



United States Department of the Interior

FISH AND WILDLIFE SERVICE

Ecological Services Field Office
10711 Burnet Road, Suite 200
Hartland Bank Bldg.
Austin, Texas 78758

MAY 21 1996

Dear Contingency Plan Cooperator:

The Revised San Marcos/Comal/Edwards Aquifer Rare, Threatened, and Endangered Species Contingency Plan has been finalized and a copy is enclosed.

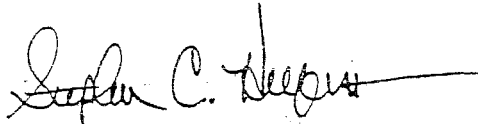
Thank you all for your generosity in discussions, meetings, and reviews. The plan incorporates a great deal of new information, and the Service feels it is workable and provides good guidance. Obviously, the document has been refined and benefitted from the expertise of all those who assisted in its preparation.

Unfortunately, we are facing a scenario of immediate implementation of the plan. Our thanks and appreciation go out to all of you for the support and cooperation you are providing for the implementation of the plan as well.

As we attempt to implement the plan and gain more experience with the protocols recommended, problems, refinements and improvements will appear. Please keep us posted about problems you may encounter and solutions or improvements that occur to you. A simple phone call can provide a great deal of information that will be invaluable in record-keeping and for future amendments or revisions of the plan.

If you have any questions at all please do not hesitate to call Kathryn Kennedy, the contingency plan coordinator or the Service lead for your taxonomic group(s) of concern.

Sincerely,


Field Supervisor

Enclosure

SAN MARCOS/COMAL/EDWARDS AQUIFER
RARE, THREATENED, AND ENDANGERED SPECIES
CONTINGENCY PLAN

SAN MARCOS/COMAL/EDWARDS AQUIFER RARE, THREATENED, AND
ENDANGERED SPECIES CONTINGENCY PLAN

I. Purpose

The purpose of this plan is to outline a strategy for preventing the extinction of listed species dependent on San Marcos and Comal Springs and the Edwards aquifer in the event that the aquifer level drops to a critical level. The species covered in this plan are dependent on aquifer, spring, or springfed aquatic habitats, all ultimately dependent on adequate water levels maintained in the Edwards aquifer. Even with normal rainfall and recharge, aquifer levels are likely to decline given the current and projected demand for water by various users. While several strategies and initiatives have been developed (and in some cases implemented) to conserve water and reduce new water demand, aquifer levels and springflows adequate to preserve the habitats of these species are by no means assured. It is possible that a water management system that can preserve springflows will not be worked out in time to prevent the drying up of Comal and San Marcos springs. If state and local governments fail to devise and implement a plan for apportionment of water supplies in such a way that springflows are assured, springflows could cease for an indefinite period of time. The springflows at Comal and San Marcos springs are currently declining and there is a high probability of spring failure in the near future.

This plan should not overshadow or de-emphasize the goal and need to conserve the ecosystem. Efforts to conserve and restore essential habitat need to continue, even as springflows decline. Active measures should be taken to maintain habitats and populations and reduce threats to these species as much as possible to maximize their potential for survival in the wild.

Actions set forth in this plan should be considered a last-ditch effort in emergency situations, as outlined in the San Marcos and Comal and Associated Aquatic Ecosystems (Revised) Recovery Plan (task 2.11). Where captive maintenance, breeding, and reintroduction techniques have been developed, captive or cultivated populations can be valuable tools because they can preserve genetic material to protect against catastrophic loss, and provide material for restoration work and non-destructive research. However, off-site artificially maintained populations cannot alone achieve recovery for the species. The purpose of the Endangered Species Act is "to provide a means whereby the ecosystem upon which endangered species and threatened species depend may be conserved," as well as to provide a program for the conservation of such endangered and threatened species. U.S. Fish and Wildlife Service Recovery Guidance states: "Captive propagation/cultivation may be a useful tool to facilitate recovery of a species in the wild, but is not a substitute for reestablishment of viable wild populations" and "The initiation of significant and costly captive propagation or cultivation programs may be necessary, but should be considered only after all other techniques to maintain or improve a species' status in the wild have failed or are determined as likely to fail."

It cannot be overemphasized that, for many of these species, establishing captive populations does not provide a high certainty of survival. The risk of loss remains high (especially for salamanders and invertebrates). Except for the fountain darter and Texas wild-rice we have little experience with captive propagation. For most of the species consistent, successful captive breeding techniques are not known and may be difficult to develop. In addition, even if the species can be conserved in captivity, real challenges also remain in attempts to restore or reintroduce the species. Reliable restoration and reintroduction techniques are not yet available.

II. Tasks Necessary to Prepare for Implementation of Contingency Plan

Implementation of this contingency plan will require some advance preparation. Written agreements for cooperation and assistance should be secured among all the cooperators for the expertise, staff time, facilities, etc. that each will contribute. All cooperators should secure any needed Federal and State permits and keep them current. Equipment must be obtained so that it is on hand and available in advance. Funding necessary to pay for equipment, maintenance etc. should be identified and secured. Pilot tests of new collection and maintenance protocols should be run. There may be other specific preparation tasks for particular species. These are outlined in the specific sections for particular species. Work on these preparatory tasks should begin immediately so that implementation, if necessary, can proceed smoothly.

III. Tracking Habitat and Species Condition and Triggers for Establishment of Captive Refugia

For each species, responsible parties are identified and a monitoring scenario has been devised to track conditions in the systems as they deteriorate. Expected "triggers" for removing organisms for captive propagation are defined.

Springflows are currently monitored by the U.S. Geological Survey and others, and cooperators will, (upon request), be sent faxes (almost daily) of Comal (and San Marcos when available) springflow discharges from the U.S. Fish and Wildlife Service Austin Ecological Services Field Office (ESFO). The ESFO has Service lead for recovery of these species and will coordinate and work closely with cooperators in implementing this contingency plan.

IV. Contingency Plan Details by Species

Coordination between the ESFO, the San Marcos National Fish Hatchery and Technology Center (Center), Texas Parks and Wildlife Department's Resource Protection Division (TPWD), and other cooperators will be regular and include information transfer on the status of the various efforts (collections, maintenance, recruitment, etc.,) for all subject species.

FOUNTAIN DARTER (Etheostoma fonticola)

The fountain darter's entire range is the Comal River and upper San Marcos River. The baseflow of these rivers consists entirely of springflows. As springflows and associated habitats decrease, the risk of fountain darter extinction in the wild increases. To minimize the risk of extinction, captive stocks should be established and maintained.

This contingency plan describes tasks needed for *ex situ* (out of the wild) conservation efforts. Other efforts should be made to manage habitats (for example, flow to the old channel of the Comal River) and darter populations in the wild as best they can given available springflow.

Adequate numbers of fountain darters should be removed from the wild to establish a genetically representative founding broodstock. Captive stocks should be maintained to conserve high genetic diversity. Cultural methods for fountain darters, to a large extent, have been developed by the San Marcos National Fish Hatchery and Technology Center (Center). Over the past several years, the Center's staff have developed methods for captive propagation of fountain darters and have invaluable knowledge that will be made available to other cooperators providing refugia for fountain darters. Provisions for the transfer of this information to other refugium facilities are needed and should proceed soon.

The Center: (1) has the staff expertise and prior experience; (2) is capable of providing near natural water conditions; (3) is logistically well positioned, being close to both spring systems/habitats; and, (4) will serve as the primary refugium and will be the facility conducting actual breeding of stock fish to maintain genetic variability through time. Uvalde National Fish Hatchery (Uvalde) will serve as a back-up facility maintaining additional stocks of fish, as needed.

Coordination between the ESFO and the Center is needed to confirm the Center and other facilities are continuously ready to receive specific stock(s). Below is a description of the areas targeted in the wild for each captive stock formation (see Figures 1 and 2):

- (1) Upper Comal = Landa Lake and upper part of new channel of Comal River, generally, exclusive of the four spring runs; ESFO sections 1, 2, 3, 4, 5, 8, & 9.
- (2) Lower Comal = Comal River, including old channel below Landa Lake and new channel below hydropower dam downstream to confluence with Guadalupe River; ESFO sections 10, 11, 13, 14, 14a, 14b, 15, 16, & 17.
- (3) Upper San Marcos = headwaters of the San Marcos River, Spring Lake; San Marcos River below Ice House dam downstream to Rio Vista dam; ESFO sections 1, 2, 3, 4, 5, & 6.
- (4) Mid San Marcos = San Marcos River between Rio Vista dam and Capes Camp dam including channel leading to Mill Race; ESFO sections 7, 8, & 9.
- (5) Lower San Marcos = San Marcos River below Capes Camp dam; ESFO sections 10, 11, 12, & 13.

Service Study Section

= Spring Run number

COMAL CO. TEXAS

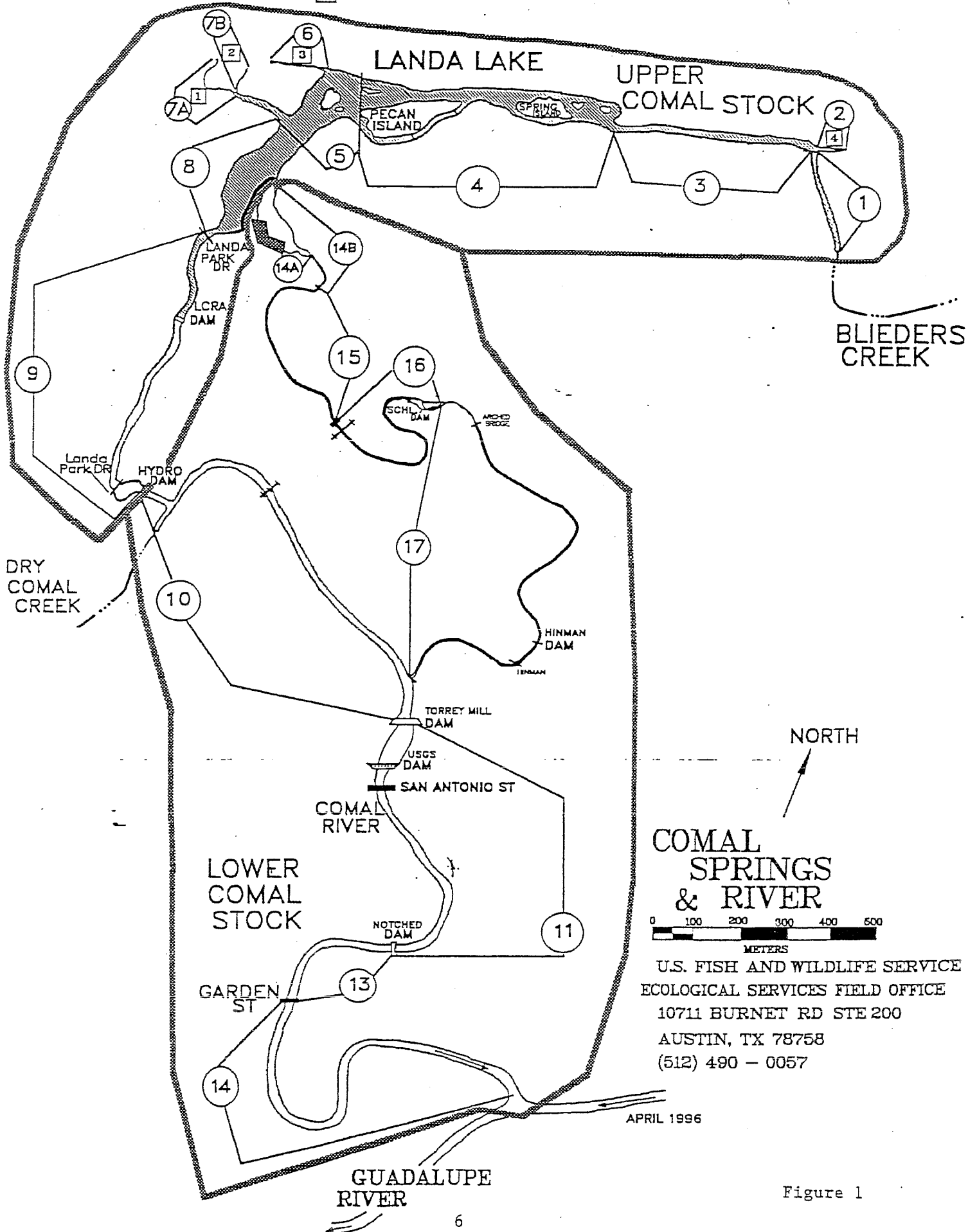


Figure 1

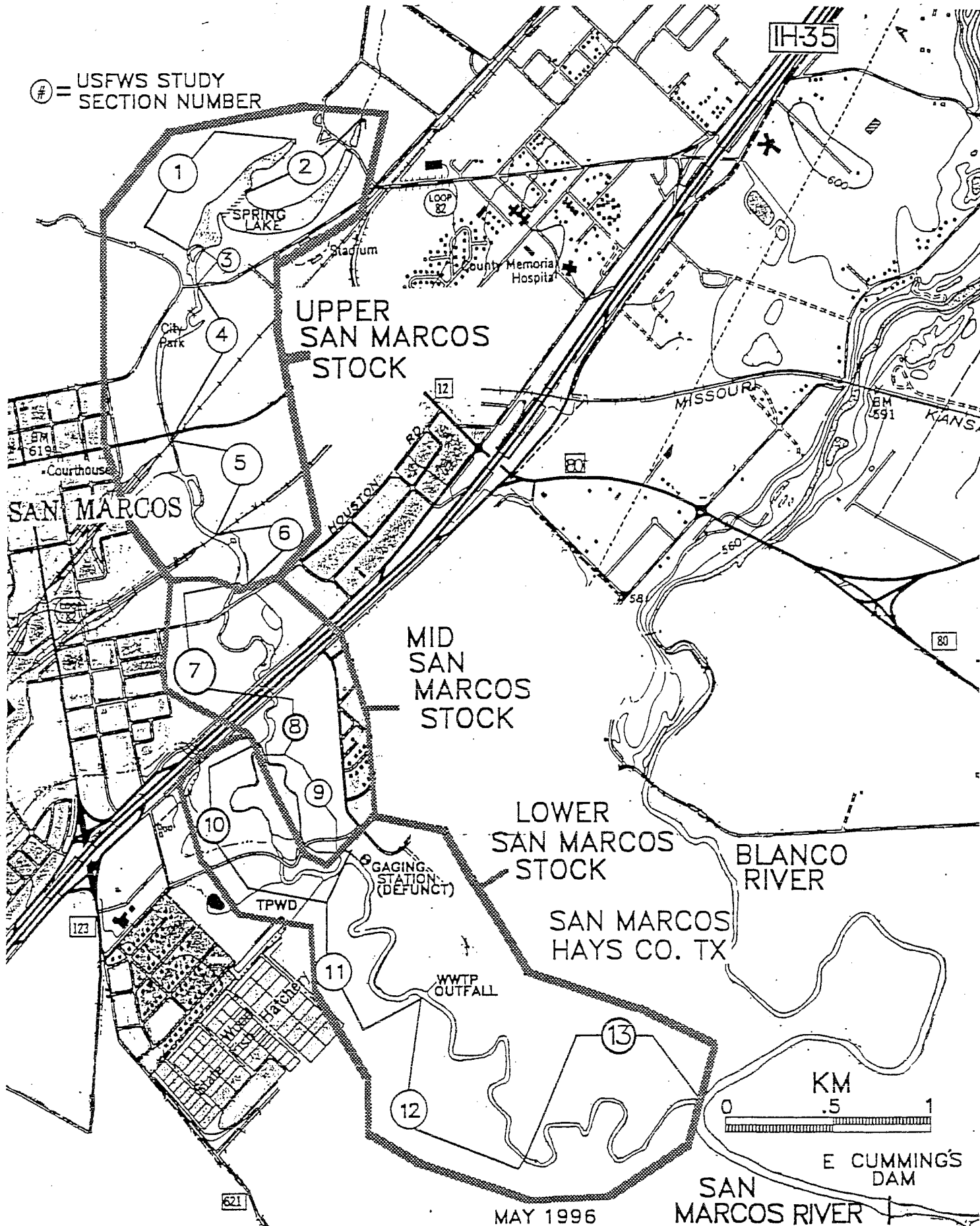


Figure 2

The contingency plan for the fountain darter is composed of three phases. Generally, each phase will be implemented in order.

Phase One involves the immediate establishment of standing stocks of adult fountain darters at two facilities. These stocks would be held in tanks or raceways and continuously maintained. This provides protection for the species from unexpected catastrophic events such as disease outbreaks and water quality impairment (such as toxicity resulting from an accident). These standing stocks also provide a back-up to the refugium stocks for captive breeding that are established under **Phase Two**.

Phase Two involves the establishment of refugium stocks, if conditions deteriorate in the river systems. These are similar in composition to the standing stocks. They consist of wild-caught adult individuals. The fish collected for the refugium stocks will be the original and primary broodstock for preservation of the specific wild stocks and are the keystone to contingency efforts. If springflows fail completely, these stocks will be maintained in a pairwise breeding program. If necessary, the progeny from this carefully controlled breeding program will be used in further controlled breeding to maintain stocks and produce fish suitable for restoration work.

Phase Three involves salvage of individuals for whom death is imminent. Salvage stocks are individuals taken from the wild when it is determined by the ESFO and cooperators that large fish kills in the wild are imminent or on-going. Individuals saved in **Phase Three** are to be kept in raceways or ponds in relatively high numbers with minimal maintenance. Salvage stocks, together with the standing stocks provide a back-up to the refugium stocks. Salvage stock subsets will also be transferred to a number of cooperators to preserve as many individuals as possible.

Monitoring of Conditions and the Decisions to Implement Specific Collections

Phase One, setting up standing stocks, should begin immediately to protect against unexpected catastrophic loss. With standing stocks in hand, the collection of refugium stocks for **Phase Two** can be deferred to the trigger conditions discussed below. An objective for collection of **Phase Two** refugium stocks is to ensure that: (1) the individuals are collected

prior to significant stress to the fish and (2) fish are still available in the lower reaches of the geographic range. Selection of trigger conditions for the collection of refugium stocks are based on prior springflow regimes. However, there is some concern that these trigger levels may be too low. Factors to consider include: (1) the impacts of Marisa sp. or other herbivores on vegetation in the Comal and (2) the potential for temperature and dissolved oxygen demand increases in the waters of the Comal or San Marcos as flow levels fall. For this reason, conditions will need to be monitored periodically as flow levels fall to avoid waiting too long to collect fish. TPWD is monitoring temperatures and dissolved oxygen in the San Marcos River. TPWD will update ESFO and Center staff on their water quality monitoring efforts. Similarly, ESFO in cooperation with the U.S. Geological Survey is monitoring water quality (temperature, dissolved oxygen, pH, and specific conductivity) in the Comal River and will keep TPWD and the Center updated as to provisional springflow discharges and water quality conditions/trends. New Braunfels Parks and Recreation Department staff (NBPARD) will be asked to monitor vegetation conditions in the Comal (with some training) starting at a flow of 100 cfs in the Comal, particularly Landa Lake and the old channel. NBPARD should communicate with the staff of the ESFO and Center if the aquatic plant community shows signs of decrease or impairment. Once refugium stocks are in place, conditions in the wild should continue to be monitored. Observations should be made concerning threats to the fish from such factors as predators, rising temperatures and variation in dissolved oxygen levels to help determine the point when salvage collections should begin.

Collection and Maintenance of Standing Stocks (Phase One)

Standing stocks should be collected as soon as possible, as they represent the protection against unexpected catastrophic events called for in the recovery plan. These standing stocks should be maintained at all times. Maintenance of standing stocks involves only adult fish. As young are produced, they will be removed regularly from these stocks before they reach adult age, so that only the original wild-caught adults remain (available for paired breeding, if needed). Mortality in the standing stocks should be closely monitored and recorded. The exact numbers of pairs in the standing stocks should be maintained at or near the specified levels. Every three months (or as needed) additional adult fish should be collected from

appropriate segments of the Comal and San Marcos river systems to replace those lost from stocks at both the Center and at Uvalde.

Collection for the standing stocks should consist of adults over 25 mm (total length) and be made as follows:

Comal Standing Stocks:

Two standing stocks will be established from the Comal system, each consisting of 75 pairs. These stocks will be taken from two distinct areas of the Comal system as described in Figure 1. These two standing stocks will be kept separately at the Center. 300

A back-up standing stock for the Comal system will also be kept at the Uvalde National Fish Hatchery, separate from Uvalde's San Marcos standing stock. Uvalde's Comal standing stock will be kept as a pooled stock of 100 pair with equal contributions of numbers of pair from each of the two Comal collection areas (50 pair from each). If additional facilities are found capable of holding stocks similar to those at Uvalde, a third standing stock should be considered. 200

San Marcos Standing Stocks:

Three standing stocks will be established from the San Marcos system, each consisting of seventy-five pairs. These three stocks will be taken from three different sections of the San Marcos system described in Figure 2. These three stocks will be kept separately at the Center. 450

A back-up standing stock for the San Marcos will also be kept at the Uvalde National Fish Hatchery. Uvalde's San Marcos stock will be kept as a pooled stock of 100 pair with roughly equal contributions of numbers of pair from each of the three collection areas. 200

Collection and Maintenance of the Refugium Stocks (Phase Two)

The following trigger condition are based on the assumption that standing stocks are being maintained.

Trigger conditions for collecting the refugium stock from the Comal system are when total springflow falls to 50 cfs or less for four consecutive days. If Landa Lake drops and its outfalls to the old channel provide/discharge less than 20 cfs sooner than this, the refugium stock should be collected at that time.

Trigger conditions for collecting the refugium stock for the San Marcos system are when springflow as measured at the University Drive gage is 75 cfs or less for four consecutive days.

If monitoring of conditions as discussed previously shows cause for concern earlier, the Austin ESFO will notify the Center's single point of contact (SPOC). The SPOC in turn will initiate collections accordingly.

Collection of fountain darters for refugium stocks from their respective systems should be completed within a few days after trigger conditions are reached.

Fish for refugium stocks should be collected in the same numbers (75 pair per stock) and segments of the river systems as for the standing stocks at the Center, and initially should be maintained in tanks (one stock to a tank) in the same manner. Refugium stocks will be held at the Center. At the same time that refugium stocks are collected for the Center, the condition of the standing stocks held in Uvalde will be evaluated and supplemented as needed.

Pairwise Breeding Protocol

Paired breeding is very labor and equipment intensive and should not be initiated prematurely. Paired breeding of refugium stock should begin within a certain timeframe (\leq one month) following loss of springflow at the stock's source. If, during the 10 months or so needed to breed 50 pairs, there is a return of adequate springflows, an evaluation of the population in the wild (and its habitat) should be made. If the wild population is stable, paired breeding efforts may be suspended.

50
50
50
50
50
50
2500

A breeding system (setup) includes the equipment described below. Paired breeding involves putting the 75 breeding pair into a divided living stream tank. This living stream consists of an insulated fiberglass trough about 1' high \times 1' wide \times 4' long, with plumbing to well water, packed column, and chiller/heater/pump. Five spawning aquaria (glass aquaria about 19 liters) are set up above it (Figure 3). Five breeding pair are selected from the stock, dispersed among the five aquaria and allowed to breed. Eggs are removed to hatching jars (glass jars about 0.5 liter), and 30 (F_1) young per pair are transferred later to a rearing tank, yielding 10 rearing tanks per breeding tank (fiberglass troughs about 1' \times 1' \times 4' long). Reared young will be maintained separately with subsequent progeny removed.

Each stock will be bred separately and maintained through time. Each run with a setup will involve 5 breeding pairs. A run is estimated to take about one month.

To go through paired breeding for a specific stock (minimum of 50 pairs bred) with one setup would involve 10 runs and take about 10 months (barring equipment failure).

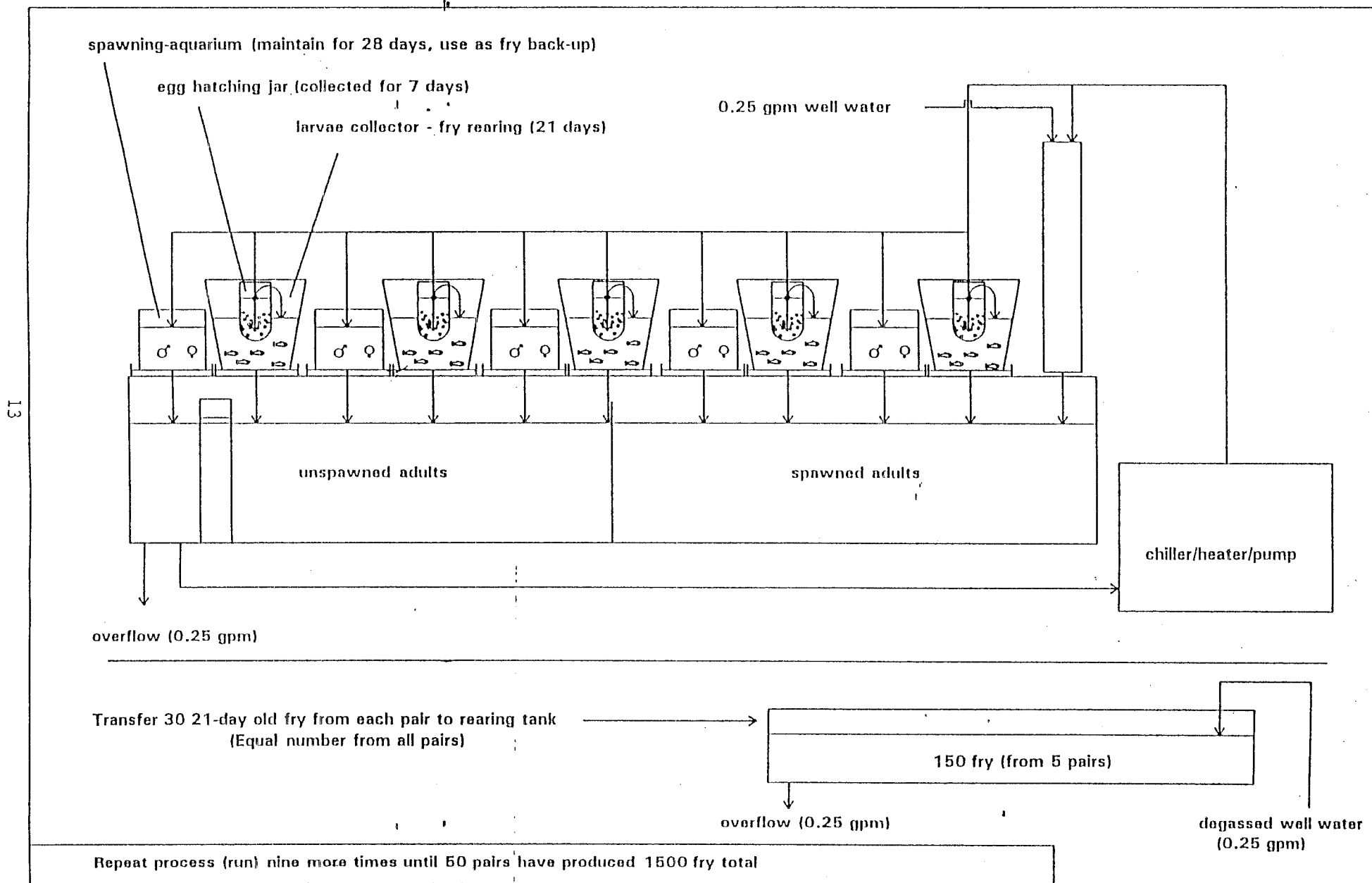
To breed all five stocks and produce 1500 F_1 progeny (each stock) will take five setups (one per stock), 10 runs each setup, and about one year. The staff time required to breed 50 pairs each from 5 stocks is enormous. Given mortality, incompatible pairings, or some form of impairment, more than 50 breeding pair may be needed if 50 unique pairings are to be made for each breeding cycle. When additional darters are needed for meeting the breeding objectives of the refugium stock, they should be taken from the standing stock.

F_1 progeny will be maintained separately in flow through systems. The F_1 s may be expected to live, on the average, two years. If springflows fail for more than one year, original refugium stocks and standing stocks may be depleted. At that time (one year after Phase Two is started), it may become necessary to use F_1 s, now sexually mature, as broodstock to maintain that stock.

Excess progeny from stocks will be transferred to researchers or museum collections to the maximum extent feasible. A list of facilities that want fountain darters for conservation or other appropriate purposes will be compiled. They will be contacted for coordination on

Figure 3

Start with minimum 75 pairs for each stock and spawn 5 pairs for 7 days



shipping, preservation protocols, etc., as fish become available. Receiving facilities will need to pay transfer expenses incurred.

Collection & Maintenance of the Salvage Stocks (Phase Three)

Trigger conditions for collecting the salvage stock will be determined by the ESFO in consultation with other work group cooperators. Generally the trigger condition will be when habitat conditions have degraded to the point that large die-offs of fountain darters in the wild are likely.

Salvage stocks of a maximum of 2,000 individuals will be taken and held pooled at the Center. They will be held in tanks or raceways provided with zooplankton and worms with protective covers. Uncontrolled breeding will be allowed. Tissue samples should be taken at initiation for baseline information with subsequent samples taken at 2 year intervals to track changes in genetic markers of the salvage stock.

In addition to this salvage stock at the Center, other facilities may be able to maintain salvage stocks. A tentative list of facilities includes Dexter National Fish Hatchery and Technology Center, Heart of the Hills Research Station, Dallas Aquarium, Tennessee Aquarium, Baltimore Aquarium, San Antonio Zoo, Fort Worth Zoo, and various other facilities.

Collection and Handling Protocol

Field collecting teams will consist of cooperating staff from the U.S. Fish and Wildlife Service (Austin ESFO), National Biological Service (NBS), Texas Parks and Wildlife Department (TPWD) and other volunteers provided they are covered by both U.S. Fish and Wildlife Service and Texas Parks and Wildlife Department scientific collecting permits. Detailed information on each team's collection effort, including the exact location/area collected, stock name, number of fish (males-females), time of day (start-end), date, and full names of collection team members, will be documented.

The Center is the primary facility for the standing stocks, refugium stocks, and salvage stocks. It is centrally located, has much of the equipment needed on-site, and must be

prepared to receive each collection before it can be made. The major responsibility for collection of the darters will reside with biologists at the Center (currently Joe Fries and Tom Brandt). Coordination for the collection teams and equipment needed will be handled by the Center, in close coordination with Austin ESFO staff, who should be assisting the collection effort. In addition to staff that may be needed for collection teams, TPWD will assist with getting GPS locations on collection sites.

Collections will be made primarily with dip nets supplemented by small seines. Extreme care needs to be taken to collect only fountain darters (to the exclusion of greenthroat darters, orangethroat darters, and dusky darters). To help avoid identification problems with smaller individuals only adult fish greater than 25 mm total length (TL) should be retained if possible. No electrofishing will be used. Fish should be handled carefully, kept in clearly labeled cured coolers, and transported immediately to the Center or other designated location.

The Center and any other refugium facilities should follow standard procedures used by the Center (for example, dilute formalin treatment for external parasites) in receiving the fish from the wild and keep stocks separated and clearly labeled.

All stocks, including standing stocks need to be carefully and regularly monitored for darters other than fountain darters. If adults are the only type put in raceways any small fish could be either progeny (or previously mistakenly introduced)

The Center will maintain detailed records of collection and breeding conditions, any problems encountered, treatments, solutions used, etc. They will maintain close coordination with ESFO and TPWD biologists who have lead for the conservation and recovery of listed species. Periodic health inspections will be made and records kept of infections, parasites, food-borne organisms, shifts in microfauna, and any treatments that may be done.

Recognized needs for implementation

The Center remains vulnerable to severe water supply problems as long as it does not have a fully operational back-up water supply. A back-up water supply, back-up electrical generation equipment, and security fencing for the exterior facilities are needed to provide protection for rare plants and animals that are provided refuge at the Center.

For its currently outlined role, Uvalde appears to have the equipment needed. Any other facilities that are identified may need to acquire equipment, and if additional large salvage stocks are determined to be needed in separate facilities additional equipment may be needed.

Additional Information Needs/Revision of the protocols

These procedures for conservation of fountain darters in captivity were developed based on our current understanding of the genetics and biology of the species and its habitat. As additional information is developed it may be possible to improve effectiveness or efficiency by changing these procedures. This plan should be considered a working document and amended as needed as new information dictates.

For example, it may be possible to maintain stocks over time with large populations, held in ponds or tanks. However, the effect of this kind of culture has not been studied. A study of the genetic structure of captive populations at the Center over long periods of time would be helpful in resolving this question.

It is very desirable to obtain a more intensive baseline sampling and analysis of current genetic structure of fountain darter populations in the Comal and San Marcos. This would provide a basis for evaluation of the success of the captive propagation program in maintaining genetic diversity and in making decisions needed for developing a restoration/reintroduction plan. If possible, a plan would need to be developed, funded, and carried out as soon as possible given current low-flow conditions so that the opportunity to collect this information is not lost.

MAY 1996

SAN MARCOS GAMBUSIA (Gambusia georgei)

An extensive search should be conducted to determine if pure San Marcos gambusia exist in the San Marcos River. This work should be initiated as soon as possible so that if the San Marcos gambusia still exists it can be located before declining spring flows affect it. A proposal for this work is included in the appendices. If the San Marcos gambusia is found, a refugia should be established immediately.

1. Collection of fish

Fish should be collected alive and any likely G. georgei should be brought back (alive) to the lab for closer examination. All those fish that are definitely not G. georgei can be returned to the river right after collection, except for exotic species, which should be removed and preserved. A refugium and captive breeding program for G. georgei should be established at the San Marcos NFH&TC (Center) if G. georgei can be obtained.

2. Maintenance of refugia gambusia

If found, G. georgei will be maintained at the Center. If a stable, reproducing refugium population can be established, a second refugium should be established.

3. Stocking of gambusia back into the river

A decision concerning the restocking of refugium gambusia back into the river will be made at the time a stable refugium population is established, instream flow conditions are adequate, and San Marcos gambusia habitat is available.

TEXAS WILD-RICE (Zizania texana)

While Texas wild-rice has been successfully cultivated at Southwest Texas State University's Aquatic Station (using Edwards aquifer waters) for many years, attempts at cultivation by researchers at other institutions have been unsuccessful. As a part of the recovery effort for Texas wild-rice, a pilot study is being conducted at present to explore reintroduction/restoration techniques for planting Texas wild-rice back into the wild. Over the last several years Texas wild-rice has been planted in various types of habitats in Spring Lake, and some plants have survived and grown larger, at least for a short time. However, there is still significant mortality in the test areas, tests have not yet been conducted in the San Marcos River itself, and long term success of present efforts is by no means assured.

Reintroduction of rare or vulnerable plants into the wild is extremely difficult, and efforts across the country to date have seldom been successful in reestablishing viable wild populations. In a survey conducted in coordination with a Center for Plant Conservation Symposium on Reintroduction (April 1993), Fielder evaluated 45 relocation/reintroduction projects with some form of follow-up monitoring in place, and concluded that fewer than 10% could be considered successful. She also noted that, similar to early efforts at reintroduction with Texas wild-rice, long-term persistence is difficult to achieve. Projects may often appear successful for the first year or two and then fail. It is obvious that reintroduction efforts are time and labor intensive and may take many years of research and management before success can be expected.

Nevertheless, there is a high probability of mortality for all plant material in the wild due to decreased flows of the San Marcos River. Bringing plants into cultivation and trying to maintain a genetically representative sample of the species with a cultivated population may be necessary to allow eventual attempts at reintroduction. Cultivated populations may represent the last hope for conserving the species.

In addition, the San Marcos and Comal and Associated Ecosystems Recovery Plan (task 2.7) has identified the need for genetically representative captive populations to be established and

maintained continuously in at least two locations as a protection against loss from catastrophic occurrences such as chemical spills. Establishing captive stocks for Texas wild-rice as outlined in this contingency plan would fulfill this recovery task as well.

If genetically representative captive populations have already been established for Texas wild-rice when emergency conditions for implementing the contingency plan arise, the adequacy and condition of the captive populations should be evaluated. The condition, extent and location of wild-rice stands present in the river at that time should be compared to conditions when the captive population material was collected, and the time interval that has passed since material was collected from the wild habitat should be reviewed. Captive populations should be expanded if substantially different stands are present under contingency conditions than are represented in the long-term captive stocks. The balance of representation of plant material from all segments of the river should also be reviewed so that all segments of the river that have stands are adequately represented in the collection. Any material that has been removed from the wild for a long time and/or has been propagated clonally many times should be evaluated and considered for possible replacement with new material from the same stands to ensure representation of the wild population in the collection.

Monitoring and Decision to collect plant material

As springflows approach 120 cfs for San Marcos Springs, Paula Power (who resides in the San Marcos area and conducts research on Texas wild-rice, frequently working in the river) will begin monitoring localities where wild-rice is expected to become stressed first. Austin staff from TPWD and the Service will assist as needed. It is believed that collection of plants from the San Marcos River will need to be initiated at about 100 cfs spring flow, but it may be needed earlier or later depending on observed conditions of the rice. As flows fall, stands likely to be impacted first will be examined periodically for changes in depth, flow, predation and other threats, and overall condition of the Texas wild-rice stands. A sample monitoring data sheet is included in the appendices.

As flows and depths decline, leaves rise to the surface where they are subject to increased herbivory by waterfowl, and floating mats of vegetation in the river increasingly snag on the

wild-rice and build up, blocking light and putting additional drag on the leaves. In some areas, as water levels fall, riffles develop and plants in these environments develop a very short-leaf growth habit. In some areas, substrate around the root systems begin to erode, roots become exposed, and eventually plants are washed out. In other areas, velocities fall to low levels. Under these conditions, herbivorous animals (probably nutria) clip or damage large amounts of the photosynthetic leaf material. In addition, Texas wild-rice plants depend on free carbon (more plentiful at higher flow velocities) rather than bicarbonate as a source of photosynthetic carbon, and under low velocities, photosynthetic efficiency is compromised. Plants begin to behave as annuals, producing reproductive culms and then dying. The reproductive culms seldom successfully set seed in these situations due to increased herbivory. In addition, impacts from recreationists appear to become more severe in shallow water.

All stands in the river are not expected to be impacted simultaneously as depths, threats, and habitat conditions differ in different segments of the river. Initially, as flows decline and the first few stands become stressed, plants from those stands impacted over more than 50% of their area will be taken into cultivation to preserve the option of including samples from those stands in the genetic collection. If conditions deteriorate to the point that stands in most segments of the river appear to be suffering potentially significant impacts, the entire collection strategy for cultivation should be implemented.

Genetic diversity present in the existing stands of wild-rice in the river is unknown. Stands of wild-rice in the San Marcos River are currently being evaluated for genetic variability, but the study is not yet complete.

Clonal species are often regarded as likely to be more genetically homogeneous within populations than non-clonal species. While this can be the case (for example, some species of Festuca cover large areas that are genetically homogeneous), studies of clonal grass species have shown that genetic diversity within a population can also be very high, particularly in older, established populations. Many species probably maintain a number of adaptive polymorphisms. Such adaptive polymorphisms may provide the foundation for successful growth in changing environments or across a variety of microsites within an

environment. They may also maximize species longevity across microsites that have variable competition from other plant species, and under scenarios of selective predation by herbivores (Harper 1977). Studies have shown another Festuca species has an average of 5.2 different genetic clones in a 15 X 15 cm quadrat, and a species of Trifolium in an old meadow has been recorded with up to five different clones intertwined in a square decimeter (Harper 1977).

Lacking full genetic analysis, to ensure that a genetically representative sample is taken of wild-rice to preserve the variability of the species, it is recommended that a series of samples be taken that cover the entire area inhabited by wild-rice. The Center for Plant Conservation has published guidelines for situations where genetic analysis is not available. They suggest as a general "rule of thumb" that collecting from 10-50 individuals in most plant populations should provide a good sample of variability (Falk and Holsinger 1991). For Texas wild-rice, boundaries between individuals within stands and genetic boundaries and degree of isolation of genetic material between stands is unknown, though it is known that wild-rice has not been flowering or reproducing sexually to any significant degree for some time. Consequently, it is recommended that the current area of distribution of wild-rice in the San Marcos River be treated as several essentially separate populations. A total of 8-11 vegetative clumps 10 cm in diameter should be collected from each of the following eight "sites" or general areas:

1. Sewell Park by the University (This is the A/B segment of TPWD monitoring plan maps)
2. between Hopkins St. and Cheatham St. (This is the C segment in TPWD monitoring plan maps), probably by Snake Island
3. between Cheatham St. and IH-35 bridge (Segments E/F of TPWD monitoring plan maps)
4. IH-35 Bridge (Segment G of TPWD monitoring plan maps)

5. between the I-35 Bridge and the county road by Thompson's Island (Cape's Road) (Segments G, H, I of TPWD monitoring plan maps)
6. between County Rd. (Cape's Road) and the old Mill Race dam (Segment J of the TPWD monitoring plan maps)
7. below the confluence with the Mill Race near the old gauging station (Segment K of the TPWD monitoring plan map)
8. between the old gauging station and the sewage treatment plant outfall, collecting in the area near the power line (Segment K - end of TPWD monitoring plan map)

When conditions necessitating the implementation of the contingency plan are reached, the genetic representation of the plant material held in the seed bank cultivated collection at Southwest Texas State University should be evaluated and additional plants added to that collection to improve/maintain genetic representation of the plants being used to produce seed for the seed-bank program.

All collected plants will be held at the Center, with a duplicate back up facility at Uvalde, and additional material at Southwest Texas State University (SWT). The State of Texas' Heart of the Hills Research Station (HOH) may be another potential site, and a pilot project should be initiated to see if Texas wild-rice can be maintained there as well. If feasible to hold a collection at HOH, material that is clonally propagated from the other collections can be provided to them.

As reliable techniques for restoring or reintroducing Texas wild-rice have not yet been developed, and maintaining large numbers of plants in captivity requires a substantial commitment (space, water, labor) and unique conditions (water chemistry, temperature, flow rate, etc), exceeding these collection guidelines for numbers of plants to be taken from the river is not recommended. It is not advisable to try to hold plants in sub-optimum conditions, even for short periods. The collection plan outlined above should provide a genetically representative sample (a total of 64-88 clonal samples at the Center, Uvalde, and

SWT, and possibly more if Heart of the Hills Research Station works out). Multiple facilities provide security back-ups, and plants can be carefully monitored and maintained, and replicated as necessary, using clonal propagation techniques currently available. Removing large quantities of rice from the river proper would be expected to be damaging to the habitat area and could preclude the possibility of the stand regenerating on its own if low-flow conditions do not persist for an extended time.

If additional research on the genetic heterozygosity of wild-rice shows that stands in the river are relatively homogeneous, or that variability is high in some areas but low in others, the collection plan should be amended to adequately sample the variability that is present in the River.

Collection and Handling Protocol

Samples should be labeled using TPWD stand designations from their ongoing monitoring project. These should be available with GPS locations in early 1996. Data regarding date, collectors, TPWD segment and stand number, and observations regarding site and plant conditions should be recorded. A sample data sheet is included in the appendices. At least one Texas Parks and Wildlife botanist who has been monitoring wild-rice should be on hand during collection to verify stand localities and supervise collection to minimize damage to wild-rice and the occupied habitat areas.

Samples should be removed using a sharpshooter shovel with a sharpened leading edge. Select vigorous vegetative (that is without signs of initiating flowering) samples from the downstream edge of the wild-rice clump in the river. Individual samples collected should be placed in a 6 in. plastic one gallon pot or azalea pot. Individual pots should be tagged immediately, and placed into a deep ice chest. **DO NOT MIX PLANTS FROM DIFFERENT SITES/AREAS IN THE SAME ICE CHEST.** Transfer ice chests to vehicle and then fill with enough water to cover at least some leaves and maintain high humidity. Do not allow plants to become heated. Transport as soon as possible, in small lots if necessary to maintain good condition. Divide the samples per site between the refugia (3-4

to the Center, 3-4 to Uvalde and 2-3 to SWT). In transporting plants to Uvalde or HOH, care should be taken to avoid desiccation or overheating.

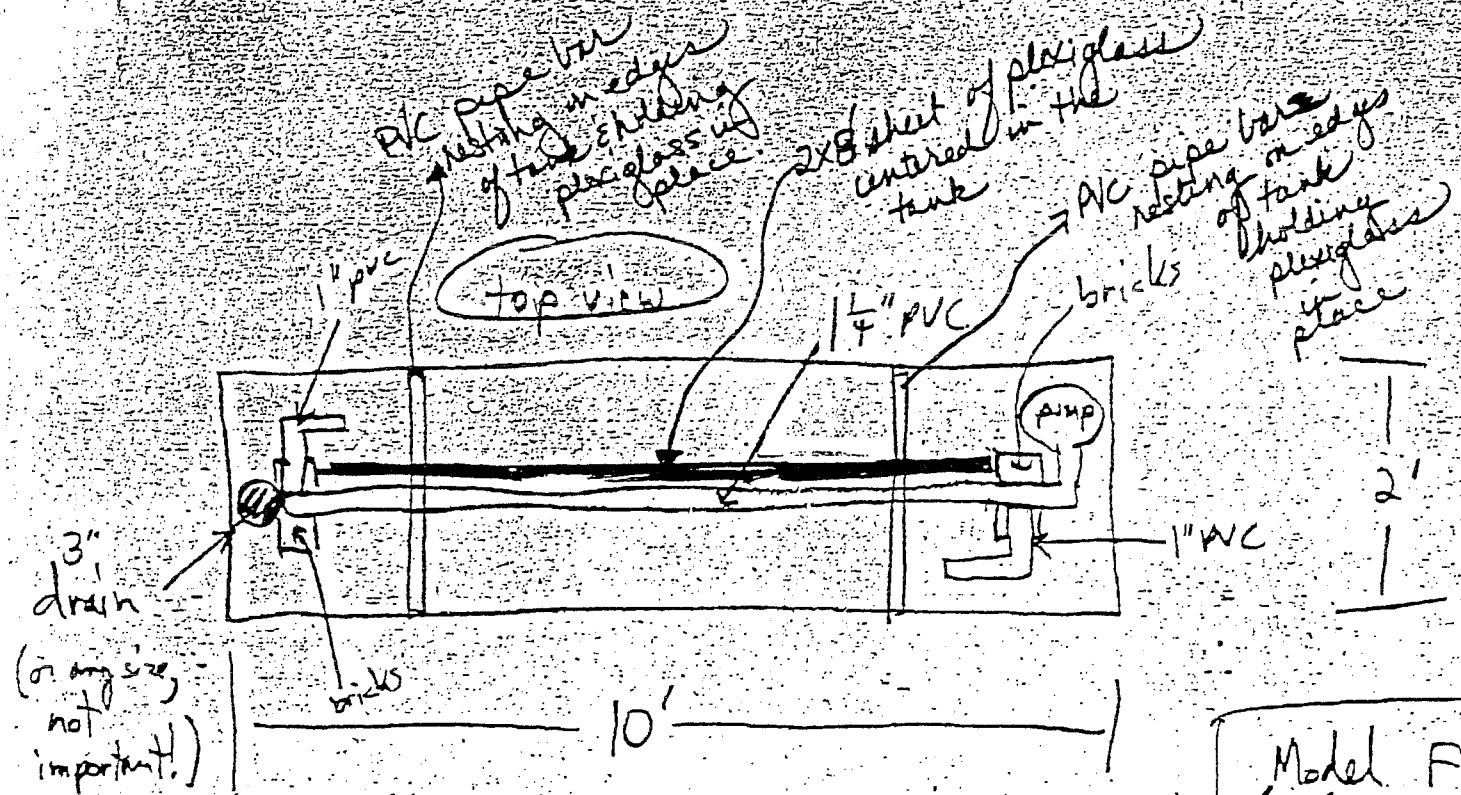
Initial Set-up and Planting

Plants from each of the eight collection sites should be maintained separately in raceways with flow-through water or in 2 X 2 X 10 foot rectangular tanks with recirculating pumps at the Center and Uvalde locations. Staff at the Center have developed and tested a protocol for the tank set-up that has sustained vigorous growth and reproductive activity in Texas wild-rice, at least in the short term (Figure 4).

To protect the genetic integrity of the plants from each site, plants from different collection sites must be separated from each other in such a way that no tillers can invade from one collection site's growing area to another, and no broken stems, tillers, uprooted segments, etc. could flow from one site's growing area to another. Raceways or tanks should also be fenced to prevent access by nutria and for security. Crayfish, turtles, or bottom disturbing fish (carp or Koi) must also be excluded.

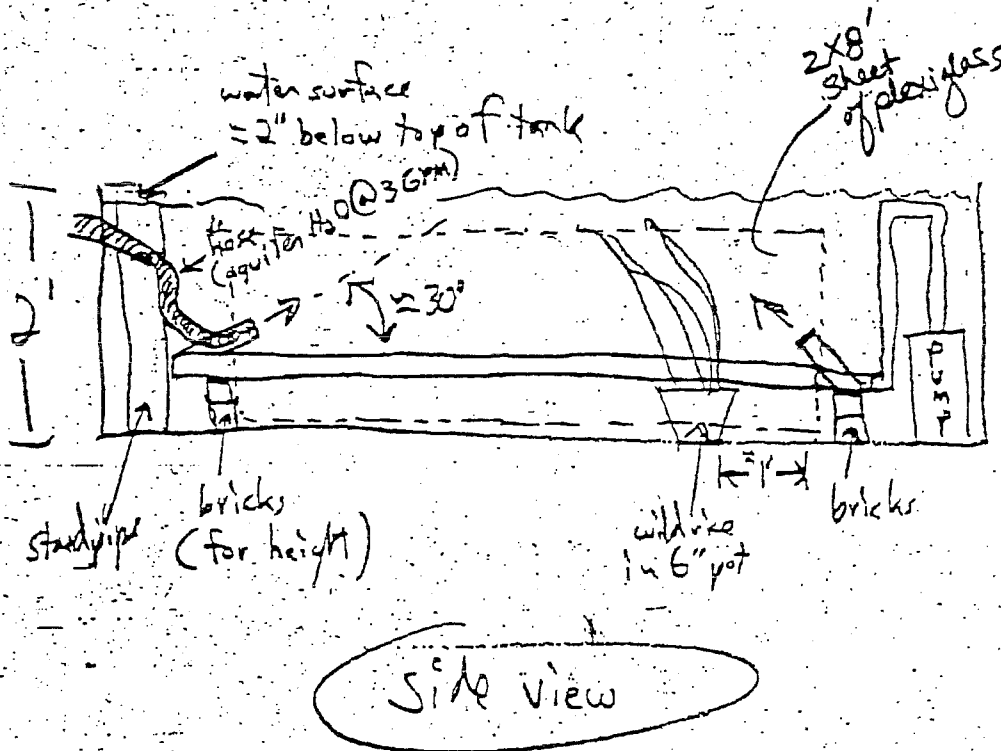
Plants should be potted in a sandy clay collected from the San Marcos River. Clay is too dense, gravel is too nutrient poor, and commercial potting mediums contain too much organic material and other materials, and will float. Keeping the plants in San Marcos River substrates may also help preserve their normal microflora and microfauna. Collection of this material from the river must be done in a way that does not impact other listed species. Uvalde (and HOH) will need a small supply of substrate material that they can keep on hand for maintenance needs for the plants. This substrate material would need to be spread out, dried and then stored and would require rewetting/submersion for at least 24 hours before use. Areas with substrates that have been successfully used to cultivate wild-rice include the area by the I-35 bridge, the confluence of Purgatory Creek and the San Marcos River, and Spring Lake near the spillway.

Water depth should be about 0.5-0.8 m. Wild-rice plants in cultivation require moving water. In a refugium it is necessary to manage for longer-lived plants, as the objective is to



Model FRT-3
2x2x10" Fiberglass
tanks

From
Red Ewell
1800-242-3525



Pump from Aquatic
Ecosystems
#POMU2A
(w/switch)

The pipe sizing seems to regulate the flow rate appropriately

preserve the existing genetic material without cross contamination. Initially, flows of 0.2-0.5 ft/s should be maintained to sustain vigorous growth over a long time. Optimum flow rates will be refined through observations of plants grown in the captive collections.

Temperature is also important. Water temperature should be kept in the temperature range of the San Marcos River water, which averages 22 degrees centigrade. Recirculating tanks may require shading if solar insolation causes water temperatures to rise.

Maintenance of Refugium Plants

Plants must be checked daily as they are susceptible to aphids, and outbreaks occur and spread very quickly. If aphids are found, spray the plants with approximately a 1:10 solution of dishwashing liquid:water. DO NOT INCREASE THE PROPORTION OF SOAP. If the soap solution is too strong, it will destroy the surface cuticle of the leaf and cause plant mortality.

Texas wild-rice plants also may get a bacterial infection known as leaf streak, but it is seldom fatal in wild-rice.

In daily plant checks, observers should note overall health and color, as well as the time of initiation of flowering, pollen release and anther drop (and the need to bag female parts of inflorescences, see below), and the ripening of fruits. If any signs of decline or disease appear, consult with wild-rice experts like Paula Power IMMEDIATELY so that corrective actions can be taken.

For many aquatic plants, adding typical horticultural fertilizers such as osmocote can be fatal. Do not add fertilizer unless the plants show symptoms of nutrient deficiency, and consult aquatic plant culturists for recommendations.

Plants growing vigorously will form tillers. Where possible, some of these should be grown out to provide new clonal duplicates of the original plants collected, especially if the original

plants show signs of senescence and/or the cultivated collection must be kept for many years. For propagating tillers, place a 4 inch peat pot filled with river substrate near the rooting tiller, gently bend the tiller stem until the roots contact the peat pot soil. Secure tiller in the peat pot with a horticultural pin. The connecting stem will eventually die away, leaving an independent clonal plant. Any plants produced by propagating from tiller material must be carefully tracked and labeled by plant of origin, and a balance of numbers of plants from various sites should be maintained.

Plants grown in cultivation at SWT commonly flower. For the cultivated collection designed to preserve the present genetic structure and diversity in the river, allowing free flowering and seed drop presents problems because of the genetic recombination involved, and the potential to recruit individuals in the tanks from fallen seed that would "contaminate" the collection by creating imbalances in the genetic material being preserved. However, the production of recombinant seed of unknown parental origin is not totally negative. Such seed could provide a valuable resource by providing back up material in seed banks to insure against loss of the cultivated material, and for some other restoration activities where a pool of genetic types with maximum variability is preferred. For this reason, blooming plants should be carefully monitored and treated as follows: No seed produced should be allowed to fall into the raceways or tanks where it could germinate and contaminate the collection of plants from the river. If flowering occurs, following the drop of the anthers, the pistillate portion of the inflorescence should be bagged with a lightweight mesh material that will allow light penetration but not let seed fall out (like fine dacron sheer curtain fabric or panty hose sections). Flowering stems may need to be supported to accommodate the weight of the bags. Seed would be allowed to mature in the bags, then be collected, labeled with date and stand information for the maternal plant, and added to the seed bank collections (see below).

Seed must be stored immediately after removal from the inflorescence in aerated water at 1-3 degrees C. Seeds are dormant for 3 weeks +/- after collection. Percent viability and germinability with seedling survival is greatest during the first 6 months of storage. After 6 months, survival of germinated seedlings drops significantly. Seed stored one year or longer should be discarded after replacement with seed from successively "newer" crops.

While a cultivated collection is the only practical technique for preserving a genetically representative sample of stands in the river, other techniques may add to the protection for the species as well. A seed bank is one technique currently available. A back-up for these plants has been established in the form of a seed bank at Southwest Texas State University and material should also be deposited with the USDA National Seed Storage in Ft. Collins, CO. The main source of these seeds will be the plants now producing seeds at SWTSU. However, it must be recognized that, since the plants producing these seeds were not collected to provide representation of all rice stands in the river, the seed collected from this source may not presently adequately represent the genetic diversity in the wild. If possible, this collection will be upgraded. Seed produced by plants in the cultivated refugia collections should also be collected. These plants should also be allowed to flower and the seed collected and stored, on site and in the seed bank(s), as noted earlier. Some clonal species may also be protected by cryopreservation of clonal material such as shoots, rhizomes, etc. The feasibility of doing this for Texas wild-rice is currently being investigated by the National Seed Storage Bank. If feasible, cryopreservation provides a relatively inexpensive means of preserving samples from all stands in the river in a facility not reliant on Edwards aquifer water, though success in regenerating them may be only partial. Additional funding would be required to support cryopreservation of clonal material for any length of time. Tissue culture may also present an option for preserving clonal material off-site, though tissue-culture techniques for the species have not yet been worked out.

Texas Blind, San Marcos, and Comal Springs Salamanders

(Typhlomolge rathbuni, Eurycea nana, Eurycea sp.)

Because a contingency plan for these salamanders has a limited potential for success, extreme caution should be exercised regarding its implementation, and every effort should be taken to avoid loss of or significant impact to wild populations. To date, captive propagation and maintenance of these species has met with very limited success, and our understanding of species needs for captive propagation is still in an infancy stage. Adults are extremely fragile and mortality occurs often. Captive individuals appear to be especially sensitive to changes in water quality and have exhibited toxic reactions to plastic containers, aged tap water, detergent residues and, if kept in a closed system, the frequency at which water is changed (Sweet, in litt., 1993). Furthermore, spawning seldom or never occurs in captivity, and eggs that are spawned may be inviable and are highly susceptible to fungal infections (Lynn Ables and Streett Coale, Dallas Aquarium; Casey Berkhouse, SMNFH&TC; Glen Longley, SWTSU, pers. comms. 1995). Until these species' reproductive requirements are better understood and maintenance techniques are fully developed, a captive propagation program runs a high risk of failure and should be considered a last resort only. Nevertheless, captive propagation may be the only alternative for attempting to conserve the species if crisis conditions develop (low aquifer levels and springflows) and may be needed for an extended time. Because of the uncertainty of success, captive stocks need to be established immediately to continue developing captive breeding and maintenance techniques and to obtain healthy individuals needed for captive refugia populations.

Texas blind salamander: The Texas blind salamander has been collected from a total of 7 locations: the artesian well at Southwest Texas State University (SWT), Ezell's Cave (EC), Rattlesnake Cave (RC), Primer's Fissure (PF), Johnson's Well (JW), Wonder Cave (WC), and Diversion Springs (DS) at Spring Lake (Russell 1976, Longley 1978). Two sites, JW and WC, no longer appear to be suitable habitat for the salamanders (Longley 1978).

Salamanders collected at the SWT and DS sites are considered "salvage organisms" because they are lost from the aquifer population whether they are collected or not. The three remaining sites are caves (EC, RC, and PF). Collection from these sites would remove animals from the aquifer population.

Though collecting salamanders from SWT and DS will not have any direct effects on the wild population (these individuals have exited the aquifer prior to collection), removing them from the cave sites might. Two salamander researchers (Andy Price, Texas Parks and Wildlife Dept., pers. comm. 1995; Paul Chippindale, University of Texas at Arlington, pers. comm. 1995) have expressed reservations over the collection of significant numbers from the cave sites. No population estimates have been attempted at any site. It is not certain if cave abundance is similar to abundance away from the caves; the populations at the caves could be larger than populations elsewhere in the aquifer, assuming food supplies are more abundant near cave openings. Collecting heavily from the caves could deplete salamander numbers at these sites. Information on population size and structure is needed before large numbers of salamanders are collected from cave sites and to estimate a level of collecting that the cave populations can tolerate.

Furthermore, there are currently almost no data on the genetic differences between salamander localities upon which to base a decision on whether each site should be represented in captive populations. Preliminary work by Chippindale (pers. comm. 1995) on a small sample (3 salamanders from RC, 1 from EC, and 1 from DS, all lumped together) found little gross genetic variation (using allozyme techniques). However, additional genetic research is needed to determine if genetic variation among the known localities exists and supports the need to collect salamanders from each site for captive refugia populations, and if collections should be separated or pooled in captivity. Until this information is obtained, collections will be maintained separately, and small numbers of salamanders will be collected from the caves prior to aquifer levels dropping to a point where collection from the caves is no longer feasible (see below).

The Dallas Aquarium and the Cincinnati Zoo have had some limited success in culturing the Texas blind salamander. Texas blind salamanders successfully reproduced four times at the

Dallas Aquarium between 1993 and 1995. This species requires high maintenance, and adults and eggs are very prone to fungal infections, particularly when handled. Curvature of the spine has been observed in both wild and captive individuals (Lynn Ables and Street Coale; Glen Longley, pers. comms. 1996). This spinal condition reversed itself in young salamanders when their diet was switched from black worms to newly hatched snails, indicating a previous calcium and/or other dietary deficiency (Lynn Ables, pers. comm. 1996).

San Marcos salamander: Despite continued efforts to culture the San Marcos salamander at the SMNFH&TC and Dallas Aquarium, no reproduction has occurred in this species to date. Adult mortality has occurred due to pump failures (Lynn Ables and Street Coale; Casey Berkhouse, pers. comms. 1995 and 1996), and disturbance and handling also appear to increase mortality (Casey Berkhouse, pers. comm., 1995). The current distribution and size of the wild populations appear to be sufficient to avoid adverse impacts from overcollecting for refugia in the numbers called for in this plan.

Comal Springs salamander: Salvaged individuals collected in 1990 when springflows fell were distributed to the Cincinnati, San Antonio, and Ft. Worth zoos and the Dallas Aquarium. The Dallas Aquarium is the only institution that still has the Comal Springs salamander in captivity and that has been successful at propagating this species, using artificial spring upwellings and an open well system (Lynn Ables, Street Coale, and Dave Roberts, Dallas Aquarium, pers. comms., 1995). This species has successfully reproduced in the artificial upwellings at the Dallas Aquarium at least once a year (three or more times) between 1993 and 1995.

Monitoring and Decision to Collect Salamanders

Until genetic research is completed, ideally salamander collections should be sufficient to adequately represent the populations at all known sites throughout each species' range, but without adversely impacting wild populations from overcollecting. In captive population collections, material from different collection sites should be kept separate.

The contingency plan for the salamanders is composed of four phases, to be implemented in order, and applies to each of the three species:

Phase I: Establishment of Breeding Stocks

Because captive breeding techniques have not been fully developed, it is critical to focus on attempts to develop captive breeding techniques as soon as possible. To do this at least 100 adults (33-50 adults per collection site) should be collected immediately, maintained in breeding setups (see below), and coordinated intensive efforts made to develop successful, reliable culturing techniques. These organisms can be spread over several facilities.

Once successful breeding techniques are established, it may not be necessary to maintain organisms in breeding setups and the original wild-caught salamanders may be subsumed into the back-up stocks.

Phase II. Establishment of Back-up Stocks

As soon as the 100 individuals are acquired for phase I, collection efforts should continue to add an additional 200-400 healthy animals (for each of the three species). At this point, there would then be 300 to 500 individuals of each of the three species in captive collections. These individuals will make up the back-up stocks for each species and provide protection against unexpected catastrophic events in the wild as well as a back-up for breeding stock. For some species, acquiring this number of individuals will be difficult to achieve, and may take time to reach these target levels without adverse impacts to the wild populations. If collections start as soon as possible, take advantage of high flow periods, and continue over several years, these targets should eventually be achieved. Because of low collection rates from DS and SWT, long-term collecting (over a period of two or more years) will probably be necessary to obtain adequate numbers of Texas blind salamanders. If possible, collecting over a period of several months to a year is also recommended for the Comal Springs and San Marcos salamanders to obtain adequate numbers and avoid impacting wild populations.

These 200-400 back-up stock individuals would be maintained in aquaria (for densities see below), and can be dispersed among several facilities. Any progeny should be removed and kept separately so that the mortality and condition of original wild-caught individuals can be tracked, and the original wild-caught genetic structure and diversity are preserved as long as possible.

The condition of both the breeding stock and back-up stocks should be evaluated quarterly for mortality and condition of the salamanders. If needed, additional collections may be made to maintain appropriate numbers and health of captive stocks.

Collections for both breeding and standing stocks should be made as evenly as possible among the following sites, with individuals from each collection site maintained separately:

Texas blind salamander: Every effort would be made to collect salvage animals from DS and SWT. Attempts should also be made to collect from a third spring outlet near DS. During phase I and II, no salamanders would be collected from the caves.

San Marcos salamander: DS, northern end of Spring Lake in the area of the Aquarena Springs hotel, and the part of the San Marcos River below Spring Lake that flows past the Clear Springs Apartments.

Comal Springs salamander: Spring runs 1 and 3.

Phase III: Supplemental Collections at Low Flows

In the event that flows drop below 105 cfs at San Marcos Springs and insufficient numbers have been collected from DS and SWT, additional collections should be made. Collections should concentrate on the most densely populated areas to ensure that at least 100 individuals are being maintained as breeding stocks and that back-up stocks are in good condition.

The San Marcos salamander is most abundant in Spring Lake near the hotel.

Under these conditions collections for the Texas blind salamander would include cave sites as well as continued well sampling. A few salamanders should be collected from at least the RC site, and preferably also the EC site. At this time, collection from the PF site is not being recommended because the PF site is privately held, and the ability to gain permission to obtain collections is unknown. Collections from caves must proceed cautiously however. Traps should be checked daily, and numbers of salamanders present should be tracked. Collectors should collect no more than 25% of the salamanders present per day. This should avoid overcollecting as the individuals present in cave samples are, in turn, undoubtedly a subset/sample of the wild population. Monitor and examine the numbers of individuals present in daily cave samples. If total sample numbers show declines this may be evidence of undesirable impacts to the wild population, and collections should cease for a week or until numbers present in daily cave samples return to more robust numbers.

Phase IV: Jeopardy and Salvage individuals

The status of the habitat will be monitored and, if it appears loss of flow is imminent in any of the segments, current genetic information about the salamanders will be reviewed, as well as the condition of captive stocks. The breeding stocks will be expanded from back-up stocks if advisable and strictly managed to maintain genetic diversity. If necessary, the progeny from this carefully controlled breeding program will be used to continue captive breeding efforts and produce individuals suitable for restoration work.

When it is determined by the Austin ESFO and cooperators that the species are at or near critical levels, additional individuals will be taken from the wild. Refugia managers should check their collections and records of adult mortality rates, and add additional individuals to meet the needs for breeding programs, and to increase the numbers of individuals in back-up stocks that are in good condition and available for use in the breeding program. Evaluations should focus on what, if any, additional salamanders may be needed to maintain a healthy refugia stock for at least 1 year.

The number of additional salamanders needed for refugia will be determined in consultation with the Austin ESFO and cooperators. Evaluations will be based on adult mortality and reproductive success in captivity, the number that can be collected without depleting wild populations, and the number of captive animals that cooperators are able or willing to hold. Enough individuals should remain in the wild to allow for the possibility of survival if conditions improve. Additional collected individuals will be distributed to as many cooperators as possible (see Appendix).

If breeding techniques are not known, additional refugia collections should be established and maintained as a short-term (at least one year) back-up to the primary breeding and back-up stocks.

Short-term numbers of wild-caught individuals to be held in refugia should be sufficient to support and to maintain genetic representation of these species as a collection of wild-caught individuals (with an upper target of 2,000 individuals).

Collection and Handling Protocols

Coordination of the refugia facilities and collection and shipment of the salamanders has been funded for 1996 - July 1998 through an interagency and cooperative agreement, executed by the U.S. Fish and Wildlife Service, National Biological Service, and Southwest Texas State University. The scope of work is attached in the appendices.

Texas blind salamanders and San Marcos salamanders will be collected from DS, SWT using drift nets. The nets should be checked daily and left in place until adequate numbers are obtained. The pump at DS will need to be running to increase collection rates from this site. Additional San Marcos salamander individuals from Spring Lake and the San Marcos River habitat, and Comal salamanders from the Comal Springs area should be collected using dip nets.

Salamanders that are collected are to be immediately placed in containers of fresh spring water and put in coolers with ice packs (the ice packs should not be in direct contact with the containers holding the salamanders) to maintain the water temperature at around 21°C. The number of salamanders per container will depend on the size of the container, but the salamanders should not be crowded and Texas blind salamanders should be separated by size class (see set-up and maintenance below) (if salamanders will be in the transport containers for more than a few hours, battery-operated air pumps should be used periodically to aerate the water).

Each container should be labeled with the name of the collector(s), exact collection site, date and time (start-end), and number of salamanders.

Salamanders should then be housed at a nearby facility for at least a week to check their condition prior to shipping to refugia. The temporary facility should have the same set up as the refugia (see below). To reduce the chance of mortality, the following is a prioritized list of shipment possibilities. Implementation of these procedures will depend on funding limitations.

1. First priority (especially for Texas blind salamanders) -hand-carry salamanders via motor vehicle (if within driving distance) or air-pressurized cabin aboard a major aircraft (if long distance).
2. Second priority - ship salamanders via Federal Express or other overnight mail service. Salamanders should be packaged as described above. The top of the shipment box should be clearly labeled "This side up".
3. Third priority - ship salamanders via air freight, with a designated person to pick salamanders up upon arrival. Shipment procedures are the same as for #2.

Set up and Maintenance of refugia salamanders

Refugia should provide an open well system with water chemistry and temperatures similar to that of the Edwards aquifer (to increase chances for reproductive success and significantly reduce maintenance costs) or be equipped to create similar conditions. Refugia should also be able to provide a reliable water supply in terms of quantity and not be vulnerable to depletion and contamination. If on a closed system, water in the tanks and artificial upwellings will need to be changed at least every other day, and susceptibility to mortality from fungus and increased handling will be greater. Adult mortalities have occurred from shipping, excessive handling, poor water quality, changes in water quality and temperatures, maintenance equipment failure (pumps), and other factors.

Back-up Stocks:

Salamanders should be stocked in glass aquaria at appropriate levels to minimize the incidence/spread of fungal infections, disease, overcrowding, etc. Stocking rates of about one salamander per every two gallons (i.e., 5 per 10-gallon aquarium, 10 per 20-gallon, 15 per 30-gallon, 30 per 60-gallon, etc.) appears to be adequate. At least two air pumps should be kept running for each aquarium in case one fails. Pea gravel and limestone rock shards should be placed in the bottom of each aquarium to provide a variety of hiding spaces for the salamanders. Any progeny noticed should be removed and maintained separately from the original wild-caught adults used to establish the standing stocks.

Until further research is conducted to determine the level of genetic variation among known localities, individuals of the same species collected at different sites should be kept separate in refugia (populations may be kept at the same refugium, but kept separate there). In addition to separating by collection sites, the Texas blind salamander should be separated into approximately four size classes (1-2 cm, 2-4 cm, 4-8 cm, and 8->11 cm) to avoid cannibalism.

At least one extra aquarium should be kept up and running for emergency backup for each species.

Care should be taken to keep lids on the tanks so that salamanders do not crawl out.

Because spinal curvature in the Texas blind salamander may be due to dietary deficiency, this species should be fed a diet high in calcium (i.e., brine shrimp, amphipods, and newly hatched snails). No black worms should be fed to this species. The San Marcos and Comal Springs salamanders should be fed a diet primarily of amphipods (these may be easily cultured in the same refugia), but may also be fed brine shrimp and black worms.

Breeding Stocks:

Set-ups for the Texas blind salamander would be the same as for the back-up stocks.

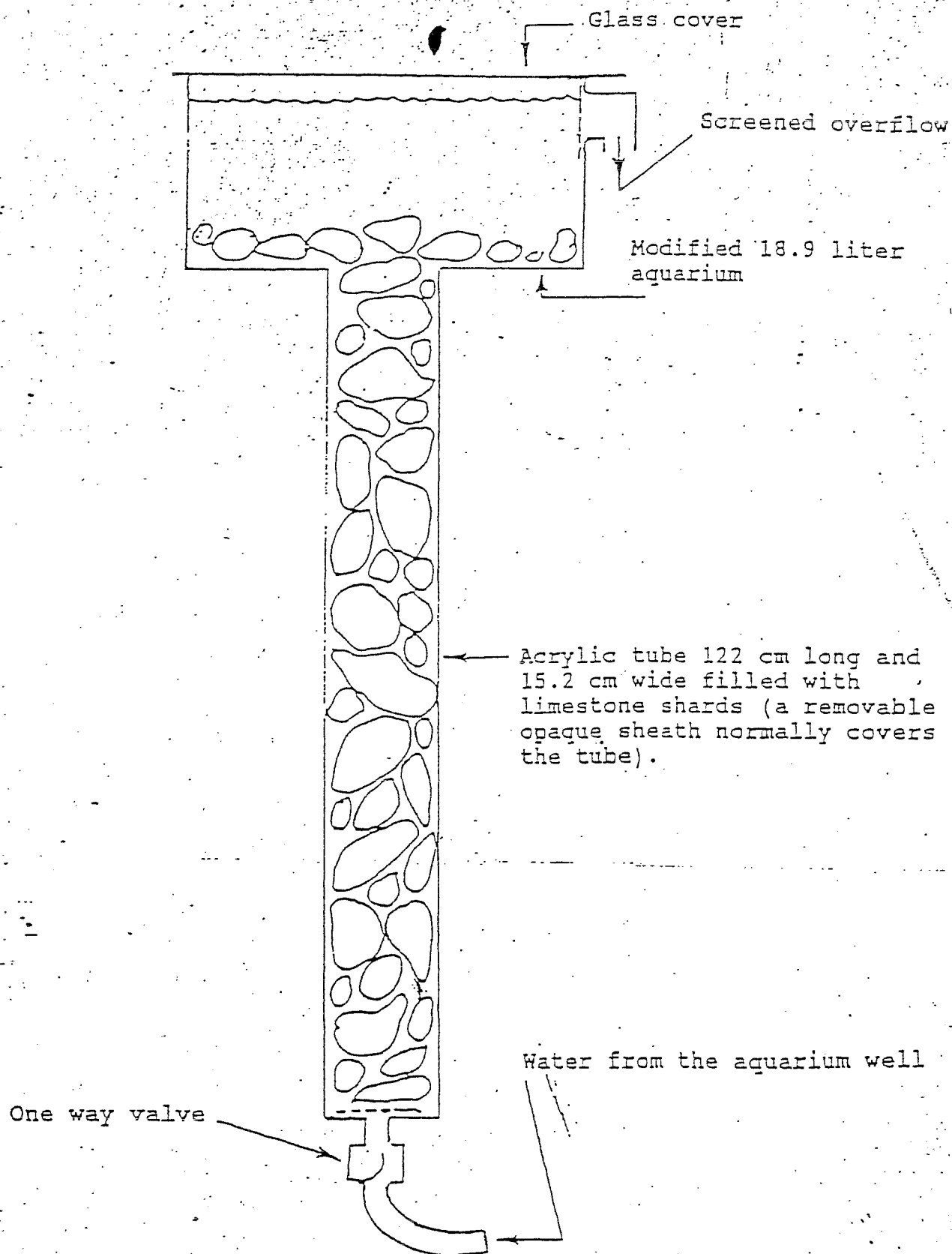
The San Marcos and Comal Springs salamanders should be placed in artificial spring upwellings (Figure 5) at a stocking rate of six salamanders per upwelling. The Dallas Aquarium maintains flows through their upwellings at about 5500 ml/min.

At least one extra aquarium or upwelling should be kept up and running for emergency backup for each species.

In addition to the Dallas Aquarium, which currently has individuals of all three species, possible refugia may include the Cincinnati Zoo, SMNFH&TC/NBS, San Antonio Zoo, and Toronto Zoo.

Schematic Design of Artificial Aquifer

Fig. 5



INVERTEBRATES

Three species of aquatic invertebrates at Comal Springs have been proposed for listing as endangered species. The Comal springs dryopid beetle (Stygoparnus comalensis) and Peck's cave amphipod (Stygobromus pecki) are both subterranean species and may have some ability to retreat into the aquifer as springflows drop below the spring outlets at Comal Springs. The third species, the Comal Springs riffle beetle (Heterelmis comalensis) is a surface species and as such is more vulnerable to drying of its springrun habitat as springflows drop. The contingency plan for the Comal invertebrates will focus on the riffle beetle.

Maintaining riffle beetles for an extended period in captivity would be an experimental effort since riffle beetles have not been maintained or bred in captivity. In the event that springflow ceases in any of the springruns that provide habitat for the beetles, the best opportunity for captive propagation attempts would be through setting up raceways or flow-through tanks at the Center in an attempt to keep a beetle population going. If undertaken, this would be a pilot project with Joe Fries at the Center and possibly an intern or graduate student from the University. This project should begin soon. The Center has the expertise to set up and maintain raceways that may support the Comal Springs riffle beetle. A raceway at the Center could provide the water quality and natural lighting that would increase the chances that the beetle could be maintained in captivity.

This pilot project should involve trying to simulate the springrun environment complete with rocky substrate obtained from Comal Springs (and probably supplemented by similar dry rocks from the margins of the springs). Three raceways 15 feet by 3 feet should be prepared prior to a drop in spring flows. This would allow time for imported substrate to age and to establish vegetation and algae from Comal Springs to increase the likelihood that transplant of the beetles would be successful.

They require a rocky substrate in cool, flowing water and this could best be simulated by setting up raceways with constantly flowing water. Adults would need to be collected before the springs go dry and as many Heterelmis as possible separated from other riffle beetle

competitors that also occur at the springs. Larvae should be collected to the greatest extent possible and would need to be separated under a scope from other riffle beetle larvae. One consideration to keep in mind while developing captive propagation techniques: often in insects taking the larvae out of their natural environment shocks them into early pupation and this may reduce the chances of survival.

Additional funding is needed to carry out this pilot project and attempts should be made to locate a source of funds.

In absence of funding for the pilot project, an effort should also be made to keep beetles alive in standard aquaria in a laboratory setting with simulated natural lighting until attempts could be made to reintroduce them into the springruns, if necessary. However, this approach is considered unlikely to succeed given the lack of facilities and resources to provide temperature-controlled, flowing water, natural lighting, actively growing vegetation, and other aspects of the springrun habitat that may be important to beetle survival.

V. Restoration or Restocking Into the Wild

The status of species of concern in the wild needs to be evaluated throughout the refugium process. Before a final decision on reintroduction is made, additional evaluations and discussion are needed. Decisions regarding restoration or reintroduction will have to be made in the future based on habitat conditions, species survivorship, success of the captive propagation program, condition of the individuals available and knowledge of the genetics and biology of the species developed up to that point. An in depth evaluation will be needed that examines how secure sustained flows are, overall river conditions, and species-specific biological issues.

Specific guidance and strategies for reintroduction will need to be developed addressing issues such as whether wild-caught individuals from captive stocks should be returned to the wild or only captively propagated materials, how to ensure organisms used are disease free, whether to use pooled or site-specific genetic stocks, criteria for success, precision of

MAY 1996

monitoring needed, and other questions. It is impossible at the present time to realistically outline a course of action.

BIBLIOGRAPHY

- Falk, D.A. and K.E. Holsinger. 1991. Genetics and Conservation of Rare Plants. Oxford University Press, New York.
- Harper, J. L. 1977. Population Biology of Plants. Academic Press, New York.

APPENDICES

APPENDIX I FISH

List of Cooperators and Contact Information

San Marcos Nat. Fish Hatchery & Tech. Center
500 East McCarty Lane
San Marcos, Texas 78666
contacts: Tom Brandt, Joe Fries, Casey Berkhouse
(512) 353-0011 - voice
(512) 353-0856 - fax

USFWS Ecological Services
10711 Burnet Road, Suite 200
Austin, Texas 78758-4455
contacts: Patrick Connor (ext 18)
(512) 490-0057 - voice
(512) 490-0974 - fax

Texas Parks and Wildlife Department
Resource Protection, Freshwater Studies Program
300 C.M. Allen Parkway, Building B
San Marcos, TX 78666
contact: Randy Moss
(512) 754-6844- voice
(512) 353-3484- fax

Uvalde National Fish Hatchery
P.O. Box 708
Uvalde, Texas 78802
contact: Manuel Ulibarri, Hatchery Manager
(210) 278-2419 - voice
(210) 278-6042 - fax

Equipment and Funding Needed

The Center currently does not have a back-up well. \$50,000 is needed to get this secondary well up and running. A generator system is needed to provide back-up power if needed. Security fencing for the refugium area is needed.

Staff Requirements

Note: All staff time needed is expected to be absorbed/donated by the participating cooperator/institution.

Depending on the number of stocks maintained, four full time equivalent positions would be needed to breed, feed, and maintain five refugia stocks for the Comal and San Marcos.

Proposals for Additional Work

- A more intensive baseline sampling and analysis of current genetic variability in fountain darter populations in the Comal and San Marcos are needed. This would provide a basis for evaluation of the success of the captive propagation program in maintaining genetic diversity and in making decisions needed for developing a restoration/reintroduction plan. It should be developed and funded as soon as possible given current low-flow conditions so that the opportunity to collect this information is not lost.
- A study of the genetics of the salvage population at the Center over time would be very helpful in resolving whether it is possible to maintain the wild genetic integrity of the darter in large (max 2,000) pooled and panmictically breeding captive populations.
- For each designated primary refugium stock, a subset of 60 fish should be frozen and placed in an ultracold freezer for isozyme comparison with the progeny after one or two years in captivity.
- San Marcos gambusia - see following proposal



DEPARTMENT OF BIOLOGY

THE UNIVERSITY OF TEXAS - PAN AMERICAN

1201 West University Drive, Edinburg, Texas 78539-2999 • (210) 381-3537 • FAX (210) 381-3657

MEMORANDUM

DATE: May 7, 1995
TO: Kathryn Kennedy, U.S.F.W.S.
FROM: Bob Edwards, David Bowles, Bobby Whiteside
and Gary Garrett
RE: "Search" for *Gambusia georgei*/
Removal of a Test Patch of Elephant Ears in the
San Marcos River

To: Kathryn Kennedy

3 pages

At the meeting in Austin concerning the Contingency Plan in the San Marcos/Comal Recovery Plan, several subcommittees were set up. One was to provide a basic research proposal (ideas) on how to go about searching effectively for *Gambusia georgei*. Below is an outline of our ideas on how to combine a removal of some of the elephant ears in a test patch in order to concentrate *G. georgei* (obviously, if they're still there) with a subsequent search for the San Marcos gambusia.

San Marcos *Gambusia* (*Gambusia georgei*) Search Study Outline

I. Remove *Colocasia esculenta* (elephant ears) from the area surrounding IH-35 downstream to Thompson's Island on both banks. Time span: One calendar year. January through December.

Rationale: There is likely some concern among local residents about "how beautiful the elephants are near the river." The area covered is used primarily by recreationists and fishermen and thus, is in a less visual region of the San Marcos River than more upstream localities and is in the center of the range of *Gambusia georgei*, whose low abundance has been attributed to increases in *Colocasia* abundance.

a) Wick *Colocasia* with a herbicide such as Rodeo in the late winter (= January, February and March) during an initial treatment phase. These months were picked to take advantage of the lessened biomass during the winter season and immediately prior to the spring-time growth surge. The manufacturer of the herbicide (Monsanto) should be contacted for advice on when the best time to wick would be. For example, if the plants are essentially dormant during the late winter months, wicking would probably not be effective at this time. (Ken Fritz, U.S. Bureau of Reclamation, El Paso Office, has checked with Monsanto about Rodeo and its uses in respect to overgrown aquatic plants in the Phantom Cave area; he should be contacted by the USFWS for additional information). Also, elephant ears have a prominent corm (tuber). It will need to be determined whether Rodeo will translocate to the corm, and if so, will the corm be damaged. A recurring suggestion is to physically remove the elephant ears in some patches by extracting the corms. This has been attempted unsuccessfully in the past; it

does result in considerable instability of the river bank and subsequent erosion of the sides of the stream and pieces of the corm are enough to initiate new plant growth. Another suggestion that vinegar (acetic acid) might be used to kill elephant ears when sprayed on their leaves. This might be attempted in a test plot to see if it really works. A mild acid, such as vinegar, would likely not impact a highly buffered carbonate stream such as the San Marcos River.

b) Keep new growth controlled by repeated "spot" wicking throughout the calendar year (i.e. March through December) to keep area clear of new growth and incompletely killed plants. This would probably necessitate approximately monthly applications.

II. Sample for *Gambusia georgei* (San Marcos gambusia) in five to six established sites.

a) Prior to the wick application of herbicide to the elephant ears, make a series of three general fish collections (using seines and approximating the methods outlined in Edwards et al., 1980) in the San Marcos River between IH-35 and Thompson's Island in order to establish a baseline for fish abundances. These should be done in the fall prior to the beginning of the wicking of the elephant ears.

b) Repeat this series of general collections shortly after the wick procedures have been accomplished in order to establish a second baseline of fish abundances after the treatment.

c) Beginning in the December (one year after the initial wicking in order to allow the gambusia to reestablish in the cleared areas), and continuing through the next December (two years after wicking), collect by seining at five to six established sites. These sites should be in areas in which there are muddy, partially shaded areas where the San Marcos gambusia has an apparent preference.

d) Sample (census) the Texas wildrice populations in the study area at periodic intervals during the study to make sure that this species is not being adversely affected by the herbicide treatment. These intervals should be bimonthly or quarterly and the USFWS would need to contact the appropriate individuals or agencies to conduct these censuses.

III. Time Frame:

Year	Section	Accomplishment	DATE
1	Ia		Jan-Mar/1996
1	Ib		Mar-Dec/1996
1	IIa		Sept-Dec/1995
1	IIb		Apr-Jun/1996
2	IIc		Dec/1996-Dec/1997
1,2	IId		Jan/1996-Dec/1997

Literature Cited

Edwards, Robert J., Edie Marsh and Clark Hubbs. 1980. The status of the San Marcos gambusia, Gambusia georgei. Endangered Species Report No. 9, U.S. Fish and Wildlife Service, Albuquerque, N.M., 34 pp.

APPENDIX II TEXAS WILD-RICE

COOPERATORS/CONTACT INFORMATION:

Texas Parks and Wildlife Department

Jackie Poole

Endangered Resources Branch

3000 So. IH-35, Suite 100

Austin, TX 78704

(512) 912-7019

possibly also Gena Janssen 912-7043, and David Hernandez or Shannon Breslen

Southwest Texas State University:

Paula Power

Aquatic Station

H.M. Freeman Aquatic Biology Building

601 University Drive

San Marcos, TX 78666-4616

(512) 245-7726

U.S. Fish and Wildlife Service:

Kathryn Kennedy

10711 Burnet Road, Suite 200

Austin, TX 78758

SMNFH&TC

Tom Brandt and Joe Fries

500 East McCarty Lane

San Marcos, TX 78666

(512) 353-0011

Uvalde National Fish Hatchery:

Manual Ulibarri
Manager, Uvalde National Fish Hatchery
P.O. Box 708 (Old Eagle Pass Road, FM 481)
Uvalde, TX 78802
(210) 278-2419

Possible Back-Up Facility:

TPWD, Heart of the Hills Research Station:

Gary Garrett
Heart of the Hills Research Station
Junction Star Route 62
Ingram, TX 78025
(210) 866-3356

FUNDING AND EQUIPMENT NEEDS, TEXAS WILD-RICE**Collection - none of this equipment is yet on hand; approximately \$150**

sharpened sharpshooter shovels (2), and knives for close work- KK has one shovel, will try to borrow another. Cost to purchase is approximately \$20. Knives should not be a problem. coolers (8-12) (about 30-50 gallons) probably purchase styrofoam coolers @ approx. \$7 each or about \$70-85

2-3 buckets to cart water to the coolers in/by the vehicles (KK has 2, should be able to borrow more if needed)

azalea pots 50-75, plastic, 1 gallon at 25cents each---approx \$20

potting media, collected from the river and stored (**any special storage requirements? how do you dry it out?**). This needs to be done in advance, probably need assistance from P. Power.

tags for the plants/pots, markers-- \$15

"accession forms" and maps to detail collection information (develop internally in cooperation w/TPWD)

Set up and maintenance - SMNFH&TC-\$3,850 Uvalde \$10,251 Total \$14,001

rectangular tanks (9 total are needed for each facility, SMNFH&TC has 6. **We would need 3 more for SMNFH&TC and 9 for Uvalde for a total of 12 additional).** w/expedited shipping to Uvalde = \$500 5,064 for Uvalde, \$2,055 for San Marcos

pumps- Uvalde needs 9 at \$200, \$1800 for Uvalde

plumbing - \$500 for San Marcos, \$700 for Uvalde

plexiglass sheets 4 per set up-\$ 250 for San Marcos, \$250 for Uvalde

plant sprayers, Tank type, dedicated to rice use (NO HERBICIDE OR OTHER PESTICIDES) **not yet procured** \$45.00 x 2 = \$90

liquid detergent dishwashing soap (should not be a problem)

inflorescence bags (these can be made from sheer Dacron curtain material or panty hose).

seed storage aeration materials, etc. (if Section 6 project does not continue, seed storage is only funded through August 1996) **not yet procured, but not a problem for immediate future.**

Thermometers (hatcheries should be able to cover this)

Possibly shade cloth and any necessary framing **not yet procured**

Uvalde \$795-screen in refugia area and get shade cloth if needed-

TEXAS WILD-RICE ESTIMATED STAFF TIME NEEDED

All staff needs will be absorbed by the agencies contributing staff or be voluntary.

collection time: allow 2 days with 4 people

probably K. Kennedy (FWS Austin ES), one TPWD person (J. Poole?), and two others (Probably Paula Power and another USFWS person or state person). Includes collection and transport to the hatcheries.

initial set up: 4 days, 1-2 people at each of 2 facilities.

maintenance: .1 to .2 FTE (at each of two facilities)

on an ongoing basis, with heaviest concentration during flowering and seed processing. **Uvalde plans on using Americorps volunteers, and SMNFH&TC has interns from the Southwest Texas State University available.**

Texas Wild-Rice Experts

For consultation on cultivation, problems, etc:

Paula Power, Southwest Texas State University

see cooperators sheet

Jackie Poole, Texas Parks and Wildlife Dept.

see cooperators sheet

Erwin Oelke

Dept. of Agronomy and Plant Genetics

411 Borlaug Hall

U. of Minnesota

St. Paul, MN 55108

(612/625-1211 or -1784)

Data Sheet for Monitoring Zizania texana
at low flows in the San Marcos River
(after Paula Power)

Date: _____ Time: _____ cfs: _____
(determined later
from: _____)

Site Description:(w/TPWD segment and area of stand numbers if possible)

Data collection by:

Current:

Depth:

```
#recreational users within 10m of stand:
```

waterfowl within 10m of stand:

Species of waterfowl:

% leaves and culms grazed:

General appearance (estimates)

leaf length

stem density

size of stand

of individual clumps in stand

Comments/Observations

MAY 1996

COLLECTION DATA FOR TEXAS WILD-RICE

Date_____

Collectors_____

TPWD segment_____

TPWD stand number_____

of collections taken

Destination of specimens

Photos taken?_____

Notes: sketches, etc.

Appendix III- Central Texas Salamanders
Contingency Plan Cooperators

SWTSU (Glen Longley, 512/245-2329)
Texas Parks and Wildlife Department (Andy Price, 512/912-7022)
Univ. of Texas at Arlington (Paul Chippindale, 817/273-2703)
SMNFH&TC (Casey Berkhouse and Joe Fries, 512/353-0011)
USFWS, Austin ES (Lisa O'Donnell, 512/490-0057)

Potential Refugia Facilities for Salamanders

San Marcos National Fish Hatchery & Technology Center (Casey Berkhouse and Joe Fries
512/353-0011)
Cincinnati Zoo (Johnny Arnett and Ed Maruska, 513/281-4701)
Dallas Aquarium (Lynn Ables and Streett Coale, 214/670-8451)
San Antonio Zoo (Russell Smith, 210/734-7184, ext. 115)
Toronto Zoo (Bob Johnson, 416/392-5968)

Manpower Estimates for Maintenance/Culturing of Salamanders

All staff time needed is expected to be absorbed/donated by the participating cooperator or institution.

The number of FTE's needed to maintain/culture each species in one refugium (based on 30-50 individuals in 3-8 separate tanks/upwellings, an average of about 20-30 minutes/day for maintenance on an open well system; 3 days for initial setup; 3 days for public relations):

0.011 (0.32 total)

FTE's needed to maintain/culture each species in one refugium (based on an average of about 1-1.5 hours/day for maintenance on a closed well system; 3-5 days for initial setup; 3 days for public relations):

0.20 (0.62 total)

ONGOING SALAMANDER RESEARCH:

- o The FWS has an interagency agreement with the NBS SMNFH&TC for \$5,260 to collect the Texas blind salamander and San Marcos salamander for refugia.
- o NBS has a cooperative agreement with SWTSU for \$15,160 (which includes the \$5,260 listed above from the FWS) to collect San Marcos, Texas blind, and Comal salamanders and to locate and coordinate delivery to refugia.
- o NBS has a cooperative agreement with Paul Chippindale, University of Texas at Arlington, for \$4,000 to help fund genetic work.

Equipment Needs/Cost Estimates for Salamander Refugia

Breeding Stocks

100 individuals of each species: approx. 1 Tx blind salamander for every two gallons (i.e., 5 per 10-gallon aquarium, 10 per 20-gallon, 15 per 30-gallon, 30 per 60-gallon, etc.); 6 San Marcos/Comal Springs salamanders per upwelling

Nine 10-gallon aquaria per refugia for Tx blind salamander to allow for separation among collection sites (2) and size classes (approx. 4: 1-2, 2-4, 4-8, 8- > 11 cm) plus at least one extra that is kept up and running for emergency backup

equipment needs/cost estimates (based on prices from local pet store)

aquaria

10-gallon glass aquarium kit	\$50.00
(includes air pump, air tubing, filters; price may be reduced to \$25.00 by using sponge filters and smaller air pumps)	

pea gravel (approx. 1/8 inch)	10.00/25 lb
(need 15lb/10-gallon tank; cheaper if obtain from quarry)	

extra air pump for each 10-gallon aquarium	7.00
(needed in case one breaks down; run both at same time)	

food	free - \$8.00/.5 lb
(will support about 40 animals for 3-4 weeks; brine shrimp, amphipods, newly hatched snails)	

limestone rock shards	for cover	free
-----------------------	-----------	------

Six upwellings per refugia for San Marcos salamander to allow for 6 salamanders per upwelling plus at least one 10-gallon aquaria for setup/emergency backup

equipment needs (based on estimates from Dallas Aquarium)

upwellings	\$250.00/ea
------------	-------------

see cost estimates above for aquaria

food	free - \$8.00/.5 lb
(brine shrimp, black worms, amphipods)	

Six upwellings per refugia for Comal Springs salamander to allow for 6 salamanders per upwelling plus at least one 10-gallon aquaria for setup/emergency backup equipment needs same as for San Marcos salamander, above

Total estimated cost (for all three refugia):

33 10-gallon aquaria kits (other sizes may also be used)	\$1,650.00
extra pumps for 10-gallon aquaria	231.00
pea gravel (approx. 495 lbs)	198.00
36 upwellings	9,000.00
food for 300 animals for one year	780-1,035.00

total	\$ 11,859-12,114.00
-------	---------------------

Back-up Stocks

200-400 individuals of each species: approx. 1 salamander for every two gallons (i.e., 5 per 10-gallon aquarium, 10 per 20-gallon, 15 per 30-gallon, 30 per 60-gallon, etc.)

Nine 20-gallon or nine 30-gallon aquaria per refugia for the Texas blind salamander to allow for separation among collection sites (2) and size classes (approx. 4: 1-2, 2-4, 4-8, 8->11 cm) plus at least one extra that is kept up and running for emergency backup

Four to six 60-gallon aquaria per refugia for the San Marcos salamander to allow for separation among collection sites (3), plus at least one extra that is kept up and running for emergency backup

Three to six 60-gallon aquaria per refugia for the Comal Springs salamander to allow for separation among collection sites (2), plus at least one extra that is kept up and running for emergency backup