LOW FLOW THRESHOLD STUDY OF AQUATIC VEGETATION

INITIAL LABORATORY TRIAL

Objective

The objective of the Low Flow Threshold Evaluation of Aquatic Vegetation study is to evaluate the effects of low-springflow, water temperature, and carbon dioxide (CO_2) on aquatic vegetation. The null hypothesis is that the high water temperature and extremely low CO_2 conditions tested will cause aquatic vegetation growth to cease with subsequent decay and death.

Study Location

The initial laboratory trials will be carried out at the San Marcos Aquatic Resources Center (ARC) located on McCarty Lane in San Marcos, TX.

Materials and Methods

Experimental design (Figure 1) will include twelve replicates of four species, *Cabomba sp.*, *Ludwigia sp.*, *Vallisneria sp.*, and *Riccia sp.* Plants will be tested at one minimal flow scenario (0.1 gpm) with one CO_2 condition (<2 mg/L) and two water temperature treatments (28 °C and 34 °C) with three replicates per treatment. This results in 12 plants x 4 species x 2 water temperature treatments x 3 replicates for a total of 288 plants (72 plants of each species) needed for the active portion of the experiment. Additionally, 10 replicates of each of the 4 species will be sacrificed following the acclimation period for baseline biomass comparisons.

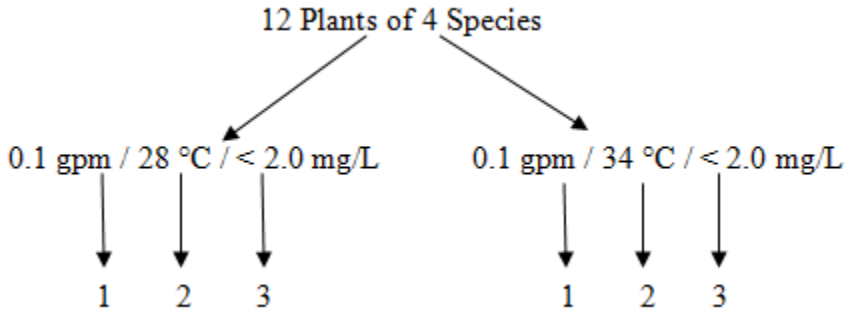

Figure 1: Experimental design diagram (Flow / Water Temperature / CO_2)

Following approval of the final experimental design, parent material for plant propagation will be obtained from stock material held at the ARC. If needed, parent material will be collected from the Comal River and treated to prevent introduced organisms per ARC protocols. Plants will be propagated in 10.16 cm diameter X 10.16 cm tall pots, referred to as “quart pots” in the plant nursery trade, with a maximum volume of 900 mL of soil. Parent material will be divided

into 20 cm long apical segments. Two segments per pot will be planted in a sandy clay loam soil mix regularly used by botanists at ARC for aquatic plant propagation. Fertilizer amendments may be added to the media as deemed necessary. A total of 350 planted pots will be produced.

All potted plants will be placed in raceways or ponds at the ARC and monitored during the acclimation period for health and growth. Once plants have sufficient top growth, they will be randomly selected and moved into the laboratory treatments. Only uniform sized plants will be used. Sickly or damaged plants will be discarded. In addition, plants with observed reproductive structures will not be selected. At the beginning of the experiment, ten additional plants from each species will be dried and weighed to get a "before-treatment" biomass measurement.

Seven 950-L fiberglass tanks (Living Stream Model MT-1024, Frigid Units Incorporated, Toledo, Ohio) are proposed for use during this experiment. The design includes constant flow (0.1 gpm) and low CO_2 (< 2.0 mg/L) with two water temperature treatments with each replicated three times for a total of six treatment tanks. As the Edwards Aquifer well water supplied to the ARC is high in CO_2, an additional preparation tank will be required. Each of the six treatment tanks and preparation tank will be connected to individual heater/chiller units. In the preparation tank, water will be re-circulated through a system of PVC pipes connected to six adjustable nozzles that will be angled to spray water just below or above the water surface to adjust the CO_2 concentration in the tank. Allowing water to spray into the air before entering the tank provides greater surface agitation and allows a considerably greater reduction in CO_2. Once water is adjusted for CO_2 it will be moved via a system of PVC pipes out of the respective preparation tank and into each treatment tank. In addition, an aerator may be placed in each treatment tank to further agitate the water surface and maintain CO_2 concentrations of < 2.0 mg/L.

Each of the six treatment tanks will be randomly assigned a water temperature treatment. The tanks will be cleaned and allowed to operate at their respective treatment conditions for 10 days to allow adjustment and equilibration of the flow, water temperature, and CO_2 concentration as necessary. Each plant/bryophyte will be randomly assigned to one of the two treatments, then to one of the three tanks within a treatment, and ultimately to a specific location within each tank. The trial will be run for an anticipated period of six weeks. Over the course of the study, 4 plants per treatment (for each species) will be collected every two weeks, with the final four collected at the completion of the study. The objective is to evaluate decay rates relative to biomass over time (=expected negative relative growth rate). During the experiment, water quality parameters and flow rate will be measured a minimum of three times per week within each treatment.

At the end of the study period, all plants will be collected, rinsed, dried in a standard drying oven and weighed. Plants will be weighed (grams) within two hours of removal from the drying oven. Growth of each species will be measured by quantifying shoot dry mass, root dry mass, number of stems, internode length and maximum stem length. In addition, the bryophytes will be heated

in a muffle furnace to a temperature of 500 ºC for four hours to allow estimation of ash-free dry mass (based on previous experience, the bryophytes accumulate inorganic sediments that cannot easily be removed prior to drying). All bryophyte ash will be weighed within 30 minutes of removal from the muffle furnace. Growth or decay of each species will be measured at each stage and the end of the experiment as changes in biomass (dry weight in grams for all plants and bryophytes and ash-free dry weight in grams for bryophytes only). That is, we will determine the relative growth rate (positive or negative) for each harvest interval. At the end of the experiment, an overall average RGR for each species will be determined from all biomass data for that species.

Statistical analyses

The specific data analysis will depend on the observed pattern of change in the vegetation. If differences in overall survival (categorical response) are observed, we will analyze those differences utilizing a Chi-Square analysis. For surviving plants, we will determine the relative growth rate (RGR's) (hypothesized to be negative) of the plants. Differences in RGR's among species will serve as an indicator of how strongly each species responds to conditions tested.

Next Steps

As highlighted in the work plan, results from the initial laboratory trial will influence the design of subsequent tasks for this Applied Research study.