What is the trophic level status, functional feeding group categorization and food source(s) of Comal Springs riffle beetle adults and larvae in their natural habitat?

- 1. Identify the trophic level status of the Comal Springs riffle beetle.
- 2. Identify the functional feeding group categorization of the Comal Springs riffle beetle.
- 3. Identify the food sources used by larval Comal Springs riffle beetle.
- 4. Identify the food sources used by adult Comal Springs riffle beetle.
- 5. Establish the stable isotope concentrations in the guts of Comal Springs riffle beetle larvae and adults.
- 6. Establish an empirical relationship between those concentrations and various food sources.

What are the long-term, dissolved oxygen and temperature tolerance ranges of the Comal Springs riffle beetle adults and larvae?

- 1. Determine the range of dissolved oxygen (DO) concentration necessary for optimum growth and development, the ranges of DO concentration which create stress in the Comal Springs riffle beetle, and the ranges of DO concentration at which the riffle beetles are intolerant.
- 2. Determine the range of temperatures necessary for optimum growth and development, the ranges of temperature which create stress in the riffle beetle and the ranges of temperatures at which the riffle beetles are intolerant.
- 3. Build on earlier studies, using more slowly-changing DO and temperatures than used in previous studies.

What is the life history of the Comal Springs riffle beetle, from egg to adult in the Comal Springs aquatic ecosystem?

- 1. Determine the phenology of the Comal Springs riffle beetle.
- 2. Descriptive study of laboratory Comal Springs riffle beetle colonies, tracking life stages and the duration of each stage.
- 3. Follow up with study of natural population.

What is the genetic taxonomic status of the Comal Springs salamander in the Comal Springs aquatic ecosystem?

- 1. Literature review of existing genetic studies, current taxonomic/genetic classification and genetic variation among samples.
- 2. What is the genetic relationships of the Comal Springs salamander to other *Eurycea* species?
- 3. Determine and describe the genetic taxonomic status and conduct phylogenetic analysis of the Comal Springs salamander and compare it to other species of *Eurycea*.
- 4. How much genetic diversity is exhibited by the population of *Eurycea* found in the Comal Springs ecosystem?
- 5. Collect samples from as varied location as possible for further genetic testing.
- 6. Comparison of current samples to existing classes.

What is the population estimate of the Comal Springs Salamander in the Comal Springs aquatic ecosystem?

- 1. Descriptive study using mark/recapture techniques.
- 2. Estimate the total population of Comal Springs salamanders in the Comal Springs aquatic ecosystem.
- 3. Estimate population size and distribution of the Comal Springs salamander within the Comal Springs aquatic ecosystem.
- 4. What it the distribution pattern of Comal Springs salamanders within the ecosystem?
- 5. Use population estimation approaches that are practical yet account for imperfect detection. Examples include spatial capture-recapture and N-mixture model methods.