Attachment 4

U.S. Fish & Wildlife Service

San Marcos Aquatic Resource Center

Low-Flow Food Source Study
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. One such condition is whether the macroinvertebrate food base that presently supports the diet of the endangered fountain darter (*Etheostoma fonticola*) will continue to persist during extreme drought conditions. To test the food base response to extreme conditions, a low-flow food source study was designed based on the available literature and information acquired during EARIP funded aquatic vegetation studies and pre-study food source investigations.

Objective

The focus of the fountain darter food source study is to determine if conditions expected to result from low- or no-flow conditions can be expected to adversely impact availability of a significant prey source of the fountain darter. It is expected that aquatic macroinvertebrates, specifically the amphipod *Hyalella azteca (proposed as a surrogate)*, will be unable to tolerate increased water temperatures that may result from low flow at some threshold. This experiment is also designed to provide results that can be linked to the submerged aquatic vegetation module and fountain darter reproduction module within the framework of the HCP ecological model(s).

Study Location and Background

Experimental trials will be undertaken at facilities provided by the USFWS San Marcos Aquatic Resources Center (ARC) located on McCarty Lane in San Marcos, Texas. Aquatic vegetation and macroinvertebrates will be collected from ARC ponds and Comal Springs, Texas.

Fountain darters are a federally listed endangered species only found in the Comal and San Marcos Rivers, Texas. Although the extreme conditions noted in 1956 (cessation of springflow) for the Comal River have not been repeated, summer flows in the Comal River have dropped below 100 cfs in 1989, 1990, and 1996 (USGS gage 0816900). The present HCP flow regime incorporates total discharge in the Comal system well below 100 cfs. As such, it becomes important to understand how elevated water temperatures due to decreased spring flow will affect the fountain darter beyond just reproductive success and organism survival.

Fountain darters typically inhabit the bottom third of the water column preferring aquatic vegetation for cover. This vegetation also provides cover for aquatic invertebrates, the preferred food of fountain darters (Bergin et al. 1995, Schenck and Whiteside 1977). Fountain darter diets are made up of many types of invertebrates, but copepods, dipterans, mayflies, and amphipods are most frequently found in their stomachs (Schenck and Whiteside 1977). The most common amphipod in the Comal Springs/River ecosystem is *Hyalella azteca*, which is widely distributed across North America (Strong 1972). These invertebrates likely reproduce most of the year due to constant water temperatures (Strong 1972). *Hyalella azteca* are shown to have inter-population variation in body size (Strong 1972, Wellborn 1994) across regions and water systems (e.g. springs [Wellborn et al. 2005]). In addition, this amphipod is known to be different morphologically in the presence of predators (e.g. fish [Cooper 1965, Strong 1972, Wellborn 1994]). As such, using experimental stock taken from the population in Comal Springs is recommended rather than solely relying on information from other watersheds.

Hyalella azteca is common in fountain darters' preferred vegetation types (bryophytes, filamentous algae, *Hygrophila*, *Ludwigia* [BIO-WEST 2012]), and therefore a widely available prey source. *Hyalella* sp. are widely distributed in North America and can tolerate a wide-range of water temperatures. While researchers have observed adverse effects of high temperatures on *Hyalella* in some regions (Pickard and Benke 1996 [>26° C], Wellborn and Robinson 1996 [30° C]), these limits are not necessarily indicative of all inhabited locations. As such, it is recommended that *Hyalella azteca* from the Comal system serve as the surrogate for fountain darter food and be tested to determine their upper thermal limit. A common method for determining an upper thermal limit is to determine the critical thermal maximum (CTM). A CTM is defined as "… the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death when heated from a previous acclimation temperature at a constant rate" (Cox 1974). The CTM will serve as a surrogate for when amphipods cease to be available as a food source to the fountain darter. This cessation of feeding is based on the literature that describes fountain darters (in laboratory studies) as only feeding on moving prey (Schenck and Whiteside 1977).

Materials and methods

CTM experiments

A CTM will be determined for the amphipod *H. azteca* which will assist in parameterization of the fountain darter food source input to the HCP ecological model(s). The CTM uses temperature as a one-variable test which will further our understanding to the ecological impact of rising water temperatures associated with low-flow conditions. Twenty-four hours prior to testing, fifty amphipods will be acclimated to each of three temperatures of 21°C, 26°C and 30°C, for a total of 150 individuals. After acclimation, trials will commence by placing 5-10 individuals in a water bath increasing in temperature at 0.3°C/minute. Amphipods will be observed constantly while temperatures are increased in order to record the temperature when ecological death occurs. Ecological death will be determined when the amphipod ceases to respond to agitation but is able to recover when placed back into its original acclimated water temperature. After twelve hours, vitality of the amphipod will be established if movement does or does not occur when placed in 70% isopropyl.

Laboratory and pond experiments

Preliminary laboratory food source trials tested amphipod survival rates at two temperature treatments. After 48-h acclimation at treatment temperatures, amphipods were separated into two treatments of 28°C and 34°C and survival was assessed incrementally. These temperatures were determined by established biological limits of the fountain darter, as 28°C has been shown to reduce offspring viability and larval survival while 34°C is approximately the critical thermal maximum for fountain darters (Bonner et al. 1998, Brandt et al. 1993). Survival of *H. azteca* was lower in the higher temperature treatment (Table 1). Using the results from preliminary trials, laboratory and pond food source study protocols were established and described herein.

Table 1. Preliminary laboratory food source trials using *H. azteca* where no surviving individuals were found at 34°C treatments eight days after acclimation.

Days after acclimation	Percent survival at 28°C	Percent survival at 34°C
4	54	8
8	29	0
16	14	0

Laboratory and pond food source experiments will use the amphipod, *H. azteca*. Half of the experimental units will include containers with only amphipods, whereas the other half will include an aquatic bryophyte, *Riccia sp*. along with amphipods. It is presumed that aquatic vegetation serves as a food source for the amphipods, thus potentially eliminating starvation as a cause of death in the *Riccia* units. In both experiments, amphipods and bryophytes will be enclosed in experimental cups (**Figure 1**) to provide a controlled study area.

Figure 1. Food source experimental cup

The cup base will be weighted down using gravel and have 5mm holes drilled into the cup base allowing water movement between the cups and the aquarium they are housed in. The cup base and lid opening will be covered with 790µm mesh using the cup lid to secure the mesh in place, completely enclosing the study area while allowing water exchange throughout the cup and the surrounding aquatic environment. Ten amphipods will be added to each cup, and bryophytes will only be added to designated cups. Gentle probing of amphipods will determine if the individual is living or deceased and will be assessed per week of the two week study.

Laboratory Experiment: Laboratory food source trials will include two replicates of three water temperature treatments (Figure 2): 21°C, 28°C, and 34°C. Amphipods and bryophytes (*Riccia sp.*) will be acclimated to their respective temperature treatments 48-h prior to testing. Each replicate will contain six total cups: three cups with amphipods and bryophytes, and three cups with amphipods but no bryophytes, for a total of 36 experimental cups.

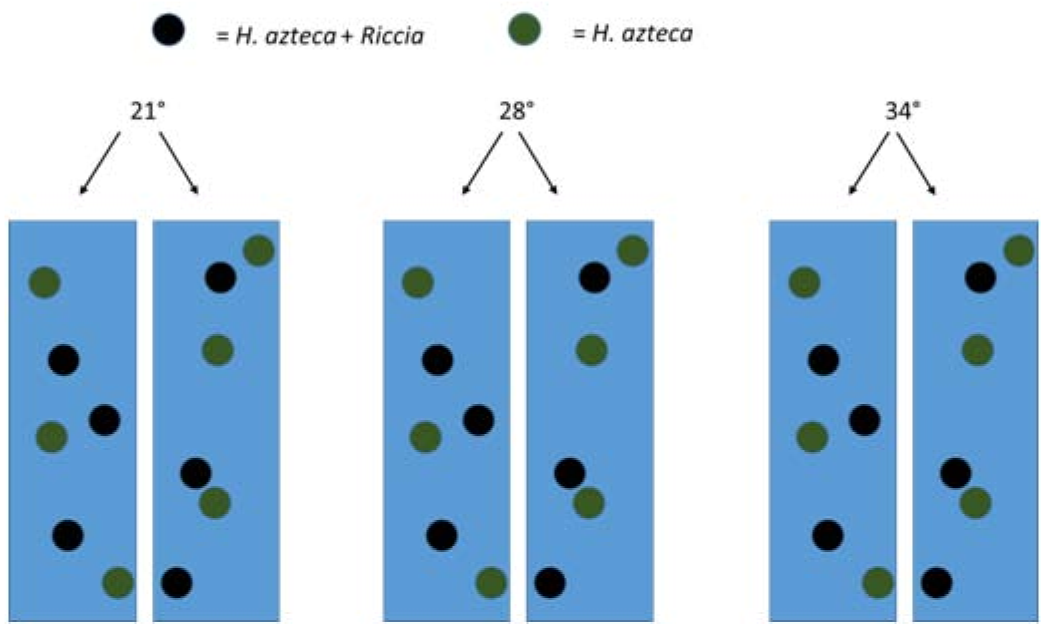

Figure 2. Experimental design of the laboratory food source experiment. Three treatment temperatures (°C), each with 2 replicates, will be used.

Pond Trial: In conjunction with the pond vegetation study, the pond food source study will include five replicates in two treatments of differing temperature regimes due to flow or lack thereof (Figure 3). Amphipods and bryophytes will be acclimated to pond conditions prior to testing. Each treatment will contain 10 experimental cups, each replicate containing one cup with amphipods and bryophytes, as well as one cup with amphipods but no bryophytes, for a total of 20 experimental cups. Amphipod cups will be randomly assigned to and placed in one of 20 experimental plant units composed of 10 individuals of either red ludwigia or eel grass. Placement within vegetation is more representative of the habitat where they would be found in the wild as well as habitat where they would likely be encountered by fountain darters. Environmental variables (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.

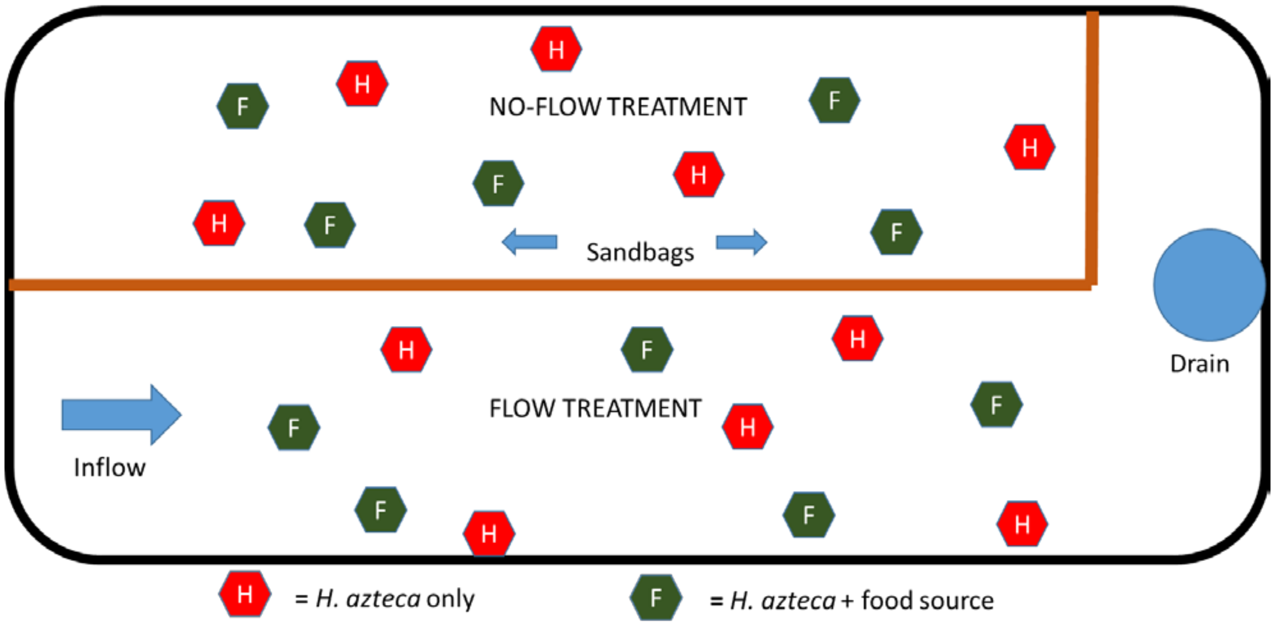

Figure 3. Representation of the experimental scheme for the low-flow food source study pond experiment. The experimental pond will be divided by a sandbag barrier, leaving approximately one half of the pond with flow-through of approximately 0.023 cfs representative of the Comal system. The remaining pond area will provide conditions representative of those expected from a loss of flow. Red and green hexagons represent experimental amphipod cups.

Data analysis

CTM: The effect of acclimation temperature on CTM of *H. azteca* will be assessed using standard analysis of variance (ANOVA) methods. Mean CTM will be determined from the mean temperatures at which individuals in each treatment were determined to exhibit ecological death.

Lab and Pond Experiments: Differences in *H. azteca* survival among treatment conditions and experimental cups with or without a food source will be analyzed using two factor ANOVA. Relationships among the measured environmental variables and *H. azteca* survival will be explored using principle components analysis (PCA).

Literature Cited

Bergin, S., T. Arsuffi, B.G. Whiteside, T.M. Brandt, and J.N. Fries. 1995. Food of the fountain darter (*Etheostoma fonticola*) in the Comal River, New Braunfels, Texas. Interim Project Report. Pgs. 1-21.

Bonner, T. H., Brandt, T. M., Fries, J. N., and B. G. Whiteside. 1998. Effects of temperature on egg production and early life stages of the fountain darter. Transactions of the American Fisheries Society 127: 971-978.

Brandt, T. M., Graves, K. G., Berkhouse, C. S., Simon, T. P., and B. G. Whiteside. 1993. Laboratory spawning and rearing of the endangered fountain darter. The Progressive Fish-Culturist 55(3): 149-156.

BIO-WEST 2012.Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2012 Annual Report. Edwards Aquifer Authority.41 p. plus Appendices.

Cooper, W. E. 1965. Dynamics and production of a freshwater amphipod, *Hyalella azteca*. Ecological Monographs 35: 377-394.

Cox, D. K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. *In* Thermal Ecology. Pgs. 158-163.

Pickard, D. P., and A. C. Benke. 1996. Production dynamics of *Hyalella azteca* (Amphipoda) among different habitats in a small wetland in the southeastern USA. Journal of the North American Benthological Society 15 (4): 537-550.

Schenck, J. R. and B. G. Whiteside. 1977. Reproduction, fecundity, sexual dimorphism and sex ratio of *Etheostoma fonticola* (Osteichthyes: Percidae). The American Midland Naturalist 98 (2): 365-375.

Strong, D. R. 1972. Life history variation among populations of an amphipod (*Hyalella azteca*). Ecology 53 (6): 1103-1111.

Wellborn, G. A. 1994. The mechanistic basis of body size differences between two *Hyalella* (Amphipoda) species. Journal of Freshwater Ecology 9 (2): 159-168.

Wellborn, G. A., and J. V. Robinson. 1996. Effects of a thermal effluent on macroinvertebrates in a Central Texas Reservoir. The American Midland Naturalist 136: 110-120.

Wellborn, G. A., R. Cothran, and S. Barthole. 2005. Life history and allozyme diversification in regional ectomorphs of the *Hyalella azteca* (Crustacea: Amphipoda) species complex. Biological Journal of the Linnean Society 84: 161-175.