Attachment 3

U.S. Fish & Wildlife Service

San Marcos Aquatic Resource Center

Low-Flow Threshold Evaluation of Native Aquatic Vegetation – Pond Experiment

August 7, 2013

Introduction

The Edwards Aquifer Habitat Conservation Plan (HCP) is built upon a foundational flow-regime that is presumed to be protective of the endangered species inhabiting the Comal and San Marcos springs/river ecosystems. However, as low flows projected in the HCP flow regime have occurred very infrequently throughout the historical record, testing of conditions anticipated to occur during extreme drought are critical during Phase 1 HCP activities. Throughout the HCP, fountain darter (*Etheostoma fonticola*) habitat (aquatic vegetation, water quality, hydraulics, substrate, etc.) is highlighted as an integral component in both systems. To protect this habitat, we must first understand the bounds of fountain darter habitat. This Pond experiment was designed based on the available literature and information learned during the Vegetation Tolerance laboratory studies presently being concluded and pond pre-study currently in progress. The Pond experiment focuses on two aspects of fountain darter habitat - native aquatic vegetation and water quality. The Pond experiment is an evaluation of extremely low-flow conditions that may be experienced in portions of the Comal and San Marcos systems within the context of the HCP flow regime.

Objective

The objective of the low-flow threshold of native aquatic vegetation pond study (Pond experiment) is to evaluate the effects of loss of water flow on survival and growth of native plant species that are components of habitat occupied by the fountain darter. It is hypothesized that loss of flow into the experimental water body will result in significant changes in water quality parameters leading to slower growth or possibly reversal of growth and potentially reduced survival of these plant species.

Study Location and Background

Aquatic macrophytes are important to the aquatic environment for many reasons including water purification, nutrient recycling, refugia for zooplankton, cover for invertebrates, cover for fish, food source, affecting flow patterns, and creating discrete habitat as physical structure in the water column (Cowx and Welcomme 1998). Aquatic macrophytes present within the San Marcos and Comal River systems have been documented by multiple ecological studies in the region (Lemke 1989, USFWS 1996, Poole and Bowles 1999, Owens et al. 2001, BIO-WEST 2002-2012). Common native, rooted species providing fountain darter habitat include eelgrass (*Vallisneria americana*), Carolina fanwort (*Cabomba caroliniana*), and red ludwigia (*Ludwigia repens*).

Studies have documented the importance of biological interactions between macrophyte species (Titus and Stephens 1983, Doyle et al. 2003), physical factors (Barko et al. 1984, Barko and Smart 1986, Madsen et al. 2001) and chemical properties of the environment (Titus et al. 1990, Pagano and Titus

2004, Engelhardt 2006, Bailey 2012) on macrophyte growth and distribution. Since the San Marcos and Comal systems are dependent upon springflow to maintain the aquatic environment, it is important to understand the responses of the macrophyte community to potential decreasing flow conditions.

Carolina fanwort is a submerged aquatic plant that grows in stagnant to slow flowing freshwater, and spreads primarily by stem fragments. Carolina fanwort prefers a warm, humid climate with a temperature range of 13-27°C and can grow well in turbid water (WSDE 2013). Red ludwigia can be found growing under a wide range of conditions including streams, ponds, wetlands and drainage ditches. It as an amphibious plant that produces both submersed and emergent leaves and can grow in wet terrestrial habitats (Godfrey and Wooten 1981). Eel grass is a submerged native aquatic plant that occurs in streams, lakes and brackish water habitats. Since it is considered an important aquatic plant for wildlife much work has been done studying responses of eel grass to environmental factors such as light availability and salinity changes. Total biomass production of *Vallisneria americana*, *Elodea canadensis*, and *Potamogeton nodosus* has been shown to increase with both increasing light and increasing temperature to at least 28°C (Barko et al. 1984), whereas Boustany et al. (2010) found salinity directly impacted growth of eel grass but that light effects were less direct.

The San Marcos and Comal systems are unique, freshwater environments in central Texas that support an aquatic macrophyte community that provides preferred habitat for native fish species. While the literature documents general habitat conditions and tolerances of these three species with respect to temperature, light, and in some cases in relation to nutrients, sediment type, and salinity, the effect of low-flow, shallow water conditions on the growth and survival of these species is not well understood. Furthermore, in preliminary studies (pH Drift Study) undertaken by Baylor University, these species have shown plasticity in regards to their method of carbon uptake depending upon the type of aquatic environment in which they inhabit (Sarah Hestor, personal communication). In this study, red ludwigia and eel grass growth will be examined under an experimental range of low-flow, shallow water conditions to simulate the effects of ponding and stagnation expected to be found after potential decrease in flow conditions in the spring environments. The low-flow threshold of this native aquatic vegetation pond study will take place in a 1,300 ft^2 rubber-lined pond at the San Marcos Aquatic Resources Center (ARC) located on McCarty Lane in San Marcos, TX.

Materials and Methods

Vegetation Tolerance Laboratory studies: Initial results of a six week greenhouse study indicate that fanwort, red ludwigia and eel grass continued to grow even when subjected to low CO2 < 5ppm and sustained water temperatures of up to 34 °C. While these conditions may be indicative of similar conditions experienced in pooled, stagnant aquatic habitats it did not subject the plants to other factors such as diurnal fluctuations in temperature, high light intensity or competition from algae. These factors may negatively impact growth of aquatic plants in a larger outdoor experiment.

Pre-Study: A pre-study trial is currently in progress at the San Marcos ARC. Existing red ludwigia and fanwort plants reared in greenhouse troughs as well as the experimental pond were used for this experimental trial. This resulted in three experimental groups based on plant source and species (Table 1). Experimental plants from each group were randomly placed into groups of ten to provide experimental units. Experimental units were placed in the pond at randomly generated gps locations with a Trimble GeoXT 6000 sub-meter gps unit (Figure 1) and protective wire cages were placed around each unit to prevent interference and damage from turtles that periodically migrate into the pond (Figure 2). Experimental plants were allowed to acclimate to pond conditions with flow (≈ 0.023 cfs) for one week. Following acclimation, ten individuals from each group were randomly selected to provide an initial biomass sample and total initial stem length was measured for all plants. After all initial data were collected; flow to the pond was terminated. These conditions are being allowed to persist for two weeks, after which total stem length of all individuals will be recorded. Ten individuals from each of the three groups will be randomly selected to provide a post treatment biomass sample. Five red ludwigia and five fanwort individuals will be selected randomly to be placed back in greenhouse troughs under optimal growing conditions (ARC well water) to investigate recovery potential. Water quality parameters (DO, temperature, pH, PAR, CO_2, pond depth) are being monitored daily throughout the study period, as they were through the acclimation period. Depth at each experimental unit location was assessed at the onset of experimental conditions.

Table 1. Distribution of plant groups into experimental units in the Pond Experiment Pre-Study. Experimental plants were grouped by source from which plants were obtained and species. The total number of plants exposed to no-flow conditions (*n*) and the number of experimental units composed of plants from each group (N) are denoted.

SOURCE	***SPECIES***	***n*/N**
GREENHOUSE	*C. caroliniana*	10/1
GREENHOUSE	*L. Repens*	30/3
POND	*C. caroliniana*	50/5

Figure 1. Randomly generated locations in the San Marcos ARC pond to which experimental units (aggregations of 10 plants) were randomly assigned.

Figure 2. Protective cages in place around experimental plants.

Pond Experiment: To provide a more formal test of the hypothesis that loss of flow will result in less favorable environmental conditions and consequently adverse effects on plant growth, the following applies. The pond will be divided lengthwise with sandbags producing a flowing treatment area that maintains flow-through of approximately 0.023 cfs for the duration of the experiment as well as a no-flow treatment area (Figure 3). The native species red ludwigia and eel grass will be used for this experiment because they are inhabited by fountain darters and likely to be present in Landa Lake during extremely low flow conditions. Plants to be used in the experiment will be grown using wild stock clippings from the Comal system. These clippings will be allowed to grow for two weeks under optimal conditions (ARC well water) in the San Marcos ARC greenhouse prior to the experiment. Twenty individuals of each species will be randomly selected at the end of the growth period to provide an initial biomass sample, with this process repeated at the conclusion of the experiment. For each species, five units of ten plants will then be randomly placed in each treatment area (Figure 3). Initial total stem length will be measured for all plants, and will be assessed again at the conclusion of the experiment. Environmental parameters (DO, PAR, CO_2, temperature, pH, flow and depth) will be measured twice daily (once in the early morning and again mid-afternoon) to capture diel fluctuation of these variables.

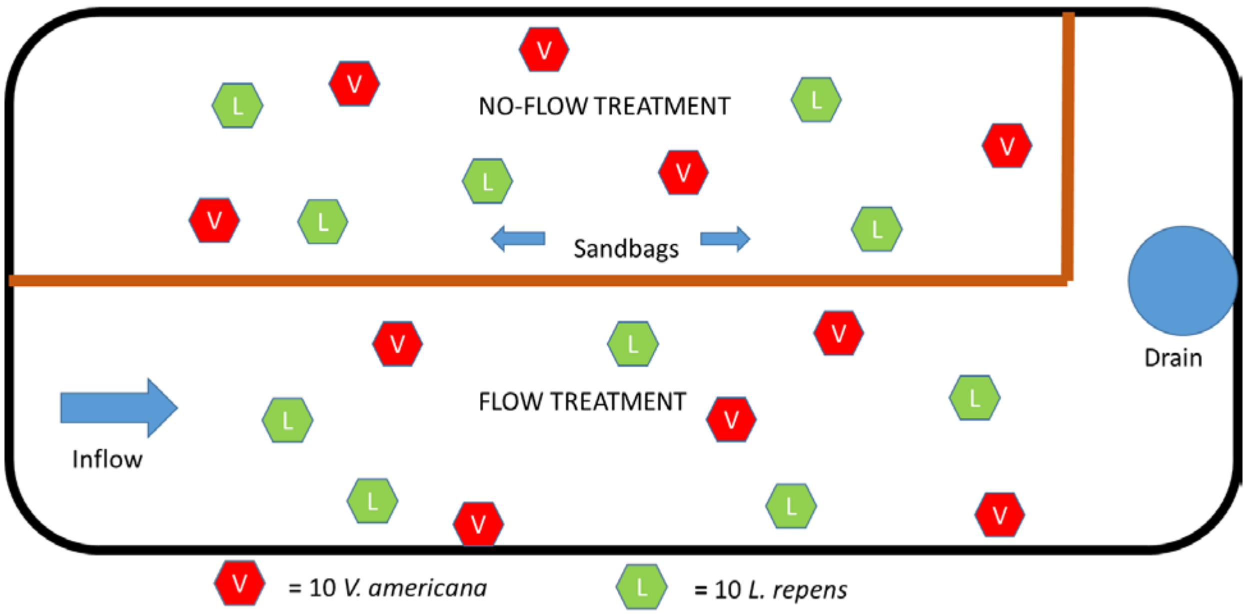

Figure 3. Representation of the experimental scheme for the low-flow threshold evaluation of native aquatic vegetation pond experiment. The experimental pond will be divided by a sandbag barrier, leaving approximately one half of the pond with flow-through conditions. The remaining pond area will provide conditions representative of those expected from a loss of flow in the Comal system.

DATA ANALYSIS

Pre-Study: Data resulting from completion of the pre-study experiment will be explored using principle components analysis (PCA) to examine the relative effect of changes in different environmental parameters on plant growth. Differences in plant growth response (change in total stem length/biomass) among species will be assessed using standard analysis of variance (ANOVA) methods. Results from these analyses will be used to further refine the design of the formal pond study.

Pond Study: Differences in the response variable (change in total stem length) for each species and each treatment will be assessed using a 2-factor ANOVA, with significant results explored using Tukey's HSD test. Relationships among the environmental variables and the response variable will be analyzed using stepwise regression. These results will be used to inform parameterization of the submerged aquatic vegetation module of the HCP ecological model.

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