- The model should be capable of simulating the dynamics of both terrestrial and aquatic ecosystems, at the appropriate time scales, and integrate both types of ecosystems on a landscape-scale where appropriate.
- The model should be capable of including plant, animal, hydrological, climatic, and management variables, and simulating interactions among all of these components.
- The model should be capable of simulations on spatial scales ranging from 1 m^2 to the entire Edwards Aquifer region.
- The model should be capable of being linked to the groundwater model(s), so that simulations can be conducted for integrated surface and groundwater systems.
- The model should be sufficiently flexible that changes in algorithms can be made as needed, based on new data and improved understanding of the ecological dynamics of the Comal and San Marcos ecosystems. Revisions to the parameter values used in the model data base should be possible in a user-friendly manner such that routine upgrades to this data base can be made as additional site-specific data become available.
- The model should be capable of being run on commercially available PC hardware and using commercially available software operating systems.
- The model should have a history of producing accurate (80-90 percent) simulations of ecological dynamics in groundwater-influenced ecosystems, as demonstrated by field validation studies.
- The model should have user-friendly interfaces such that it can be used by a range of experienced personnel, upon completion of some degree of specific training on the use of the model.

6.3.4 Applied Research Facility Experimental Channel at the USFWS National Fish Hatchery and Technology Center

6.3.4.1 Description of the Applied Research Facility

As discussed throughout this HCP, applied research coupled with ecological modeling is a valuable component of the Phase I package. During Phase I, applied research will be conducted to better understand the ecological dynamics of the Comal system, particularly under low flow conditions. Initially, an on-site research channel at Landa Park in the Comal system for conducting these experiments was considered to limit costs and maximize effectiveness. BIO-WEST (2011c). However, an appropriate site could not be obtained in the Comal ecosystem. Accordingly, an applied research experimental facility will be constructed at the USFWS National Fish Hatchery and Technology Center (NFHTC) in San Marcos, Texas. The NFHTC has the existing infrastructure (Aquifer exempt wells, ponds, containment areas, recirculation and reuse capabilities, etc.) to allow for construction and operation of an applied research facility to inform Phase II decisions regarding the Covered Species and, to the extent possible, adjustments to conservation Measures during Phase I.

Although termed "applied research facility," the conceptual design is a series of man-made channels with earthen substrate intertwined with the existing ponds available at the NFHTC. This will allow water use and reuse through the plumbing already in place while allowing the flexibility to pump water through several research channels for experimentation. To recreate the natural environment to the extent possible, considerable effort will be needed to simulate channel configuration, substrate, instream debris, riparian zone structure (trees, shrubs, grass), aquatic vegetation, and other natural and anthropogenic conditions present in the Comal River. These components will be carefully designed and constructed to provide the most authentic simulation practicable. A riffle beetle upwelling and spring run area (similar to that proposed in BIO-WEST 2011c) will be created at the headwaters of two of the research channels.

The EAA will support and coordinate the NFHTC's construction and maintenance of the Applied Research Center. EAA will contract for the research activities in the Applied Research Center identified in this Section or developed as part of the AMP. The Program Manager will coordinate, supervise and oversee the implementation of all such research.

6.3.4.2 Research in the Experimental Channels

The main focus of the research channels will be to evaluate the effects of low-flow on Covered Species and their habitat. This evaluation will include springflow conditions that bracket the range of 5 cfs to 100 cfs. Considering the Phase I schedule and the need to first get this facility designed, permitted, and constructed, it is likely that only five years will be available for Phase I experimentation. As such, key questions will need to be addressed during this time period, which will require a strict schedule and intense focus. 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 low-flow impacts directly on the fountain darter and Comal Springs riffle beetle; 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). The experimental design for the research will be prepared prior to the initiation of the reseach. The experimental design for the research will receive input from the Science Committee prior to its initiation and on issues that arise during the conduct of the reasrch. (FMA Section 7.13.2),

Tier A – Fountain Darter Habitat and Food Supply

- ***Low-flow effects on native aquatic vegetation***

A key unknown is the tolerance of native aquatic vegetation to reduced flow conditions in these systems. The timing and duration of these low-flow events will be studied relative to the native vegetation, starting with the plant species identified in the long-term biological goals for the fountain darter. Decay of the above ground and below ground biomass will be measured over time. Above ground biomass is important for Covered Species habitat while below ground biomass is critical for root establishment and holding the plant in place during any subsequent pulse event. Water quality will be continuously measured to evaluate the before, during, and after effects of vegetation decay on water temperature, dissolved oxygen, carbon dioxide, and pH. Additional water quality parameters such as nutrients may also be studied. In addition to studying the effect of vegetation decline, decay and ultimately death, studies will be designed to evaluate recovery of native vegetation following various stages of aquatic vegetation decline and decay.

- ***Low-flow effects on macroinvertebrates (fountain darter food source)***

Another critical component of fountain darter habitat that is presently unknown is the relationship of macroinvertebrates (fountain darter's main food source) to low-flow conditions. Studies will be designed to evaluate the simulated effects of changing water quality conditions and aquatic vegetation composition on the macroinvertebrate (mainly amphipods) community. It may be that the amphipods are affected much earlier than actual vegetation decline or decay which would mean impacts to the darter from reduced food supply could potentially occur prior to even vegetation decline. Conversely, it may be that decomposing vegetation provides ample habitat for macroinvertebrates to the point of near vegetation death and as such the food source would not be the limiting factor to the fountain darter during periods of extremely low flow. Similar to the aquatic vegetation study, not only will simulated impacts be assessed during extended periods of simulated low flow, but recovery following these periods will be studied to learn response time (amphipod recovery) following a severe event.

Tier A – Comal Springs Riffle Beetle Habitat Associations and Movement

- **Effects of flow levels on Comal Springs riffle beetle movement**

Upon completion of the artificial upwelling and spring run habitat within the created channel, Comal Springs riffle beetles will be collected from the wild and introduced into the artificial habitats. The first step will be to assess the survival success of adults. Once an adult population is established, flow manipulations will be performed to study the affinity of riffle beetles to flow and to track movement from surface to subsurface habitats and vice versa. The immediate goal is not to establish a reproducing riffle beetle population but to evaluate movement patterns of riffle beetles during periods of varying springflow. This study will be complicated by uncertainties in the ability to replicate food sources for the riffle beetle similar to what is experienced in the wild, so considerable trial and error is likely.

- **Extended Low-flow period effects on Comal Springs riffle beetles**

Once a population is established in the experimental habitat, extended periods of low-flow will be tested to evaluate the effect of these periods on riffle beetle survival and habitat use. Surface habitat will be completely removed for extended periods of time, water quality will be altered to simulate extreme conditions, and other factors adjusted (*e.g.*, reductions in leaf material or detritus, etc.) to simulate conditions that might be experienced in the wild during these conditions. As with other proposed Tier A efforts, recovery following impacts will also be investigated.

- ***Test spring run connectivity***

Once a population is established and the above two Tier A riffle beetle studies performed, the concept of spring run connectivity will be tested. This will involve simulating subsurface habitat cutoff from surface habitat and riparian detritus, and subsurface habitats that are connected to surface habitats via the trickling of water across the surface habitat. This is a key study to assess the value of this concept as an additional protection measure in Spring Run 3 of the Comal system as discussed in BIO-WEST (2011).

Tier B – Direct Impacts to Covered Species

- ***Low-flow effects on fountain darter movement, survival, and reproduction***

A series of low-flow experiments with various timing and durations will be evaluated while examining direct impacts to fountain darters. A whole host of questions can be addressed under this topic with just a few examples including:

- when and where do darters move as vegetation decays and water quality deteriorates;
- when does reproduction stop or does it;
- does compensatory reproduction get triggered, and if so, when and what causes it; and
- what is the effect of predation on fountain darter population size?

Since the fountain darter is a visual predator, and turbidity from stormwater run-off and recreational activities both increase turbidity, behavioral impacts of the fountain darter under different turbidity levels will also be examined in relation to feeding success. An endless number of scenarios are available to discuss under this heading which highlights the importance of a focused study design and schedule.

- ***Low-flow effects on Comal Springs riffle beetle survival and reproduction***

A series of low-flow experiments with various timing and durations will be evaluated while examining direct impacts to Comal Springs riffle beetles. A core question is: when are reproduction and survival compromised as physical habitat (surface and subsurface) declines and water quality deteriorates? The reproduction component assumes that a reproducing population can be established in the study habitat during Phase I. If a reproducing population is successfully established, this flow manipulation research could be expanded to include evaluation of desirable and threshold environmental conditions for larval and pupae stages.

Tier C – Testing repeat occurrences of low-flow or combination of effects.

- ***System Memory***

Upon completion of Tier A and B studies certain components and parameters will likely show impacts and some will not. Tier C is designed to take those components or parameters that do show impacts at varying springflow levels and to evaluate potential additive effects of repeat occurrences. As with all other studies, careful study design will be needed to maximize the efficiency of any system memory studies.

- ***Ecological Model Validation***

Existing information and data gathered during Tiers A and B applied research and through continued ecological monitoring and on-site studies will be entered into the ecological models developed for these ecosystems. Towards the end of Phase I, specific studies will be designed and conducted to test the validity of ecological model results. This may involve simple or complex parameters and single or multiple low-flow events depending on Phase II questions that may be relevant at that time.

Regardless of what Tier is involved, to be useful, studies will need to be designed to achieve an endpoint that can provide input to the ecological model or directly answer specific questions for the Phase II decision-making process or refinement of Phase I measures.

6.3.4.3 Additional Studies

Additional physical habitat activities/studies will be performed in the field. The following activities will be conducted within the Comal and San Marcos systems as part of the implementation of minimization and mitigation measures. Although not specifically covered under Applied Research at the NFHTC these activities have the potential to directly influence study design at the applied research facility and, thus, are included to close this section.

Aquatic Vegetation Restoration and non-native plant removal

- Evaluate transplant methodologies for various types of native aquatic vegetation
- Evaluate success of transplants over extended time period
- Evaluate methodologies for removal of non-native plants
- Track maintenance required to keep non-native species from re-establishing

Old Channel ERPA

- Evaluate the need for channel manipulation for the enhancement of fountain darter habitat in the Old Channel. (Section 5.2.2.1).

Other biological interaction studies such as an evaluation of non-native animal species interactions with the fountain darter or gill parasite/snails/fountain darter interactions cannot be conducted at the NFHTC and thus will also be tied directly to on-site activities associated with those HCP conservation measures.

6.3.5 Texas Wild-Rice Enhancement

As discussed in Sections 5.3.1 and 5.4.1, restoration and enhancement of Texas wild-rice will be conducted during Phase I of the HCP. Initially, these activities will involve an applied research component. Methods for Texas wild-rice enhancement will need to be investigated to understand the potential for increased areal coverage of Texas wild-rice through implementation of this measure. Non-native vegetation mixed in with Texas wild-rice or surrounding existing Texas wild-rice plants but still located within optimal habitat areas will be removed to see if areal coverage of Texas wild-rice will expand in those areas. The specific areas chosen for evaluation will include only areas that would be suitable over the full range of discharges between the long term average and Phase I minimum flows. Once proven successful or not, this information can be beneficial for the Strategic Adaptive Management Decisions.

6.3.6 Monitoring and Reduction of Gill Parasites

A major concern in the Comal Springs ecosystem is the continued presence of an Asian trematode, *Centrocestus formosanus*. This parasite was first discovered on fountain darters in the Comal River during October 1996. The parasite attaches to the fish's gill filaments causing extensive gill tissue proliferation and damage (Mitchell *et al*. 2000) with mortality in the wild being reported following the discovery in 1996 (Tom Brandt, USFWS, personal communication). A non-native snail, *Melanoides tuberculatus*, that has been in central Texas since 1964 (Mitchell *et al*. 2005) has been confirmed as its central Texas first intermediate host (Mitchell *et al*. 2000). Parasite monitoring via examination of presence on fountain darter gills to determine *C. formosanus* levels in the Comal River has been ongoing since the late 1990s by the USFWS, Texas State University, and BIO-WEST (EAA Variable Flow Study).

Through the EAA Variable Flow Study monitoring, the USFWS NFHTC sampled three sites in the Comal River during two sampling periods; first during 2006–2007, and again during 2009–2010. Two of the sites were located in the Upper Spring Run reach, and the third site was located downstream of Landa Lake in the Old Channel of the river. A significant decline in cercarial density was observed between the first and second sampling periods. Abiotic factors, such as total stream discharge and wading discharge, did not change significantly ($p>0.05$)[1] between sampling periods. Abiotic factors do not adequately explain the observed long-term decline in cercarial density (Johnson *et al*. 2011). Johnson *et al*. (2011) speculates that observed decline over time is likely a reflection of the typical pattern followed by most invasive species as they gradually become integrated into the local community following an initial explosive growth in population. Johnson *et al*. (2011) concluded that although cercarial densities may be abating, fountain darters in the Comal River are still threatened by the parasite, and conservation efforts will focus on reducing levels of infection pressure from the parasite whenever possible.

[1] Statistical level of significance.

Informal observations suggest that the density of *C. formosanus* cercariae in the water column increases as stream discharge decreases and vice versa (T. Brandt, USFWS, personal communication), but there has been little definitive proof of this. If this relationship does exist between *C. formosanus* cercariae and discharge in the Comal River, there are concerns that increased levels of infection pressure would exacerbate the other stresses of low-flow periods on the fountain darter. Elimination of the parasite from the river probably cannot be accomplished. However, a possible practical approach to managing the parasite in the Comal River might be to control the parasite's snail host, *M. tuberculata*. USFWS and U.S. Environmental Protection Agency (EPA) authorizations to use chemicals known to be lethal to the snail likely cannot be obtained for the Comal River. Therefore, alternative methods need to be explored for decreasing abundances of *M. tuberculata* and the associated parasite.

In 2010, the EARIP funded a study (USFWS NFHTC and BIO-WEST 2011) to determine the effectiveness of *M. tuberculata* removal by physical methods on lowering drifting gill parasite numbers in the Comal River. The results from the study support the hypothesis that removing *M. tuberculata* from the Comal River correlates with a decrease in *C. formosanus* in the water column. These results support *M. tuberculata* control as an important HCP measure. However, there are several management and research questions still unanswered that may play a role in snail/parasite control and the relationship between the snails and the cercariae they release. The following activities to address these uncertainties will be conducted.

The initial activity will be the evaluation of alternative methods for snail removal so that removal can be accomplished in the most effective, yet least destructive manner. The second activity deals with understanding the magnitude of snail removal necessary to affect downstream cercaria concentrations in the water column. Once the magnitude of snail removal for effective control of water column cercaria is identified, a study is necessary to evaluate the long-term benefits of that removal. For instance, it is important to understand if the snails repopulate the area within a short period of time and cercaria concentrations quickly return to near original levels, or if both snail populations and cercaria counts stay suppressed for an extended period of time.

Additionally, although cercarial densities may be abating in the Comal system (Johnson *et al*. 2011), *C. formosanus* still poses a threat to fountain darters in the Comal River, especially during low-flows. As such, continued monitoring is essential and the following activities are included within this HCP conservation measure:

- A system-wide survey of snail population density and cercarial concentrations will be conducted to provide a baseline condition;

- Based on that system-wide survey, a decision will be made following the process set out in the AMP Agreement as to whether an initial system-wide removal effort is necessary, and if so, how to facilitate the performance of that effort;

- Based on the system-wide survey, a gill parasite monitoring program will be designed and implemented. Cercarial concentrations will be monitored in multiple areas along the Comal River on at least a semi-annual basis, and more frequently when spring flow drops initially below 150 cfs or other springflow triggers that are developed. Corresponding fountain darter sampling to examine correlations between cercariae densities and fountain darter impacts in the wild will also be part of that monitoring effort.

6.4 Core Adaptive Management Actions

This section outlines the AMP actions to protect habitat and populations of Covered Species in both the Comal and San Marcos Springs/River ecosystems in the event of limited recharge.

6.4.1 Risk Assessment, Estimation of Take, and Drought of Record

Because biological data typically has great variability and there are many habitat and population parameters that potentially affect the population dynamics of a species, it is very difficult to assess the threat of extirpation. In any natural setting, the unpredictability of the effects of an individual event (*e.g.*, extended low flow period) are often highly correlated with conditions immediately prior to the event complicating development of target conditions necessary to maintain habitat. For the AMP outlined here, a range of parameters is used to assess biological risks associated with deviating from the objectives set forth in Chapter 4 above for the individual species.

Although protection of springflows to minimize the level of take is incorporated into the Phase I package, it is possible that conditions may reach or exceed the level of take during a repeat of conditions similar to those in the drought of record. This plan provides a framework for addressing such conditions, if they should occur, by providing measures to mitigate effects of such droughts on the species. The Phase I package should limit the time at and magnitude of impacts, but in the event that discharge falls to these levels, additional measures need to be in place to monitor changes closely and further protect habitat and the Covered Species.

The AMP proposes a conservative approach that incorporates regular biological monitoring before and after and frequent monitoring during such events. It is important to accurately define dynamic ecosystem conditions prior to the onset of a limited recharge period to assess potential threats during an extended period under those conditions. Biological monitoring during a period of declining spring discharge will permit a close examination of actual population and habitat conditions when flow declines to or below modeled levels of concern. This approach differs from the traditional one of establishing one fixed number for total discharge below which the species is at risk and above which it is not. Instead, fixed numbers of total discharge are used to trigger additional sampling and used in conjunction with those sampling results to more accurately define biological risk and population changes. Fixed sampling outcomes coupled with fixed discharge levels elicit specific management responses. This is a more dynamic process that takes into account actual conditions rather than predetermined hypotheses of what conditions might be expected at certain discharge levels based on limited data. It is also anticipated that the ecological modeling discussed above will prove instrumental in projecting potential impacts allowing for informed and timely management decisions.