

HCP Measure 6.3.4 - APPLIED RESEARCH AT USFWS NATIONAL FISH HATCHERY AND TECHNOLOGY CENTER (NFH&TC)

Long-term Objective: The long-term objective of this measure will be to conduct applied research relative to the ecological dynamics of the Comal system, particularly under low flow conditions. The ultimate objective will be to inform Phase II decisions regarding the Covered Species and, to the extent possible, adjustments to conservation Measures during Phase I.

Introduction/Overview: As part of the AMP, there are several HCP measures designed to track the condition of the ecological systems over time (biological and water quality monitoring), and to improve habitat and Covered Species conditions (restoration and non-native controls, etc.) to prepare the systems for future low-flow conditions. However, these activities alone will not be sufficient to address the questions of whether the flow-regime and long-term biological goals presented for Phase I of the HCP are protective and/or appropriate for the Covered Species. As such, additional applied research is critically necessary to support answering these overarching questions. Simply stated, are the HCP measures and long-term biological goals protective or not? Without this answer, limited guidance if any regarding adjustments for Phase II will be available which would likely be viewed as unacceptable for HCP continuance. These overarching questions cannot be answered alone by the applied research proposed for the HCP, but the knowledge gained through this applied research will be used in conjunction with information gathered via the aforementioned monitoring and restoration activities to drive the mechanistic ecological model (HCP Measure 6.3.3), and thus, to assess the questions regarding potential HCP effectiveness.

The main focus of the applied research will be to evaluate the effects of low-flow on Covered Species and their habitat. Considering the Phase I schedule and the need to first get this facility designed and constructed, it is likely that only five years will be available for Phase I experimentation in the actual experimental channels. As such, key questions will need to be addressed during this time period, which will require a strict schedule and intense focus. During the design and construction period, several studies using existing NFH&TC facilities will be initiated to inform the AMP as well as future studies in the research channels. As described in the HCP (6.3.4.2), the applied research at the NFHTC facility for Phase I will focus on the fountain darter relative to Comal (although research should be transferable to the San Marcos system) and the Comal Spring riffle beetle, as these are the two species with the greatest potential for impact relative to the Phase I package. This applied research will be further divided into three tiers. Tier A will focus on habitat requirements and responses; Tier B will focus on direct impacts of low-flows; and Tier C will investigate the implications of the timing, frequency, and duration of multiple events in varying sequences and include specific research efforts designed to assess ecological model predictions (e.g., model validation).

Target for 2013: The target for 2013 is to complete detailed landscape and construction designs for the applied research channel(s) and initiate construction during the later portion of the

year. This schedule will be driven by any Year 0 activities conducted to support design activities. Additionally, Tier A research will be conducted including 1) the effects of flow, temperature, and CO₂ on the development and subsistence of native aquatic vegetation over extended periods of low-flow conditions, and 2) how low-flow conditions and changing water quality might affect fountain darter food sources (aquatic macroinvertebrates).

Protocol: As described above, the main focus in 2013 will be to get the research channel(s) designed and under construction, along with initiating applied research at the NFH&TC with existing facilities.

- Design and construction plans - Although a conceptual design is currently envisioned for the applied research channels, a formal landscape and construction design will need to be prepared during winter/spring of 2013 or sooner (Year 0 activities are presently proposed to jumpstart the design process). Either way, a series of meetings/workshops with scientists will be conducted in Year 0 or January 2013 (if Year 0 activities are not funded) to solicit input for design components that will be necessary to conduct the research outlined in the HCP within the operational constraints of the NFH&TC. From those meetings/workshops a preliminary design will be completed for review by the HCP Implementation committee in early 2013 or sooner. Following review and comments, a final construction plan will be prepared in Spring 2013 or sooner.
- Initial construction - Upon receipt of the final construction plans, on the ground activities will be initiated in 2013 to start modification of existing NFH&TC facilities where necessary and start construction on new research channel components to the degree supported by the remaining 2013 funds. The primary focus of 2014 will be to complete the construction phase of the research channel(s).
- Applied Tier A research to be conducted with existing NFH&TC facilities is an independent task relative to design and construction and will be initiated starting in January 2013. Several of the studies proposed can be conducted simultaneously depending on available space within the NFH&TC greenhouse and ponds. Tier A studies proposed for initiation in 2013 are described below. Final details and methodologies for 2013 studies will be vetted with a group of scientists familiar with the Comal Springs ecosystem and biostatisticians (where appropriate) and modified from the below descriptions as deemed appropriate.
 - *Laboratory versus field comparison of flow conditions for native and non-native aquatic vegetation.* The specific purpose of this study is to test flow velocity as the driving variable in aquatic vegetation growth as well as investigate the nuances between laboratory and in-situ results. For this evaluation, potted plants including replicates for *Ludwigia*, *Cabomba*, *Sagittaria*, and *Hygrophila* will be placed in up to four select flow conditions (eg. Zero flow, 0.5 gallons per minute [gpm], 2 gpm, and 5 gpm) within the laboratory, and then replicated within similar velocity fields in the Old Channel. Potted plants will be used in

both the laboratory and field investigations. Water quality conditions (dissolved oxygen, temperature, pH, conductivity, and CO_2) measured within the Old Channel study area will be replicated within each of the laboratory flow treatments via the use of heater/chiller units, aerators, etc.. To the degree possible, plants will be obtained from the NFH&TC greenhouse and/or raceways. Any plants collected from the Comal system will be treated and held as per NFH&TC protocol to ensure all snails and other organisms are removed prior to placement in experimental tanks. All species will be placed in the containers to be used during experimentation and moved into tanks prior to initiation of the experiment for acclimation and observation. Only plants of similar length with green, crisp, unbroken leaves will be used. In addition, plants with observed reproductive structures will not be selected. At the beginning of the experiment, five plants from each species will be dried and weighed to get a "before-treatment" biomass measurement. Since the biomass of experimental plants cannot be measured before the experiment, similar sized plants will be used to estimate the mean before-treatment biomass. These plants will be placed in the same conditions in the acclimation tank prior to drying and weighing. The study will be conducted for a period of six weeks. During this time frame, water quality parameters and depth/velocity will be measured three times per week within each treatment. At the end of the six-week study period, growth of each species will be measured as changes in total, above-ground, and below-ground biomass (dry weight in grams for all plants).

- *Closed system pH drift experiment to evaluate bicarbonate utilization of *Hygrophila*, *Ludwigia*, *Sagittaria*, and bryophytes under CO_2 -stressed conditions.* Bicarbonate use of Texas wild-rice has been previously studied by Power and Doyle (2004), but no information has been collected on other important native or non-native vegetation from these systems. Bicarbonate utilization under CO_2 stressed conditions can serve as an indicator of the tolerance that a plant may have under extreme low-flow conditions. Understanding the tolerance of aquatic vegetation to environmental conditions is a critical step in predicting system response to low-flows. In particular, *Hygrophila* is targeted for non-native removal in the AMP but will likely remain in each system at some capacity. Considering that *Hygrophila* may be extremely tolerant and the fact that it does provide suitable habitat for fountain darters, additional study of low-flow tolerance of this species is warranted. As such a pH drift study following the same protocols of Power and Doyle (2004) will be performed using this non-native and the aforementioned native species.
- *Low-flow thresholds evaluation of native and non-native aquatic vegetation conducted in aquaria and ponds.* This study will focus on the effects of low-flows on water temperature, CO_2 , and algal buildup on aquatic vegetation. The

initial trials will be conducted within the greenhouse, followed by a revised trial using potted plants within one of the available NFH&TC ponds. An initial laboratory experiment will use replicates of *Ludwigia*, Bryophytes (*Amblystegium* and *Riccia*), and *Comal Vallesneria*. Extreme conditions anticipated to be expected during a repeat of the drought of record (or worse) will be simulated over time to investigate the threshold at which, and the rate at which, each species begins to decline and ultimately die. Replication and starting plant numbers will be sufficient to sacrifice individual plants over time to evaluate decay rates relative to biomass. A follow-up laboratory experiment using a larger variety of aquatic vegetation (e.g. replicates of *Ludwigia*, *Sagittaria*, Bryophytes [*Amblystegium* and *Riccia*], Texas wild-rice, *Cabomba*, *Hygrophila*, and *Comal Vallesneria*) will be conducted. Finally, during summer 2013, a NFH&TC pond will be used to conduct a summer time experiment to evaluate minimal or no flow conditions over time and the effects on aquatic vegetation growth at a larger scale.

- *Laboratory study to evaluate the effects of low-flow on amphipod populations within select aquatic vegetation types.* This experiment will be built upon the results obtained from the aquatic vegetation studies described above and the aquatic macroinvertebrate sampling as part of the Variable Flow study during spring 2013. As such, the first step in this study will be to complete the formal study design in late spring 2013. It is anticipated that aquatic vegetation most suitable for amphipod growth and survival (e.g. bryophytes, *Ludwigia*, *Hygrophila*, and *Cabomba*) will be used for testing. Sustainable populations of amphipods will be established in each aquatic vegetation type, followed by a series of experiments regarding low-flow and/or changing water quality to evaluate impacts to amphipod populations.

Allocated funds for 2013: \$750,000

Estimated 2013 Budget broken down per activity:

- Design and construction plans
 - Total Cost: \$150,000
- Initial Construction
 - Total Cost: \$350,000
- Tier A applied research
 - Total Cost: \$250,000 - costs include labor and non-labor expenses, and operational costs associated with water and electricity use to support the specific study activities.
 - Laboratory vs. field comparison
 - \$57,500

- pH drift experiment
 - \$17,500
- Low-flow thresholds evaluation
 - \$115,000
- Food-source threshold study
 - \$60,000

Allocated Amount for 2013	\$750,000
• 2013 Budget for Design and construction plans	\$150,000
• Less amount advance for conceptual design under Zero Year Work Plan	\$25,000
Total Amount Available in 2013 ¹	\$725,000

¹ The \$25,000 advanced by EAA in 2012 will be reimbursed to EAA out of Design and Construction subcategory leaving \$125,000 for design work in 2013 and \$725,000 in the Allocated Amount for 2013.