NARRATIVE & BUDGET

I. **Fountain Darter** HCP Measure 5.1.1 San Marcos NFHTC, Uvalde NFH, and Inks Dam NFH-Refugia

Long-term Objective: A series of refugia, with back-up populations at other facilities, will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event such as the unexpected loss of springflow or a chemical spill.

Target for 2013: House and protect adequate populations of Covered Species and expand knowledge of their biology, life histories, and effective reintroduction techniques.

Protocols:
1. *Genetics*-In order to ensure that conservation goals are met in accordance with the regulatory and guiding conservation documents (e.g., 1996 USFWS; EARIP HCP 2011) additional and potentially routine genetics analyses are needed to ensure that fountain darter populations held at the NFHTC refugia represent the wild population in both allelic richness and diversity. More specifically, as refugial stocks decline and are replaced genetics will need to be evaluated to ensure that the held populations are appropriate. Additionally, there is a significant need for a genetic management plan for the fountain darters that details collection procedures (i.e., specific proportion of individuals held from specific collection locations and the sequence of salvage efforts), molecular data collection protocols, pedigree reconstruction, relatedness estimation, and recommendations for specific fish crosses annually under various instream flows. The genetic management plan should highlight or recommend specific NFHTC efforts aimed at: 1) minimizing mean kinship, 2) limiting inbreeding in the captive population, and 3) equalizing founder representations held in refugia with the ultimate goal to maximize the captive effective population size. Finally, the hatchery genetic management plan should highlight genetically appropriate stocking protocols for both the San Marcos and Comal rivers.

2. *Maintain Populations Held in Refugium*-We propose that partial support be used to purchase, install, maintain, and upgrade equipment within "Holding House" located at the NFHTC that isolates and houses various stocks from the upper, middle, and lower San Marcos and Comal rivers (see 1996 USFWS for exact collection locations). In addition, we propose that work continue examining the survival and growth of fish held in refugium, including health and physiological diagnostics. This work is critical to the quality of mature adults maintained that intuitively will in turn result in high quality offspring. More specifically, work will include rigorous examination of parasite treatments as well as monitoring and evaluation of trematode loads *in situ* and effects on reproductive success. Work will continue with the reovirus such as *in situ* prevalence and its effect on reproductive success, specifically examining maternal inheritance and its implications on egg health and offspring survival. Work will continue on diet development in relation to broodstock growth rates and reproductive success. We also propose to continue work

examining holding facility conditions on broodstock survival and reproductive success, specifically the implications of flow rates and pond rearing on bioenergetics and reproductive success.

3. *Optimize Culture*-In order to effectively maintain fountain darter condition and genetic diversity while simultaneously reducing costs, we propose to initiate pond culture. Although fish can be held within tank systems holding them in ponds may provide the capacity to hold and produce far more individuals than is currently possible. This is of critical importance given that theoretical models suggest that 750 to 1,000 individuals be held in refugia in order to curtail the loss of genetic diversity if restocking is an ultimate goal. Modeling suggests that most of the heterozygosity in the NHFTC population is lost early (in the first 5-15 generations) depending upon the refugium population size and the population growth rate. This result reflects the fact that we assume the populations continue to grow at a steady rate. If growth is slower or declines, the loss of heterozygosity will be greater. Second, the loss of heterozygosity is lowest when the growth rate is largest. Less than 4% of heterozygosity is lost in the smallest refugium population (N = 300) if the population doubles each generation until reaching carrying capacity in the wild (N = 200,000). This result suggests that small refugium populations may be adequate provided the re-introduced population grows rapidly. On the other hand, over 8% of the heterozygosity is lost in the smallest population if the population growth rate is 25% per generation. Third, the variation in loss of heterozygosity is lowest (1.0-2.5% for growth rates of 1.25-2.0%) for the largest refugium population (N = 1,000). This result indicates that the larger refugium populations in this analysis are influenced less by growth rate of the re-introduced population than are the smaller refugium populations. As a result of these scenarios, we propose to stock varying densities of genetically appropriate individuals in replicate ponds. Multi-locus DNA genotype information from the brookstock will be used to estimate genetic change. The selectively-neutral, DNA markers will be used to determine the amount of genetic change associated with captive rearing of wild-caught fountain darters, spawning, and subsequent hatchery rearing of their hatchery origin progeny.

Allocated funds for 2013: $50,000

Estimated Budget: $50,000

a. Genetics - Sample hatchery stocks (HS) and wild stocks (WS), genetic analyses via 29 microsatellite loci. Compare diversity of HS vs. WS $20,000
b. Hatchery Genetic Management Plan - Assemble Core Team Draft Outline $2,500
c. Maintain hatchery populations - (Misc. research supplies; nets, PVC) $2,500
d. Optimize culture - Collect specimens, maintain pond systems, collect genetic samples, compare pond populations to HS and WS $25,000

II. **Texas Wild Rice** HCP Measure 5.1.1 San Marcos NFHTC, Uvalde NFH, and Inks Dam NFH-Refugia

Long-term Objective: A series of refugia, with back-up populations at other facilities, will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event such as the unexpected loss of springflow or a chemical spill.

Target for 2013: House and protect adequate populations of Covered Species and expand knowledge of their biology, life histories, and effective reintroduction techniques.

Protocol:
1. *Genetics*-Since these original collections were established genetic analyses has revealed that allelic diversity appears to be concentrated in relatively large, demographically stable stands; whereas, stands smaller than $2m^2$ contributed no unique alleles (Richards et al. 2007). Although these original collections have been maintained as individual specimens, specific individuals that were collected from small (less than $2m^2$) ephemeral stands, have succumbed to mortality as the plants have aged. In addition, the spatial distribution and sizes of various stands of Texas wild rice *in situ* appear to have changed since the last genetic evaluation was conducted over 10 years ago. In order to ensure that conservation goals are met in accordance with the regulatory and guiding conservation documents (e.g., 1996 USFWS; EAA 2011) additional and potentially routine genetics analyses are needed to ensure that stock plants held at the NFHTC refugia represent the wild population in both allelic richness and diversity. It is also imperative that genetic analyses also be conducted to evaluate how the current wild stands and refugial stock compare to the historical estimates of genetic diversity as reported by Richards et al. (2007). Genetic analyses of current refugial and wild stock plants will be completed by collecting leaf tissues from representative plants from all stocks for shipment to an appropriate laboratory. Individual samples will be genotyped. Comparisons to Richards et al. (2007) reported data will be completed for both wild and refugial stocks using genetic indicators such as gene diversity, observed heterozygosity, and loss of heterozygosity. More specifically, genetic analyses will detail what current and historical locations (i.e., stands) are or are not represented by the refugial population at NFHTC and wild stands. Additionally, there is a significant need for a genetic management plan for the Texas wild rice refugial population that details molecular data collection, pedigree reconstruction, relatedness estimation and recommendations for specific plant crosses annually under various instream flows. The genetic management plan should highlight or recommend specific NFHTC efforts aimed at: 1) minimizing mean kinship, 2) limiting inbreeding in the captive population, and 3) equalizing founder representations held in refugia with the ultimate goal to maximize the captive effective population size. This includes the establishment of a working group for the Genetic Management Plan. The working group would be charged with defining goals and components that are to be included in the plan as well as editing and revising the final draft prior to submission and review by the USFWS.

2. *Maintain Populations Held in Refugium*-We propose that partial support be used to purchase, install, maintain, and upgrade equipment within "GreenHouse" located at the NFHTC that isolates and houses various stocks from the upper, middle, and lower San Marcos (see 1996 USFWS for exact locations). In addition, we propose that work continues to examine the survival and growth of plants held in refugium. More specifically, work will include rigorous examination of thermal, flow, and CO_2requirements of Texas wild rice and other native aquatic plants. We also propose to continue work examining holding facility

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conditions on broodstock survival and reproductive success, specifically the implications of air temperatures on seed production and reversal of morphology from sexual to asexual.

3. *Optimize Culture*- To meet restoration goals set out by the Edwards Aquifer Recovery Implementation Plan Habitat Conservation Plan (see EAA 2011) native plant propagation practices will be explored to increase progeny production for stocking *in situ*. These practices will employ Texas wild rice stolons and seeds as a means of mass producing genetically appropriate stocks for future restoration and enhancement efforts. This is particularly important since the collection of mature plants from the wild is both costly and fraught with inherent demographic and genetic risks. The use of seeds and stolons provide a number of benefits that include the production of relatively large numbers of plants while simultaneously maintaining genetic integrity of propagated plants since seed production can be controlled and stolons arise from the plant's base asexually (i.e. genetic clones). Mass production of genetically appropriate Texas wild rice and other native plants for future restoration and enhancement efforts as outlined in Edwards Aquifer Recovery Implementation Plan Habitat Conservation Plan. Mass production of plants will entail but not be limited to the collection of wild stock, potting, and grow-out within pond systems. Multiple ponds will be utilized in an attempt to maximize plant production.

Allocated funds for 2013: $50,000

Estimated Budget: $50,000

a. Genetics - Sample hatchery stocks (HS) and wild stocks (WS), genetic analyses via microsatellite loci. Compare diversity of HS vs. WS $20,000
b. Hatchery Genetics Management Plan - Assemble Core Team Draft Outline $2,500
c. Maintain hatchery populations - (Misc. research supplies; pots, soil) $2,500
d. Optimize culture - Collect specimens, maintain greenhouse & pond systems, collect genetic samples, compare pond populations to HS and WS $25,000

III. **Comal Springs Riffle and Dryopid Beetles and Peck's Cave Amphipod** HCP Measure 5.1.1 San Marcos NFHTC, Uvalde NFH, and Inks Dam NFH-Refugia

Long-term Objective: A series of refugia, with back-up populations at other facilities, will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event such as the unexpected loss of springflow or a chemical spill.

Target for 2013: House and protect adequate populations of Covered Species and expand knowledge of their biology, life histories, and effective reintroduction techniques.

Protocol:
Genetics - Pecks Cave Amphipod and Comal Springs riffle beetle have had some preliminary genetics work done with wild-caught organisms; however, captive propagation has not been adequate to provide genetic material of captive-bred juveniles or adults. Wild Peck's cave

amphipods had an even distribution of haplotypes while Comal Springs riffle beetles had an uneven distribution. Although no genetics work has been done on the dryopid beetle, wild populations will be evaluated using genetics. We will produce advanced stages of all these species to compare genetics of captive-bred with those of wild-caught organisms. New genetics techniques, involving analyses of millions of short DNA sequences, allow for accurate, high resolution characterization of population genetics. From this, management plans for long-term maintenance of genetically appropriate captive invertebrate populations will be developed. Additionally, population estimates and effects of historic floods and droughts on population genetics (as hinted at by comparative population-level genetic variation) of these and related species will be determined.

Maintain populations held in refugium - All work involves maintenance of refugium populations as per Recovery Plan and as a source for experimental animals. We will collect invertebrates as needed to maintain wild stock Comal Springs riffle and dryopid beetles and Peck's cave amphipods in refugium and an adequate number for maintenance in captivity and research.

Optimize culture - Although reproduction has occurred and young have been reared to reproducing adults, success (survival, growth, reproduction, and hatch) has been somewhat variable and minimal (e.g., only about 0.1% of larval Comal Springs riffle beetles have become potentially reproductive adults). We will try various food types at different life stages to determine best diet, determine optimum density for growth and reproduction, test a variety of cover types and vegetation for different life stages to determine ideal "tank furniture" for survival and reproduction, and determine optimal water quality, flow, and light levels. Optimal culture conditions allow for increased survival, reproduction, and likely promote evenness of genetic contribution from parental stock. Additionally, life history information gained would be useful for development of a sound genetic management plan for the refugium populations.

Reproduction - While reproduction in captivity has occurred for these species, we've had very limited success completing their life cycles. We will test a variety of methods to consistently complete life cycles, a necessity for long-term maintenance in captivity. For Comal springs riffle and dryopid beetles, environment for pupation (e. g., substrate type and moisture, food type and quantity, light levels, temperature) will be determined. For Peck's cave amphipods, environment to minimize cannibalism (e. g., substrate type, screening materials, habitat separation of young and adults) will be determined. Controllable reproduction is a requisite for the development of a functional genetics plan for captive propagation and for potential repatriation.

Temperature effects - No temperature work has been done with these species. We will conduct tests on effects of elevated temperatures on growth, reproduction, survival, and behavior for both species. This information will be useful for culture, for management of wild habitat to maintain appropriate temperatures, and for determining triggers for collection of salvage invertebrates.

Allocated funds for 2013: $475,000

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Estimated Budget: $475,000

a. Genetics - Sample hatchery stocks (HS) and wild stocks (WS), genetic analyses via microsatellite loci. Compare diversity of HS vs. WS for all three species $75,000
b. Hatchery Genetics Management Plan - Assemble Core Team Draft Outline for all three species $50, 000
c. Maintain hatchery populations - (Misc. research supplies, pumps, tanks, chillers etc) $115,000
d. Optimize culture - Collect specimens, maintain systems, collect genetic samples, diet analyses, NATURES rearing $100,000
e. Reproduction - Manipulation of various biotic and abiotic variables believed to influence reproduction and pupation $70,000
f. Temperature effects - Determine thresholds that positively and negatively affect growth, reproduction, behavior and survival $65,000

IV. **San Marcos and Texas Blind Salamanders** HCP Measure 5.1.1 San Marcos NFHTC, Uvalde NFH, and Inks Dam NFH-Refugia

Long-term Objective: A series of refugia, with back-up populations at other facilities, will preserve the capacity for these species to be re-established in the event of the loss of population due to a catastrophic event such as the unexpected loss of springflow or a chemical spill.

Target for 2013: House and protect adequate populations of Covered Species and expand knowledge of their biology, life histories, and effective reintroduction techniques.

Protocol:
Genetics - A recent study on the San Marcos salamander indicated that captive-bred salamanders had less genetic diversity at some loci but similar diversity at others compared to wild-caught salamanders. There has been no work comparing captive-bred Texas blind salamanders with wild-caught salamanders. New genetics techniques, involving analyses of millions of short DNA sequences, allow for accurate, high resolution characterization of population genetics. From this, management plans for long-term maintenance of genetically appropriate captive salamander populations will be developed. We will collect samples for genetic analyses, provide funding for lab analyses and interpretation of the samples, and aid in the development of a practical genetics management plans for captive salamanders. Additionally, population estimates may be possible with the information available from the genetic analyses.

Maintain populations held in refugium - All work involves maintenance of refugium populations as per Recovery Plan and as a source for experimental animals. We will collect salamanders as needed to maintain at least 156 wild stock San Marcos salamanders in refugium and as many Texas blind salamanders as we can passively collect with nets over artesian flow sources for maintenance in captivity and research.

Optimize culture – Although reproduction has occurred and young have been reared to reproducing adults, success (survival, growth, reproduction, and hatch) has been somewhat variable. We will try various food types at different life stages to determine best diet, determine optimum density for growth and reproduction, and test a variety of cover types and vegetation for different life stages to determine ideal "tank furniture" for survival and reproduction. Optimal culture conditions allow for increased survival, reproduction, and likely promote evenness of genetic contribution from parental stock. Additionally, life history information gained would be useful for development of a sound genetic management plan for the refugium populations.

Reproduction - Consistent and predictable reproduction of captive salamanders has been elusive. However, recent work with short-term separation by gender of the Barton Springs salamander has been promising – salamanders are separated by gender for 2 months, combined for 2 weeks, and have reproduced in 10 of 13 separation/combination cycles. We will conduct similar, preliminary 6-month tests with the San Marcos and Texas blind salamanders to attempt to trigger reproduction. If results are positive, we will conduct replicated studies for both species. Controllable reproduction is a requisite for the development of a functional genetics plan for captive propagation and for potential repatriation.

Temperature effects - Preliminary work indicated that elevated temperatures may be detrimental to San Marcos salamanders but no work has been done with Texas blind salamanders. We will conduct tests on effects of elevated temperatures on growth, reproduction, survival, and behavior for both species. This information will be useful for culture, for management of wild habitat to maintain appropriate temperatures, and for determining triggers for collection of salvage salamanders.

Allocated funds for 2013: $150,000

Estimated Budget: $150,000

a. Genetics - Sample hatchery stocks (HS) and wild stocks (WS), genetic analyses via microsatellite loci. Compare diversity of HS vs. WS for all three species
$50,000
b. Hatchery Genetics Management Plan - Assemble Core Team and Draft Outline for all three species
$25, 000
c. Maintain hatchery populations - (Misc. research supplies, pumps, tanks, chillers etc)
$25,000
d. Optimize culture - Collect specimens, maintain systems, collect genetic samples, diet analyses, NATURES rearing $20,000
e. Reproduction - Manipulation of various biotic and abiotic variables believed to influence reproduction and egg viability
$15,000

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f. Temperature effects - Determine thresholds that positively and negatively affect growth, reproduction, behavior and survival $15,000

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