ft) elevation (Fig. 7). A similar number are located in Yosemite National Park (YOSE) and Sequoia/King's Canyon National Park (SEKI), and some others are located between the two parks in the John Muir Wilderness (JMW). A handful of lakes in the JMW are elevationally and physically similar to SEKI lakes. Lakes in YOSE span a large elevation range, but those in SEKI are all within 300 m (1000 ft) elevation.

The subset of SEKI lakes contains 31 lakes in which 1 or more Rana sierrae tadpoles were observed in the initial survey of the lake. There are 23 in which no amphibians were observed in the initial survey. The elevations of these frog-containing (frog+) and frogless (frog-) lakes are similar (Fig. 9). Elevation can sometimes serve as a proxy for habitat complexity, productivity, or temperature.

I will first analyze these data to evaluate the extent to which physical factors determine differences in the benthic macroinvertebrate communities observed by Roland. These relationships have been evaluated before for larger sets of lakes (Knapp and Matthews, 2001, and Knapp, Hawkins, Ladau, McClory, 2005). Following that, I will examine differences between benthic macroinvertebrate communities based on the presence or absence of frogs. I will perform a similar analysis on the full 125 lake data set.

To find lakes to survey in future field seasons, I will use Roland's recent data on frog abundance and Bd status to select lakes that have changed state (frog+ to frog-, or vice-versa) since the initial survey. I will resurvey these lakes to compare macroinvertebrates before and after frog extinction (or recovery). We can also determine the duration a lake has been frogless and examine differences in communities that recently lost frogs and those that lost frogs further in the past.

I discussed with Roland the data from his long term monitoring of lakes in Humphrey's Basin (about 7 lakes in JWM, including 50170 and 50183 in Table 1). Initial surveys of these lakes were performed in 1996, and Roland collected 3 benthic macroinvertebrate samples per season for the next 7 seasons, then one per year most years since then (2004-2009). This makes a 13-year time series, however, most of these lakes were subjected to fish eradications which were shortly followed by both successful and unsuccessful frog reintroductions. The loss of fish would confound the introduction or absence of frogs in these lakes as fish-presence is a strong driver of community dynamics, so they may not be the best data for my frog-centric interests.

There seem to be enough lakes for a frog-frogless comparison. It remains to be seen whether there are enough data to look at community dynamics over time in relation to duration frogless. I might be able to look at resistance, but perhaps not resilience, of lakes to the loss of mountain yellow-legged frogs.