



EDWARDS AQUIFER HABITAT CONSERVATION PLAN PROGRAM

CONTRACT NO. 16-822-HCP

BETWEEN THE

EDWARDS AQUIFER AUTHORITY

AND THE

UNITED STATES FISH & WILDLIFE SERVICE,
REGION 2, FISH AND AQUATIC CONSERVATION OFFICE

FOR THE

IMPLEMENTATION OF THE REFUGIA PROGRAM UNDER THE
EDWARDS AQUIFER HABITAT CONSERVATION PLAN

THIS CONTRACT is made and entered into by and between the EDWARDS AQUIFER AUTHORITY (“EAA”), a political subdivision of the State of Texas, with its principal place of business located at 900 E. Quincy, San Antonio, Texas 78215, and the United States Fish & Wildlife Service, Region 2, Fish and Aquatic Conservation Office (“USFWS”), a bureau within the U. S. Department of Interior and an agency of the federal government with its principal place of business located at 500 Gold Avenue SW, Room 9100, Albuquerque, New Mexico 87103-1306. Each of these entities is, at times, referred to in this Contract individually as a “Party,” and both are referred to collectively as “Parties.”

RECITALS

WHEREAS, the EAA was created by the Edwards Aquifer Authority Act, Act of May 30, 1993, 73rd Leg. R.S. ch. 626, 1993 Tex. Gen. Laws 2350, as amended (“Act”); and

WHEREAS, the USFWS issued to the EAA, among others, the ITP under Section 10(a) of the ESA, requiring it to implement the EAHCP; and

WHEREAS, Paragraph K of the ITP, and Sections 5.1.1 and 6.4 of the EAHCP relate to the Refugia Program, which generally provide for the maintenance of a refugia population of the

Covered Species including the propagation and restocking of the species if extirpation occurs, and which is required to be implemented by the EAA; and

WHEREAS, this Contract provides that the USFWS will provide for the maintenance of a refugia population of the Covered Species, including the propagation and restocking of the species if extirpation occurs, with funding to be provided by the EAA; and

WHEREAS, Section 7 of the Fish and Wildlife Act of 1956 Act (16 U.S.C. 742f(a)(4)) authorizes the USFWS to provide for the development, advancement, management, conservation, and protection of the fisheries resources, and to take steps as may be required for the development, advancement, management, conservation, and protection of fish and wildlife resources, including through research; and

WHEREAS, the P.L. 106-113 Appropriations Act for the Department of the Interior and Related Agencies, 2000 authorizes the USFWS to provide reimbursable specialized or technical services to State or local governments; and

WHEREAS, the EAA Board of Directors approved this Contract on November 8, 2016 by the adoption of Resolution and Order No. _____; and

WHEREAS, it is in the public interest that the EAA and the USFWS enter into this Contract.

AGREEMENT

NOW THEREFORE, for and in consideration of the mutual promises and agreements set forth in this Contract, the EAA and the USFWS agree as follows:

ARTICLE 1 – DEFINITIONS

Section 1.1. Definitions. The following terms as used in this Contract have the meanings provided for in this section:

(a) “Aquifer” means the Southern (or San Antonio) Segment of the Edwards Aquifer within the boundaries of the EAA, which is that portion of an arcuate belt of porous, water-bearing, predominately carbonate rocks known as the Edwards and Associated Limestones in the Balcones Fault Zone extending from west to east to northeast from the hydrologic division near Brackettville in Kinney County that separates underground flow toward the Comal Springs and San Marcos Springs from underground flow to the Rio Grande Basin, through Uvalde, Medina, Atascosa, Bexar, Guadalupe, and Comal Counties, and in Hays County south of the hydrologic division near Kyle that separates flow toward the San Marcos River from flow to the Colorado River Basin.

(b) “Covered Species” means those species identified in Paragraph G of the ITP.

(c) “EAHCP” means the conservation plan required by Section 10(a)(2)(A) of the ESA, entitled “Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan (November 2012),” as amended, which was approved by the USFWS as part of the application for the ITP.

(d) “ESA” means the federal Endangered Species Act of 1973, 16 U.S.C. §§ 1531 - 1544, as amended.

(e) “Refugia Facilities” or “Facilities” means the facilities, plants, works, improvements, land, easements, rights of way, any other interests in land, and water rights owned by the USFWS and set forth and described in the Refugia Facilities, which is attached as Exhibit A, where the Services will be performed.

(f) “FMA” means that certain Funding and Management Agreement, effective January 1, 2012, as amended, to which the EAA, the City of New Braunfels, the City of San Marcos, the City of San Antonio, acting by and through its San Antonio Water System Board of Trustees, and Texas State University – San Marcos are parties, and which provides for the funding and management of the EAHCP, incorporated herein by reference for all purposes.

(g) “ITP” means Incidental Take Permit No. TE63663A-1 originally issued by the USFWS on February 5, 2013, to be effective on March 18, 2013, pursuant to Section 10(a) of the ESA, as amended.

(h) “Petitioned Covered Species” means the Texas Cave Diving Beetle (*Haideoporus texanus*) (also sometimes referred to as the Edwards Aquifer Diving Beetle), the Comal Springs Salamander (*Eurycea sp.*), and the Texas Troglotic Water Slater (*Lirceolus smithii*).

(i) “Refugia Program” means the off-site refugia activities described in Paragraph K of the ITP and Sections 5.5.1 and 6.4 of the EAHCP, and any approved associated applied research activities.

(j) “TPWD” means the Texas Parks and Wildlife Department.

ARTICLE 2 – TERM

Section 2.1. Term. This Contract is effective and commences on January 1, 2017 (“Effective Date”), and terminates on March 31, 2028 (“Expiration Date”).

Section 2.2. Renewal. This Contract may be renewed and extended for such period as may be agreed to in writing by the Parties.

ARTICLE 3 – SERVICES TO BE PERFORMED BY THE USFWS

Section 3.1. Services. Subject to the terms and conditions of this Contract, the EAA engages the USFWS to perform the work set forth and described in the Scope of Work, which is

attached as Exhibit B (“Services”). The USFWS accepts this engagement and will furnish any and all labor, personnel, machinery, equipment, appliances, materials, tools, facilities, plants, works, improvements, land, easements, rights of way, any other interests in land, water rights, and permits, licenses or other approvals, necessary for its performance of the Services. The EAA will reimburse the USFWS for furnishing these items as provided for in Article 4 of this Contract.

Section 3.2. Performance. The USFWS warrants and represents that it will devote its best efforts and abilities to the performance of the Services and will perform them in a good and workmanlike manner, in conformance with the requirements of this Contract, and in accordance with applicable federal, state, and local law.

Section 3.3. Commencement of Services. The USFWS will commence performing the Services immediately upon the date of receipt of the written notice to proceed issued by the EAA’s General Manager.

Section 3.4. Completion of Services. All Services will be completed and delivered to the EAA by the Expiration Date, and shall be completed during the term of this Contract in compliance with the schedule set forth and described in the Project Schedule, which is attached as Exhibit C. It shall be the USFWS's sole responsibility to ensure that the completion times for the Services set forth in Exhibit C are met.

Section 3.5. Time of the Essence. Time is of the essence in the performance of the Services.

ARTICLE 4 – COMPENSATION

Section 4.1. Total Compensation. (a) Subject to subsections (b)-(d), the total compensation payable to the USFWS for the performance of the Services shall in no event exceed \$ 18,876,267

(b) The amounts paid to the USFWS for the performance of the Services shall in no event exceed the amounts budgeted for specified tasks set forth and described in the Budget, which is attached as Exhibit D, unless previously approved in writing by the EAA.

(c) The amounts paid to the USFWS for the performance of the Services in a particular year during the term of this Contract shall in no event exceed the overall budgeted amounts, or the amounts budgeted for specified tasks, as set out in the Application for Program Funding approved by the EAA Board of Directors for that year, unless previously approved in writing by the EAA. The role of the USFWS in the preparation and processing of Applications for Program Funding is set forth in Subtask 5.2 of the Scope of Work in Exhibit B.

(d) Without the prior written approval of the EAA, the USFWS may not provide any Services that exceed the amounts described in subsection (a)-(c). The USFWS will be solely responsible for the payment of any and all fees, expenses, construction, or other costs incurred by it that exceed these amounts. The USFWS may not hold the EAA accountable for the payment of

any unauthorized work performed, or funds expended by the USFWS in excess of these amounts in the performance of the Services.

Section 4.2. Fees for Labor and Personnel. Subject to Section 4.1, the EAA agrees to pay the USFWS fees for labor and personnel necessary for the performance of the Services. The fees to be paid are those set forth and described in the Annual Work Plan and Cost for each year as described in Subtask 5.2 of the Scope of Work in Exhibit B and made a part of the Application for Program Funding approved by the EAA Board of Directors for that year. The USFWS may invoice the EAA for its personnel direct and indirect costs according to Exhibit 2 of USFWS Policy 264 FW 1 (09/24/15).

Section 4.3. Expenses. Subject to the provisions of Section 4.1, the EAA agrees to reimburse the USFWS for any expenses incurred that are necessary for the performance of the Services as set forth and described in the Annual Work Plan and Cost Estimate for each year as described in Subtask 5.2 of the Scope of Work in Exhibit B and made a part of the Application for Program Funding approved by the EAA Board of Directors for that year.

Section 4.4. Machinery and Equipment. Subject to the provisions of Section 4.1, the EAA agrees to reimburse the USFWS for the acquisition of any machinery, equipment, appliances, materials, or tools that are necessary for the performance of the Services. Acquisition costs for the purchase of machinery, equipment, etc. may be invoiced to the EAA only if: (i) the machinery, equipment, etc. has been delivered to the USFWS and is in its title, custody, and possession; and (ii) the USFWS has furnished evidence satisfactory to the EAA that the delivered machinery, equipment, etc. conform to the specifications described in the Annual Work Plan and Cost Estimate for each year as described in Subtask 5.2 of the Scope of Work in Exhibit B and made a part of the Application for Program Funding approved by the EAA Board of Directors for that year.

Section 4.5. Facilities. Subject to the provisions of Section 4.1, the EAA agrees to reimburse the USFWS for the design, construction, replacement, or rehabilitation, and operation and maintenance of any new, replaced, or rehabilitated facilities, plants, works, or improvements that are necessary for the performance of the Services. Design, construction, replacement, or rehabilitation, and operation and maintenance costs may be invoiced to the EAA only if the USFWS has furnished evidence satisfactory to the EAA that the costs conform to the plans, specifications, and standards described in the Annual Work Plan and Cost Estimate for each year as described in Subtask 5.2 of the Scope of Work in Exhibit B and made a part of the Application for Program Funding approved by the EAA Board of Directors for that year.

Section 4.6. Permits. Subject to the provisions of Section 4.1, the EAA agrees to reimburse the USFWS for the acquisition of any permits, licenses or other approvals that are necessary for the performance of the Services. Acquisition costs for permits, licenses, or other approvals may be invoiced to the EAA only if the USFWS has furnished evidence satisfactory to the EAA that the permit, licenses or other approvals described in the Annual Work Plan and Cost Estimate for each year as described in Subtask 5.2 of the Scope of Work in Exhibit B and made a part of the Application for Program Funding approved by the EAA Board of Directors for that year.

Section 4.7. Invoices; Payment; and Retainage. Subject to the provisions of Section 4.1 all invoices from the USFWS to the EAA for the Services shall be billed on the last business day of the month and sent monthly and shall provide an itemization of the: (i) fees for the Services rendered; (ii) expenses incurred; (iii) costs of any machinery, equipment, appliances, materials, or tools acquired; (iv) Facility design and construction costs; (v) Facility operation and maintenance costs; and (vi) permit acquisition costs incurred during the service period. If the USFWS utilizes a subcontractor to perform part of the Services, a completed “Subcontractor Utilization Report” on a form prescribed by the EAA must accompany the applicable monthly invoice submitted to the EAA. The EAA will pay the USFWS within thirty (30) calendar days upon EAA receipt and approval of that invoice and supporting documents such as receipts, contracts (for the total amount of award), and subcontractor invoices etc.

Section 4.8. Disallowed Costs. The USFWS may not invoice, nor will the EAA pay, for any costs or expenses associated with the following activities:

- (a) Acquisition of any land, easements, rights of way, or any other interests in land, or water rights;
- (b) Production, treatment, distribution, or discharge of any water source other than from the Aquifer to house, conduct research on, sustain, manage, and propagate the Covered Species at the Refugia Facilities, unless previously approved in writing by the EAA;
- (c) Construction, completion, or support of water reuse infrastructure or capabilities at the Refugia Facilities, unless previously approved in writing by the EAA;
- (d) Depreciation;
- (e) Public education, public relations, advertising, or other outreach, unless previously approved in writing by the EAA;
- (f) Consultancy fees for legal, biological, geologic, hydrogeologic, auditing, legislative advocacy, public relations, or any other professional or non-professional services, unless previously approved in writing by the EAA;
- (g) Enforcement or compliance penalties, fines, or other assessments;
- (h) Debt service;
- (i) Unless rediscovered, any activity to research, investigate, collect, house, sustain, or propagate the San Marcos Gambusia (*Gambusia georgei*);
- (j) The disposition of individuals or populations of the Covered Species that are deemed unfit for release into the wild because they pose a risk to wild populations of the Covered Species as determined by the USFWS or the TPWD, unless such unfitness is foreseen in the management of the Species under the approved species management, propagation, genetics, and/or disposition plans, or other approved applicable plans or protocols, which are incorporated as a part

of any federal, state, or local permits that authorize the USFWS to perform the Services;

(k) Any activity associated with a Covered Species that may inadvertently or accidentally escape or be released from the Refugia Facilities due to the actions of the USFWS regardless of where the species may be found and regardless of whether it survives and/or breeds in the wild;

(l) Report preparation that is not required by the ITP, EAHCP, or the FMA, and this Contract;

(m) Publication or peer review of reports.

ARTICLE 5 – OWNERSHIP OF THE REFUGIA FACILITIES

Section 5.1. Equipment. All machinery, equipment, appliances, materials, and tools acquired by the USFWS to perform the Services will be owned by and be the property of the USFWS.

Section 5.2. Refugia Facilities. (a) The Refugia Facilities constructed or rehabilitated under this contract may only be used for performance of the Services to implement the Refugia Program. Unless required by federal law, or approved by the EAA, the USFWS may not repurpose any newly constructed buildings or rehabilitated buildings for uses other than those Services expressed herein while this Contract is in effect. If this Contract is renewed, or a new contract for the implementation of the Refugia Program is entered into with the USFWS, the USFWS must continue to use the Refugia Facilities to implement the Refugia Program. If the USFWS determines it needs to change the mission of the Refugia Facilities or is required to dispose of these lands, the USFWS agrees, within their best efforts, to provide the EAA with a minimum of one year of advance notice.

(b) All facilities, plants, works, improvements, land, easements, rights of way, other interests in land, and water rights, acquired or constructed by the USFWS at the Refugia Facilities to perform the Services will be owned by and be the property of the USFWS.

ARTICLE 6 – DOCUMENT MANAGEMENT

Section 6.1. Ownership of Documents. All data, information, documents, updates, plans, reports, studies, applications, or any other electronic or written materials (“documents”) produced, created, or supplied under this Contract by the USFWS, and whether finished or unfinished or in draft or final form, will be owned by and be the joint property of the USFWS and the EAA.

Section 6.2. Disclosure of Documents. Documents may be disclosed by the USFWS to any third party as may be required by federal law without the prior written consent of the EAA. Documents may be disclosed by the EAA to any third party as may be required by state law without the prior written consent of the USFWS. Both Parties shall promptly advise the other Party of any such document requests and fully cooperate with the other Party in the processing of such requests.

Section 6.3. EAA Review and Comment. Prior to preparing a final version of any reports or studies, and the publication of any report or study related to this contract, the USFWS shall first present the document to the EAA in draft form for review and comment. The EAA will submit reviews and comments to the USFWS no later than thirty (30) days. The USFWS will make all efforts to incorporate the comments of the EAA prior to going final and resolve any substantive issues in a good faith manner after which the USFWS may proceed, publish, or apply without prior written consent of the EAA. Specifically, with regard to applications for permits, licenses, or other approvals of an application, the USFWS is authorized to file such application with the relevant federal, state, or local agency to whom the application is required to be filed. Promptly after filing, the USFWS will provide a copy of the application to the EAA.

Section 6.4. Periodic Reports. The Parties agree to furnish all required periodic reports and consider requested special reports or information pertaining to this Contract as may be reasonably requested in writing. The EAA will reimburse the USFWS for the actual cost of time and expenses of preparation and production of a special report not otherwise required to be prepared by this Contract.

Section 6.5. Record Copies. The USFWS shall retain a record copy of all documents developed to implement this Contract for up to six years. Upon request of the EAA, copies of such documents will be promptly supplied to the EAA, including documents retained no more than six years after the Expiration Date or the termination of this Contract. The EAA will reimburse the USFWS for the actual cost of time and expenses of reproduction of such requested documents.

Section 6.6. Delivery of Documents upon Termination. Upon termination of this Contract, the USFWS will promptly deliver to the EAA a copy of all documents developed under this Contract that are not already in the possession of the EAA.

ARTICLE 7 – MISCELLANEOUS

Section 7.1. Authority to Contract. Each Party represents and warrants for the benefit of the other Party that: (1) it has the legal authority to enter into this Contract; (2) that it has the legal authority to perform the duties and responsibilities of each respective Party; (3) this Contract has been duly approved and executed; (4) no other authorizations or approvals are or will be necessary in order to approve this Contract or to enable that Party to enter into and comply with the terms and conditions of this Contract; (5) the person executing this Contract on behalf of each Party has the authority to bind that Party; and (6) the Party is empowered by law to execute any other agreement or documents and to give such other approvals, in writing or otherwise, as are or may hereafter be required to implement and comply with this Contract.

Section 7.2. Entire Agreement. This Contract and the attached Exhibits constitute the entire agreement between the Parties and there are no representations, warranties, agreements, or commitments between the Parties except as set forth herein.

Section 7.3. Amendments. (a) This Contract may be amended. No amendment to or rescission of this Contract shall be binding on the Parties unless reduced to writing and signed by the Parties.

(b) The Board of Directors of the EAA delegates to the EAA General Manager the authority to approve and enter into amendments to this Contract without further authorization by the Board consistent with the General Manager's authority to enter into contracts under Section 4.01 of the EAA's Bylaws.

Section 7.4. Officers or Agents. No officer or agent other than the Parties' authorized representatives is authorized to waive, amend, or modify any provision of this Contract.

Section 7.5. Notices. (a) All notices or communications under this Contract to the EAA shall be in writing and shall be sent to the EAA's principal place of business as follows, unless and until the USFWS is otherwise notified:

EDWARDS AQUIFER AUTHORITY
900 E. Quincy Street
San Antonio, Texas 78215
ATTENTION: ROLAND RUIZ, GENERAL MANAGER

(b) All notices or communications under this Contract to the USFWS shall be in writing and shall be sent to the address of the USFWS as follows, unless and until the EAA is otherwise notified:

SAN MARCOS AQUATIC RESOURCES CENTER
500 East McCarty Lane
San Marcos Texas 78666
ATTENTION: KENNETH OSTRAND, CENTER DIRECTOR

(c) Any notice or other communication provided for in this Contract to be given by a Party to the other Party will be in writing and may be given by depositing the same in the United States mail, certified with return-receipt requested, properly stamped and addressed to the Party to be notified. Notice may also be given by personal hand delivery, overnight delivery service, or facsimile. Notice deposited in the mail in the manner described herein will be conclusively deemed to be effective, from and after the expiration of three (3) business days after it is so deposited. Notice given by personal or overnight delivery or facsimile will be deemed to be effective upon the day of delivery. Notice may also be given by electronic communication but such communication is effective only upon the effective date of one of the other forms of delivery discussed above.

Section 7.6. Response Time. The Parties will use reasonable efforts to respond to written communications from the other Party within thirty (30) days.

Section 7.7. USFWS Independent Contractor. (a) The Parties understand and agree that this Contract does not create a fiduciary relationship between them, that they are separate entities, that the USFWS is an independent contractor with respect to the performance of the Services and is not subject to the direct or continuous control or supervision of the EAA, and that nothing in this Contract is intended to make either Party a subsidiary, joint venturer, partner, employee, agent, servant, or representative of the other Party for any purpose whatsoever. Except as provided in this section, the EAA shall have no right of direction or control of the USFWS.

(b) The EAA shall have the right of direction or control of the USFWS only relative to compliance with the terms and conditions of this Contract, and the results to be obtained in accordance with the Services. The EAA may inspect the progress of the Services, and receive reports as agreed to herein. The USFWS shall accommodate all reasonable requests from the EAA to allow EAA employees, agents, or representatives to accompany and inspect, observe, and monitor the USFWS in carrying out the Services.

(c) At the cost of the EAA, the USFWS will provide any and all labor and personnel necessary for its performance of the Services. The USFWS will be responsible for its employees and agents in all respects, including their compliance with all applicable laws and their safety, including without limitation, applicable Occupational Safety and Health Administration (“OSHA”) standards, requirements, and regulations.

(d) The USFWS may retain and utilize all subcontractors necessary for its performance of the Services. The USFWS, shall have the right to terminate, limit, or alter, at any time, the participation of any subcontractor if it reasonably determines that the subcontractor is not devoting its best efforts and abilities to the performance of the Services, or is not performing its activities in a good and workmanlike manner. The USFWS will be responsible for its subcontractors in all respects, including their compliance with all applicable laws and their safety, including without limitation, applicable OSHA standards, requirements, and regulations.

Section 7.8. Assumption of the Risk; Indemnification. (a) The USFWS shall assume all risks associated with and shall waive any claim against the EAA regarding the following:

(1) The USFWS’ or its subcontractors’ performance of the Services under this Contract;

(2) Damages arising out of the performance of the Services;

(3) The inadvertent, accidental, or otherwise unauthorized escape or release of any individual or population of Covered Species from the Refugia Facilities due to or as a result of the actions of the USFWS or its subcontractors.

(b) The USFWS shall indemnify, and hold harmless the EAA, its directors, employees, and agents from any and all claims, damages, loss, or liability of any kind whatsoever, including the costs of litigation and attorney’s fees arising from:

(1) The USFWS’ or its subcontractors’ performance of the Services under this Contract;

(2) Any contract or other arrangement between the USFWS and any third parties entered into in order to perform the Services;

(3) The inadvertent, accidental, or otherwise unauthorized escape or release of any individual or population of Covered Species from the Refugia Facilities due to or as a result of the actions of the USFWS or its subcontractors.

(c) It is specifically intended by the Parties that this assumption of the risk and indemnification is for Services to be performed by the USFWS in favor of the EAA as specified only in this Contract. Nothing contained in this Contract is intended to limit the authority of the USFWS to seek civil or criminal penalties or otherwise fulfill its enforcement responsibilities under the ESA and other applicable laws.

Section 7.9. Unauthorized Escape or Release of the Covered Species. The USFWS shall use best management practices to prevent the inadvertent, accidental, or otherwise unauthorized escape or release of any individual or population of Covered Species outside of the Refugia Facilities. Any authorized release of the Covered Species shall be limited to the spring systems at Comal Springs or San Marcos Springs at which the species were originally collected.

Section 7.10. Default – Notice and Opportunity to Cure. If any Party fails to perform any obligation or make any payment in the required amount when due under this Contract, the other Party shall provide written notice of default to the nonperforming Party. The nonperforming Party has thirty (30) business days to reply to the notice, and sixty (60) days from receipt of the notice within which to remedy the default, unless another time frame is agreed to by the Parties.

Section 7.11. Remedies. (a) The Parties recognize that failure in the performance of any Party's obligations hereunder may not always be measurable solely in money damages. Each Party, therefore, agrees in the event of any default on its part that each Party will have available to it, in addition to all other legal remedies, the equitable remedy of injunction, mandamus and/or specific performance, and, except as provided by Section 7.13, not termination, as long as the ITP is outstanding and in effect. It is the intent of the Parties that any default may be subject to the remedy of injunction, mandamus and/or specific performance to the extent that injunction, mandamus and/or specific performance is possible to remedy the existing circumstances.

(b) In the event of termination of this Contract under Section 7.13, such termination shall not constitute a waiver of any rights or remedies available at law or in equity to a Party. All remedies, either under this Contract or at law or in equity, or otherwise available to a Party, are cumulative and not alternative and may be exercised or pursued separately or collectively in any order, sequence or combination. In addition to these provisions, applicable provisions of this Contract shall survive any termination of this Contract.

Section 7.12. Mediation. (a) Either Party may request informal consultation with the other Party at any time to resolve a current or anticipated controversy arising under this Contract. Any such request will not be unreasonably refused by the other Party. The consultation shall be undertaken by both Parties in good faith and with due diligence to effect the purpose of this Contract.

(b) In the event any current or anticipated controversy arising under this Contract is not resolved by informal consultations between the Parties within thirty (30) business days after any Party requests consultations, then, upon the request of any Party, the controversy will be referred to mediation. The mediation process will continue until the sooner of: (i) no more than thirty (30) business days from the designation of the mediator; (ii) until the controversy is resolved; (iii) the mediator makes a finding that there is no possibility of settlement through mediation; or (iv) either Party chooses not to continue the mediation further. All costs and expenses of the mediation (including the mediator's fees) will be shared equally by the Parties, provided however, that costs incurred individually by each Party will be costs solely of such Party.

Section 7.13. Termination. (a) Except as provided in this section, neither Party may terminate this Contract.

(b) The EAA may, in its sole discretion, terminate this Contract at any time upon thirty (30) days prior written notice to the USFWS if: (i) the ITP terminates, and is not renewed or replaced by another ITP that requires the implementation of the Refugia Program; (ii) the ITP is amended such that it no longer requires the Refugia Program to be implemented; or (iii) the ITP is amended such that it no longer requires the EAA to implement the Refugia Program.

(c) The EAA may terminate this Contract at any time upon one year prior written notice to the USFWS if the Refugia Program is modified by the USFWS through the ITP, the EAHCP, or other permits issued under Section 10(a) of the ESA, and such modification results in a material conflict with this Contract, and the Parties, after attempting to resolve the issue in a good faith manner, are unable to renegotiate a mutually acceptable modification hereto that is consistent with the modified Refugia Program within 180 business days from the date the USFWS gives notice to the EAA that the Refugia Program has been modified.

(d) In the event the USFWS takes final action not to list a Petitioned Covered Species under Section 4 of the ESA, this Contract immediately terminates relative to such species upon such final action by the USFWS. The USFWS shall promptly give written notice to the EAA of such final action not to list a Petitioned Covered Species. Applicable provisions of this Contract shall survive any termination of obligations to Petitioned Covered Species under this Contract.

(e) This Contract shall immediately terminate if the ITP is no longer in full force and effect for any reason.

(f) Subject to Section 7.10, the USFWS may terminate this Contract if the EAA fails to make payment as required by Article 4.

(g) In the event for any reason the USFWS believes it has become legally unable to perform its obligations under this Contract due to an executive, administrative, legislative, or judicial action occurring after the effective date of this Contract, the USFWS may terminate this contract and shall attempt to give no less than one year advance written notice to the EAA of the effective date of such action.

(h) Upon receipt of a termination notice, the USFWS shall take all appropriate steps to

stop all work in progress, including all work performed by its subcontractors. Insofar as possible, all work in progress will be brought to a logical termination point. Within 60 days of the final invoice following termination, the EAA shall pay the USFWS all moneys then due and owing for the Services rendered, and expenses reasonably incurred up to the time of termination, plus termination costs. If a termination notice is issued under this section, the USFWS, in winding down its performance of the Services, shall devote its best efforts and abilities to remain in, or, if necessary, to come into compliance with all applicable federal laws, regulations, rules, and permits, and, if applicable, assist the EAA in the transition of the performance of the Services.

Section. 7.14. Damage or Destruction of the Refugia Facilities. (a) If all or part of the Refugia Facilities shall be damaged by wind, fire, flood, or any other casualty, or if the USFWS or the USFWS's employees, agents or subcontractors cause a casualty as a result of gross negligence or intentional misconduct, the USFWS must promptly notify the EAA of such casualty.

(b) The Parties expressly agree that the EAA benefits from and gains an advantage by the continued existence and operation of the Refugia Facilities for the purpose of implementing the Refugia Program, and that it would suffer a loss and be disadvantaged by the destruction of such Facilities in that the EAA would have a continuing legal duty to implement the Refugia Program. This loss would be compounded by the fact that the EAA would have paid a significant sum of public funds to design, construct, replace, and/or rehabilitate such Facilities. Accordingly, the EAA shall, at its cost, obtain property casualty insurance protecting the Refugia Facilities from damage for certain risks in a form determined to be suitable in the sole judgment of the EAA, and in so doing protect the EAA's interest in the Refugia Facilities. The insurance would provide that EAA has the right to direct payment for a loss payout under the insurance policy at the time an insured loss under the policy occurs. The policy will provide that the EAA will be free to use the proceeds to repair, replace, or rebuild the Refugia Facilities, or if the USFWS is unable to continue to perform the Services under this Contract, construct other facilities as in its judgment are suitable and appropriate to implement the Refugia Program. Not later than the date of receipt of the written notice to proceed under Section 3.3, the EAA will provide the USFWS with copies of the insurance policy and, if necessary, certificates of insurance.

(c) To the extent the Refugia Facilities cannot be occupied, or are unsuitable for the performance of the Services due to such casualty, the USFWS shall not invoice the EAA for any costs under Article 4 relative to the unoccupied or unsuitable facilities, until the USFWS has completed repairs and restorations such that the damaged facilities are once again suitable for the performance of the Services.

(d) Neither Party shall have the right to terminate this Contract due to the occurrence of a casualty under this section.

Section 7.15. Multiple Original Counterparts. This Contract may be executed in two or more counterparts, each of which shall be deemed an original but all of which together shall constitute one and the same instrument.

Section 7.16. Further Instruments. Each of the Parties will, promptly upon the request of another Party, execute, acknowledge, and deliver to the other Party any and all further instruments as are reasonably requested or appropriate to evidence or give effect to the provisions

of this Contract.

Section 7.17. Rights Regarding Books and Records. (a) Upon reasonable prior written notice, each Party will permit the other Party's authorized representatives to examine and copy all the books and records kept by the Party pertaining to this Contract.

(b) Upon reasonable prior written notice, a Party may conduct a complete audit of all books, accounts, and records of any kind kept by the other Party pertaining to this Contract, or any payments made hereunder, as well as the information and documentation used to prepare the books and records. Any such audit will be at the requesting Party's sole expense and will be prepared by a certified public accounting firm and/or registered professional engineer, or other appropriate professional such as USFWS Administrative officer as may be appropriate. If the audit report discloses actual errors in the books and records such that the charges assessed to the other Party are in error, then such error will be corrected for the period up to four years after the erroneous charge was paid, and all payments will be reconciled during the subsequent twelve-month period after the date of completion of the audit. If the error identified in the audit is greater than the cost of the audit, the audited Party will reimburse the requesting Party the cost of the audit.

Section 7.18. Access to Property. Upon reasonable prior notice, the USFWS agrees to allow the employees and duly authorized agents of the EAA to enter any facilities, plants, works, improvements, land, easements, rights of way, or other interests in land used by the USFWS to perform the Services, at any reasonable time for the purpose of inspecting and investigating conditions relating to the implementation of or compliance with the terms and conditions of this Contract. EAA employees and/or agents, while on the Refugia Facilities, shall observe the USFWS's rules, policies, and procedures concerning safety, internal security, bio-security and fire protection.

Section 7.19. Non-Waiver. No delay or failure by either Party to exercise any right under this Contract, nor any partial or single exercise of that right, shall constitute a waiver of that or any other right.

Section 7.20. Headings. Headings in this Contract are for convenience only and shall not be used to interpret or construe its provisions.

Section 7.21. Governing Law. This Contract shall be deemed to have been executed and performed in the State of Texas and shall be construed in accordance with and governed by the laws of the Federal Government and the State of Texas.

Section 7.22. Binding Effect; Delegation. The provisions of this Contract shall be binding upon and inure to the benefit of the Parties and their respective successors and assigns, provided, however, that the USFWS may not assign any of its rights nor delegate any of its duties under this Contract without the EAA's prior written consent.

Section 7.23. Validity. The invalidity of any provision(s) of this Contract shall not affect any other provision of this Contract, which shall remain in full force and effect, nor shall the invalidity of a portion of any provision of this Contract affect the balance of such provision.

Section 7.24. Non-Waiver of Immunity. Nothing in this Contract is intended as any waiver by the Parties of any immunity from suit to which they may be entitled under law.

Section 7.25. Attachments. The Exhibits or other documents attached hereto or referred to herein are incorporated herein and made a part of this Contract for all purposes. As used herein, the expression "Contract" means the body of this Contract and such Exhibits or other documents, and the expressions "herein," "hereof," and "hereunder" and other words of similar import refer to this Contract and such Exhibits or other documents as a whole and not to any particular part or subdivision thereof.

Section 7.26. Costs; Attorney's Fees. If any action at law or in equity, mediation, or other proceeding is brought to enforce or interpret a provision of this Contract, or because of an alleged breach or default relating to this Contract, the prevailing Party shall be entitled to recover from the other Party reasonable attorney's fees, costs, and other necessary litigation disbursements, in addition to any other legal or equitable relief to which it may be entitled. It is the intent of the EAA that the Contract be construed to be a written agreement that expressly authorizes the prevailing Party in an adjudication under Subchapter I of Chapter 271, Texas Local Government Code, as may be amended, or its successor statute, to recover its reasonable and necessary attorney's fees by specific reference to Section 271.159, Texas Local Government Code.

Section 7.27. Federal, State or Local Laws. (a) Except as provided herein, this Contract is subject to all applicable federal, state, and local laws and any applicable permits, ordinances, rules, regulations, and orders of any federal, state, or local governmental authority having jurisdiction. However, nothing contained herein will be construed as a waiver of any right to question or contest any such law, ordinance, rule, regulation, or order in any forum having jurisdiction.

(b) Rules, regulations, and orders adopted by the USFWS or the EAA shall not and shall never be construed to preempt, preclude, override or interfere with the provisions of this Contract.

(c) Each Party represents that, to the best of its knowledge, no provisions of any applicable federal, state, or local law, nor any applicable permit, ordinance, rule, regulation, or order of any federal, state, or local governmental authority having jurisdiction will limit or restrict the ability of either Party to carry out its respective duties and obligations under or as contemplated by this Contract.

(d) Each Party warrants and represents that it will comply with all applicable federal, state, and local laws when performing any activities under this Contract. Moreover, in its performance of any activities under this Contract, each Party warrants and represents that, to the best of its knowledge, it is or will be in compliance with all applicable federal, state, or local law, including any applicable permit, ordinance, rule, regulation, or order, and that it has obtained any and all permits, licenses, or other approvals as may be required by law to perform such activities to accomplish the objectives of this Contract.

Section 7.28. No Third-Party Beneficiaries. This Contract is only intended to confer any rights, privileges or causes of action upon the Parties. Without limiting the applicability of the rights granted to the public pursuant to the provisions of 16 U.S.C § 1540(g), this Contract shall not create any right or interest in the public, or any member thereof, as a third party beneficiary hereof, nor shall it authorize anyone not a party to the Contract to maintain a suit for personal injuries or property damages pursuant to the provisions of this Contract. The duties, obligations, and responsibilities of the Parties with respect to third parties shall remain as imposed under existing federal or state law as applicable.

Section 7.29. Goods and Services. The Parties agree that the mutual commitments stated in this Contract to provide operational and implementation services in the execution of the ITP and EAHCP constitute an agreement by each Party to provide goods and services to the other Party, that payments due from the EAA and the mutual provision of goods and services, are amounts due and owing under this Contract, and that this Contract is subject to Chapter 271, Subchapter I, Texas Local Government Code, as may be amended, or its successor statute.

Section 7.30. Force Majeure. If by reason of Force Majeure any Party will be rendered unable wholly or in part to carry out its obligations under this Contract, such Party will give notice and full particulars of such Force Majeure in writing to the other Party within a reasonable time after the occurrence of the event or cause relied on. After providing such notice, the obligation of the Party giving such notice, so far as its performance is prevented by such Force Majeure, will be suspended during the continuance of the inability then claimed, but for no longer period, and any such Party will endeavor to remove or overcome such inability with reasonable dispatch. The term “Force Majeure” as used herein will mean those situations or conditions which are beyond the control of the Party and which, after the exercise of due diligence to remedy such situation or condition, render the Party unable, wholly or in part, to carry out the covenants in this Contract. Such Force Majeure events are limited to: Acts of God other than drought, strikes, lockouts or other industrial disturbances, acts of public enemy, orders of any kind of the government of the United States or the State of Texas, regulatory restrictions imposed on the EAA by the Texas Legislature, regulatory restrictions imposed on the Refugia Program by any governmental authority of any nature, subject to the limitation in Section 7.27(b), any civil or military authority, insurrection, war, terrorism, riots, epidemics, landslides, lightning, earthquake, fires, hurricanes, tornados, storms, floods, washouts, restraint of government and people, civil disturbances, explosions, extraordinary breakage or accidents to machinery, wells, pipelines or canals, partial or entire failure of water supply, or water production infrastructure, or on account of any other causes insofar as any of the foregoing are beyond the reasonable control of the Party claiming such inability, or its employees, agents, contractors or subcontractors. Neither Party shall be deemed in default hereunder for any failure to perform due to any Force Majeure event.

Section 7.31. Limitation on Funding Source. The USFWS agrees and understands that the cost of implementation of this Contract will be paid solely from Program Aquifer Management Fees, or funding contributions from third parties, as provided by Sections 5.1, 5.3, 5.4, 5.5, and 5.6 of the FMA, and that the EAA will not be obligated to provide funding from any other sources.

Section 7.32. No Construction of Works by the EAA. The USFWS has no right to require the EAA to acquire, install, or construct any facilities, plants, works, improvements, land,

easements, rights of way, other interests in land, or water rights to implement this Contract.

Section 7.33. Relation to EAHCP Documents. This Contract is not intended to, and shall not be construed, to change, amend, or otherwise alter the terms and conditions of the ITP or the EAHCP.

Section 7.34. Cooperation. The Parties agree to fully cooperate with each other in the implementation of this Contract. The USFWS also agrees to fully cooperate with other contractors of the EAA that are conducting applied research relative to the Refugia Program. The EAA shall give written notice to the USFWS of the names of any such contractors and provide copies of the contracts between the EAA and the contractor. The USFWS agrees to participate in any meetings necessary to develop, coordinate, and implement all Refugia Program-related applied research in a manner to promote efficiency, quality of results, and cost effectiveness.

Section 7.35. Warranty of Services. The USFWS will make every reasonable effort to provide the Services described in this Contract. However, as a Federal agency, it cannot provide either an express or implied warranty for the Services.

Section 7.36. Availability of Funds. Implementation of this Contract by the USFWS is subject to the requirements of the Anti-Deficiency Act and the availability of appropriated funds. Nothing in this Contract will be construed by the Parties to require the obligation, appropriation, or expenditure of any money from the U.S. Treasury. The Parties acknowledge that the USFWS will not be required under this Contract to expend any federal agency's appropriated funds unless and until an authorized official of that agency affirmatively acts to commit to such expenditures as evidenced in writing.

IN WITNESS WHEREOF, this Contract is executed as of the day and date first written above in Section 2.1.

EDWARDS AQUIFER AUTHORITY

U.S. FISH & WILDLIFE SERVICE

By: _____
Roland Ruiz date
General Manager

By: _____
Stewart Jacks date
Assistant Regional Director,
Fish and Aquatic Conservation

ATTEST:

By: _____
Jennifer Wong-Esparza date
Assistant to the Board Secretary

APPROVED AS TO FORM:

Darcy Alan Frownfelter date
General Counsel
Edwards Aquifer Authority

EXHIBIT A

REFUGIA FACILITIES



Figure 1. An aerial view of the San Marcos Aquatic Resources Center located in San Marcos, Texas. The red squares represent the approximate location of the new proposed structures to house Edwards Aquifer Covered Species.



Figure 2. An example of what the proposed Refugia and Quarantine building would look like at the San Marcos Aquatic Resources Center.



Figure 3. An example of what the interior of the proposed Refugia and Quarantine building would look like at the San Marcos Aquatic Resources Center.



Figure 4. An aerial view of the Uvalde National Fish Hatchery located in Uvalde, Texas. The red squares represent the locations of the proposed building renovations required to house Edwards Aquifer Covered Species.



Figure 5. Existing space at Uvalde NFH to be renovated for the fountain darter and salamander refugia.



Figure 6. Existing space at Uvalde NFH to be renovated for invertebrate refugia.



Figure 7. Existing space Uvalde NFH to be used for the Quarantine Facility.

EXHIBIT B

SCOPE OF WORK

INTRODUCTION

The overarching goal of this Contract is to ensure EAA's compliance with the ITP. As stated in Section K of the ITP: *"...The support of the refugia will augment the existing financial and physical resources of these facilities, and provide supplementary resources for appropriate research activities, as necessary, to house and protect adequate populations of Covered Species and expanded knowledge of their biology, life histories, and effective reintroduction techniques. The use of this support will be limited to the Covered Species in the EARIP HCP."*

The refugia will be operated in accordance with guidelines identified in the EAHCP, Section K of the ITP, and the USFWS "Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act" (65 Fed. Reg. 56916 (Sept. 20, 2000)). USFWS, in coordination with EAA, will manage all aspects of the Refugia Program through March 2028. All Services contracted for in this Contract shall be completed by December 1, 2028 (the "Completion Date").

USFWS will collect and maintain standing stock and refugia stock in a manner that preserves genetic integrity of the Covered Species. USFWS will also develop the Refugia Program standing stock, refugia stock, and salvage stock (if triggered) in a manner that allows for the adaptation of target numbers and management units as new information about the species becomes available.

Task 1. Refugia Program Operations

USFWS will provide fully functioning Refugia operations for all of the Covered Species, except the San Marcos Gambusia¹. Refugia operations will include, but are not limited to, the following:

- One main "off-site"² refugia facility located at the San Marcos Aquatic Resources Center, and one redundant off-site refugia facility located at the Uvalde National Fish Hatchery;
 - To include construction and/or rehabilitation of all facilities necessary to provide full refugia operations;
 - Salvage capabilities will be fully operational no later than January 1, 2017;
- A refugia program staffed with qualified and permitted individuals for all Covered Species; and
- Collection, establishment, and maintenance of standing stocks, refugia stocks, and salvage stocks for the Covered Species.

¹ Presumed extinct

² Off-site is defined as "away from the principle area of activity". For the Refugia program, "off-site" simply refers to a location far enough away from the springs ecosystems (Comal and San Marcos), so that if the springs' ecosystem is compromised by some action (flood, fire, natural or man caused disaster, etc.), the offsite facility is not compromised by the same action

In accordance with USFWS Ecological Services, USFWS will take all appropriate steps to collect and propagate standing stock, refugia stock, and salvage stock to reach target numbers as described below to maintain compliance with the ITP for each Covered Species.

Species Triggers and Numbers										
Species	Standing	Stock	Refugia		Stock	Salvage				Stock
	Trigger	Target #	Trigger		Target #	Trigger				Target #
Fountain Darter (Comal)	ASAP	1,000	<	150 cfs	1,000 minus standing stock	<	50% mean aquatic vegetation (Landa lake and Old Channel) and darter presence system wide; or <20% mean aquatic vegetation (Landa Lake and Old Channel) and <30% darter presence system wide			2000
Fountain Darter (San Marcos)	ASAP	1,000	<	80 cfs	1,000 minus standing stock	≤	75 cfs for four (4) consecutive days			2500
Texas Wild-Rice	ASAP	430	<	120 cfs	430 minus standing stock	<	3,500 m ² total coverage in the San Marcos River; or Texas wild-rice exists at < three (3) of the seven (7) distinct sections (Biological Monitoring sections)			1500
Texas Blind Salamander	ASAP	500	<	105 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%				500

Species	Standing	Stock	Refugia		Stock	Salvage	Stock
	Trigger	Target #	Trigger		Target #	Trigger	Target #
San Marcos Salamander	ASAP	500	< 120 cfs		500 minus standing stock	< 50% suitable habitat (Biological Monitoring locations) and < 20% salamander density; or < 25 % suitable habitat (Biological Monitoring locations) and < 30% salamander density	500
Comal Springs Salamander	ASAP	500	< 120 cfs		500 minus standing stock	< 50% suitable habitat (Biological Monitoring locations) and < 20% salamander density; or < 25 % suitable habitat (Biological Monitoring locations) and < 30% salamander density	500
Peck's Cave Amphipod	ASAP	500	< 30 cfs		500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by $\geq 10\%$	500
Comal Springs Riffle Beetle	ASAP	500	< 120 cfs		500 minus standing stock	< 30 cfs when only one (1) of three (3) monitored sites continues to have six (6) or more adult Comal Springs riffle beetles collected in a 24 hr sample period using cotton lures	500
Comal Springs Dryopid Beetle	ASAP	500	< 30 cfs		500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by $\geq 10\%$	500

Species	Standing	Stock	Refugia		Stock	Salvage	Stock
	Trigger	Target #	Trigger		Target #	Trigger	Target #
Edwards Aquifer Diving Beetle	ASAP	500	< cfs	30	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by $\geq 10\%$	500
Texas Troglobitic Water Slater	ASAP	500	< cfs	50	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by $\geq 10\%$	500

*Since the Gambusia is considered extinct, it is not included in this list.

- Preparation of species-specific Propagation, Genetic Management, and Reintroduction Plans to be used for the duration of the ITP, with specific goals and milestones. These plans are considered dynamic in that as new relevant information is generated plans will be modified;
- Conduct research, identified within the plans, with the intent to further refine refugia animal rearing methods and captive propagation techniques, provide monetary and logistical efficiencies, and enhance species survival and fitness;
- Propagate Covered Species, when triggers occur;
- Stock affected species, as defined in the Reintroduction Plan, if triggered; and
- Program permitting and reporting, as required by: the ITP, USFWS Ecological Services, TPWD, the EAHCP Annual Report, EAHCP Annual Work Plans and Cost Estimates.

USFWS will provide disease testing and treatment as required, and provide biosecurity for all species held in refugia. USFWS biosecurity and health procedures will include:

- Quarantine of all wild caught individuals until deemed healthy for incorporation into general refugia population;
- Isolation plan for appropriate species, stocks of species, and species from each spring system;
- Follow biosecurity plan protocols (see Exhibit E);
- Appropriate treatment of effluent waste water;
- Prevent escapement of all Covered Species at all life stages; and
- Test species' lots prior to any stocking efforts.

Organisms collected during salvage operations will be maintained at the Refugia Facilities for a limited duration (up to one year) or until their disposition has been determined. If EAHCP salvage

triggers are reached the Refugia Facilities would accommodate salvaged organisms no more than two times during the 12-year period.

Production of species will be limited to no more than two times during the 12-year period once species extirpation is determined. Species produced will be held for a limited time (up to one year) or less if stocking is required.

Maximum Task 1 Budget for the Term of the Contract - \$12,376,686

Task 2. Research

Subtask 2.1 Literature Review

USFWS will conduct a literature review, including EAHCP Applied Research reports, to assist in refining methodologies, study parameters, etc.

Subtask 2.2 Research Plan

USFWS will complete a draft Research Plan due to the EAA within 180 days of the Notice to Proceed issued by the EAA General Manager under Section 3.3. USFWS will present the methodologies discussed in the plan to the EAHCP coordinator. Research outlined in the plan will be presented to the EAHCP Adaptive Management Science Committee (“Science Committee”) for review and comment. EAA approval of final draft of the Research Plan is required prior to implementation. Research will begin no later than 365 days from Notice to Proceed. Research on the Covered Species will be conducted based on the priority ranking in Table 1 below:

TABLE 1 Species Federally Listed as Threatened or Endangered:	Priority Ranking
Comal Springs Riffle Beetle (<i>Heterelmis comalensis</i>);	1
Comal Springs Dryopid Beetle (<i>Stygoparnus comalensis</i>);	1
Pecks Cave Amphipod (<i>Stygobromus pecki</i>);	1
Texas Blind Salamander (<i>Eurycea</i> (+ <i>Typhlomolge</i>) <i>rathbuni</i>);	2
San Marcos Salamander (<i>Eurycea nana</i>)	2
Fountain Darter (<i>Etheostoma fonticola</i>)	3
Texas Wild Rice (<i>Zizania texana</i>)	3
Species Petitioned for Listing as Endangered	
Texas Cave Diving Beetle (<i>Haideoporus texanus</i>)	4
Texas Troglotic Water Slater (<i>Lirceolus smithii</i>)	4
Comal Springs Salamander (<i>Eurycea sp.</i>)	4

Research proposals will describe the methodology used to conduct research on the topics listed below, timelines and milestones, and will provide justification for the selection of the methodologies selected. For each Covered Species, research will be conducted based on the priority ranking in Table 2 below:

TABLE 2 Research Topics	Priority Ranking
Collection Methods and Techniques	1
Species Husbandry	2
Species Propagation	3
Species Genetics	4
Species Reintroduction Methods	4

For example, the research topics listed in Table 2 are to be conducted on the Comal Springs Riffle Beetle, the Comal Springs Dryopid Beetle, and the Peck's Cave Amphipod before beginning the same research on the Texas Blind Salamander and other species with lower priority as described in Table 1.

Subtask 2.3 Covered Species Research

USFWS will conduct research as necessary to expand knowledge of the Covered Species for the Refugia Program. At the direction of the EAA, USFWS will conduct research including but not limited to, species' physiology, environmental requirements, health and disease issues, life histories, genetics, and effective reintroduction techniques.

Research may be suspended or terminated if space is required for salvaged organisms or, if space is required to house cultured species.

Performance of this task will be suspended in the event that the triggers initiating Task 4 are reached.

Maximum Task 2 Budget for the Term of the Contract - \$5,225,581

Task 3. Species Propagation and Husbandry

USFWS will develop and refine Standard Operating Procedures (SOP) for animal rearing methods and captive propagation techniques for the Covered Species. USFWS will document the success and failures of each rearing method and captive technique. These species-specific SOPs will contain photo documentation and provide step-by-step directions for the Covered Species' husbandry. Delivery of SOPs will be as provided in Task 5.

Performance of this task will be suspended in the event that the triggers initiating Task 4 are reached.

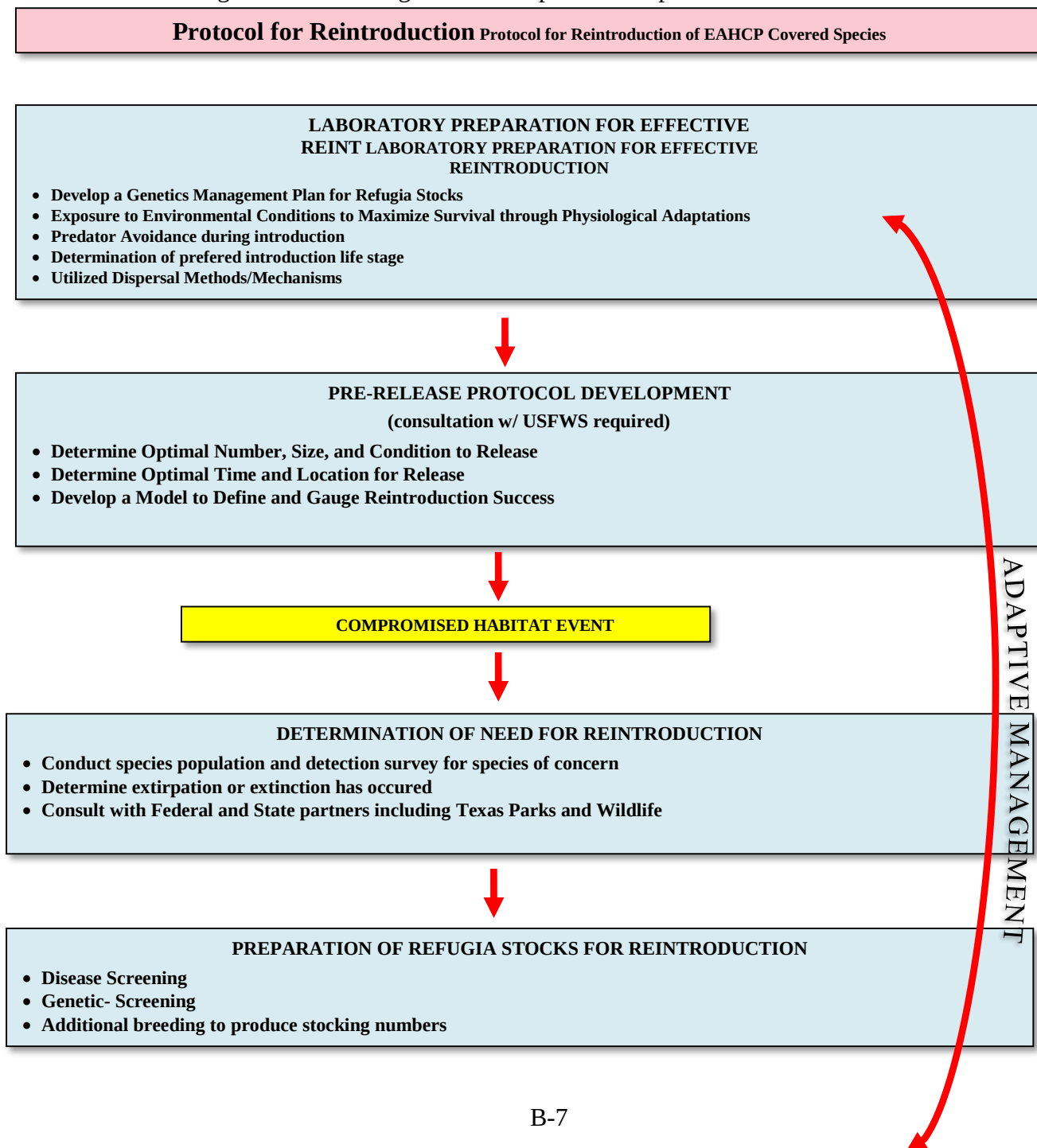
Maximum Task 3 Budget for the Term of the Contract - \$0 (Funding for this task is contained in Task 1)

Task 4. Species Reintroduction

USFWS will prepare, in cooperation with and approval from the EAA, a specific Reintroduction Plan for each Covered Species describing the process and methods for reintroduction of affected species including the monitoring of recovery in their native environment due to the EAA within 180 days of the Notice to Proceed.

USFWS will work closely with the TPWD and other entities as necessary to develop reintroduction plans, reintroduction, and monitoring of recovery of the Covered Species.

See the flow diagram below for a general concept of the steps in reintroduction.





POST-RELEASE MONITORING AND EVALUATION

- **Initiate Intense Monitoring for Reintroduction Evaluation and Modification**
- **Reintroduction Approaches will be Evaluated and Revised as needed**



CONTINGENCY PLAN

- **Modify stocking plan (location, timing, lifestage, etc.) if necessary**
- **Additional Stockings**

Maximum Task 4 Budget for the Term of the Contract - \$100,000 (These funds are reserved for 2027 and 2028; Task 4, if triggered, will be funded with Task 2 funds from 2017-2026)

Task 5. Reporting

Subtask 5.1 Annual Reports

USFWS's annual report of activities under this Contract shall contain the following information:

(a) A description of USFWS's activities under this Contract for the reporting year to include:

- An itemized list of all equipment and expendables purchased for this project during the reporting period;
- Methods used for collection, maintenance, and restocking including SOP's developed and other plans composed;
- Descriptions and photographs of procedures from collections to restocking will be provided as photographic record;
- Descriptive statistical results for any applied methodologies including any novel collection and culture tools developed;
- Summary of data analyses will include but will not be limited to the results for genetic analyses, disease records and treatment, and culture methods; and
- Electronic versions of all raw genetic and pathogenic data, in a commonly useable format such as Microsoft Excel be will submitted to the EAA. The EAA will provide a database structure to USFWS to accommodate EAA's existing data needs.

(b) A description of the terms and conditions under this Contract or any federal, state, or local permit that has been issued to USFWS in order to perform the Services that were required to be met during the reporting period, and a self-evaluation of whether such terms and conditions were met;

(c) A description of the terms and conditions under the ITP and EAHCP relevant to the Refugia Program that were required to be met during the reporting period, and a self-evaluation of whether such terms, conditions, goals, and objectives were met; and

(d) Any other data or information that in the judgment of the EAA is reasonably relevant and necessary to review and assess the efficacy of the performance of the Services.

USFWS will provide a draft annual report to the EAA no later than December 31st of the year of the reporting period for review and comment. The EAA will have ten (10) business days to review and comment on the draft report.

After USFWS receives EAA's comments, USFWS will review such comments and incorporate any changes that are reasonable and appropriate within 10 business days. USFWS will provide a written response to any comments that are not incorporated, and the reasons therefor, after which the annual report may be finalized and the report filed with the EAA

Subtask 5.2 Annual Work Plans and Cost Estimate

For each year during the term of this Contract, USFWS will cooperate with the EAA in the preparation, processing, and approval of the Annual Work Plan and Cost Estimate ("WP/CE") and Application for Program Funding ("APF") for the Refugia Program under the timelines and conditions provided for in Articles V and VI of the FMA.

USFWS will be responsible for the initial preparation of the annual draft WP/CE, which should include:

- (1) the Services expected to be performed;
- (2) labor and personnel costs,
- (3) expenses;
- (4) costs to acquire machinery, equipment, appliances, materials, and tools;
- (5) costs for the acquisition of land, easements, rights of way, other interests in land, and water rights;
- (6) costs to acquire permits, licenses, or other approvals;
- (7) costs for the design and construction, replacement, or rehabilitation of facilities, plants, works, and improvements;
- (8) operation and maintenance costs facilities, plants, works, and improvements; and
- (9) a detailed explanation of research projects and methodologies.

The Parties agree to attempt to resolve any issues that may arise in the preparation of the WP/CE and APF in a good faith manner.

The draft WP/CE are due to the EAHCP Chief Science Officer on or before March 1st of each year for the term of the Contract. The EAA comments will be provided to USFWS by March 15 of each year. USFWS shall review the comments of the EAA and consult the EAA on the comments, and prepare a final WP/CE to the EAA no later than March 25th of each year. The WP/CE shall be approved by the EAA no later than March 31 of each year.

Subtask 5.3 Status Reports

USFWS will submit monthly electronic reports (email) outlining activities for the previous month. Reports should discuss activities such as:

- Construction
- Species Collection
- Issues and Concerns
 - Propagation
 - Research
 - Budget

These reports will be submitted via email to the EAHCP Chief Science Officer. Monthly reports are due each first Monday of the month by 5 pm for the previous month's activities.

Upon completion of the collection phase of Task 1, USFWS may begin monthly reporting of project status.

Maximum Task 5 Budget for the Term of the Contract - \$945,001

Task 6: Meetings and Presentations

USFWS will attend meetings and give presentations to the USFWS Ecological Services personnel, Science Committee, EAHCP Implementing Committee, and EAA Board of Directors, as requested by the EAHCP Program Manager.

Additionally, at a minimum of once a quarter, if needed, USFWS may be required to meet at the Refugia Facility with EAHCP staff. Together, USFWS and EAHCP staff will review the facility, current research, and discuss any contractual compliance issues or budgeting concerns. The EAA, with reasonable notice to USFWS, may visit/access the Refugia Facilities at any time.

Maximum Task 6 Budget for the Term of the Contract - \$228,999

EXHIBIT C

PROJECT SCHEDULE

[illegible]

EXHIBIT D **TASK BUDGET**

	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	Total
Task 1. Refugia Operations	\$5,515,263	\$592,572	\$616,457	\$612,562	\$627,011	\$634,422	\$656,248	\$678,729	\$701,884	\$725,734	\$750,299	\$265,505	\$12,376,686
Task 2. Research	\$467,391	\$438,039	\$445,900	\$444,176	\$453,282	\$462,660	\$472,321	\$482,271	\$492,519	\$503,075	\$513,947	\$50,000	\$5,225,581
Task 3. Species Propagation and Husbandry *	-	-	-	-	-	-	-	-	-	-	-	-	-
Task 4. Species Reintroduction †	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$100,000	\$0	\$100,000
Task 5. Reporting	\$83,522	\$78,190	\$79,617	\$79,303	\$80,959	\$82,664	\$84,421	\$86,230	\$88,094	\$90,012	\$91,989	\$20,000	\$945,001
Task 6. Meetings and Presentations	\$12,000	\$13,100	\$14,310	\$15,641	\$17,105	\$18,716	\$20,488	\$22,120	\$24,551	\$25,937	\$26,531	\$18,500	\$228,999
Totals	\$6,078,176	\$1,121,901	\$1,156,284	\$1,151,682	\$1,178,357	\$1,198,462	\$1,233,478	\$1,269,350	\$1,307,048	\$1,344,758	\$1,482,766	\$354,005	\$18,876,267
* Funding for this task is contained in Task 1													
† Maximum Task 4 Budget for the Term of the Contract - \$100,000 (These funds are reserved for 2027 and 2028; Task 4, if triggered, will be funded with Task 2 funds from 2017-2026.													

EXHIBIT E

SAN MARCOS AQUATIC RESOURCES CENTER BIOSECURITY PLAN

**Specimen Collection, Hazard Analysis Critical Control Points, Quarantine, & Specimen
Transfer: San Marcos Aquatic Resources Center Standard Operating Procedure**

Prepared By:

United States Department of the Interior
Fish and Wildlife Service
San Marcos Aquatic Resources Center
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San Marcos, Texas 78132

Revised: August 15, 2014

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INTRODUCTION

The San Marcos Aquatic Resources Center (SMARC) undertakes a broad array of tasks ranging from maintaining standing stocks to hypothesis driven research on a wide variety of animals and plants. As a result, live specimens are commonly collected from their native environments and transported to the SMARC. In doing so inadvertent pathogenic, parasitic, and exotic species could be introduced and become established at the SMARC or outside of their endemic range. To minimize the risk to the SMARC and inadvertent release to the wild all specimen collection, maintenance, and transfer follows biosecurity measures outlined herein.

Biosecurity at the SMARC consists of a number of procedures that are aimed at minimizing the risk of introducing an infectious disease, parasite, or non-native species and spreading it to animals held in refugia, other facilities, and other drainages. Biosecurity procedures at the SMARC are cost effective in that they proactively contribute to the positive physiological performance of maintained organisms by reducing stress and optimizing health and immunity thereby reducing the economic losses from mortalities and treatments. Biosecurity will only be successful if managers, staff, and visitors understand and follow the necessary practices. As such all new employees, volunteers, and visiting researchers must be trained in SMARC biosecurity procedures prior to beginning work. The biosecurity program and employee compliance will be included in all EPAP's and reviewed during evaluations. Employees and visitors who do not follow established protocols will increase the risk of disease, parasites, and unwanted organisms. Access to sensitive areas will be restricted to authorized personnel only. Visitors must be accompanied at all times. Visitors who come to the SMARC immediately after visiting another aquaculture facility should be considered a serious risk. Detailed and species-specific steps in the SMARC biosecurity process fall under five overarching categories. These categories include 1) actions prior and 2) during specimen collection, 3) quarantine procedures, 4) daily routines, and 5) transfer off SMARC grounds.

ACTIONS PRIOR TO SPECIMEN COLLECTION

Justification to bring on station.- Prior to any tissue samples, seeds and eggs and specimens being collected, including but not limited to State and Federally-listed fish, salamanders, invertebrates, and plants, justification should be documented and communicated to the immediate supervisor and Project Leader to ensure that efforts fit with the SMARC and Service mission. Justification to collect specimens used to supplement standing stocks at the SMARC is already documented within species recovery plans, contingency plans, hatchery management, genetic management, and stocking plans. Thus, no written documents will need to be composed for these standing stock supplementation efforts excluding the appropriate documentation such as transport permits, health reports, etc. Alternatively, specimens collected for independent studies that deviate or are not covered under current permits, reimbursable agreements and contracts, graduate student research, or trial and error pilot studies need a pre-approved study plan (Appendix A) that clearly details the number of individuals to be collected, how they will be maintained or treated while at the SMARC, and their deposition.

Permit coverage.- Prior to any specimen collection both state and federal permits need to be reviewed to ensure that coverage exists. Do not assume that the “take” amount requested is all that has or will occur during the year. Instead confirm your proposed “take” coverage with permit holder (i.e. Project Leader) to ensure that it is under the designated limit listed on the permit when all other “take” is considered. In addition, if collection efforts involve non-Service employees make sure the permit allows those individuals to collect and transport specimens. If specimens are to be collected and immediately transferred off station, ensure that appropriate documentation has been obtained by all parties for the possession of State and Federally-listed species. Procedures for providing another lab with endangered species for research purposes when number of specimens or duration of study would not warrant the laboratory obtaining their own permit are listed below:

- Federal and State Permit
 - Prepare short proposal that contains, purpose of research, person who will be doing the work, source (wild, F1, F2, etc.) and number of organisms to be used in research, and disposition of organisms after project completed (e.g. organisms destroyed or organisms donated to museum).
 - Contact by e-mail (cc copy to Marty Tuegel – RO HCP/Permits) individual(s) at Austin ES and TPWD that have or are considered the lead for the species.

- Provide a study proposal and ask them to review it and receive concurrence, documented in an email, that the research has scientific and recovery value.
- Prepare a letter from the SMARC Center Director stating that researcher can work under the SMARC federal and State permit. This letter must always accompany the samples/specimens during transport to the researcher, during the research, during transfer to and storage at a museum or other facility or until samples destroyed.
- Request an annual report for the project, completion report, and a copy of any resulting publications associated with the project.
- Send two signed copies of a TPWD transfer receipt and instruct the researcher (recipient) to sign both and return one.
- The SMARC annual endangered species report should contain a current summary of the project until it is completed. An appendix containing the final report should be included in the annual report upon project completion.

HACCP plan composition.- Prior to any specimen collection complete a (Hazard Analysis and Critical Control Point (HACCP; see <http://www.fws.gov/fisheries/ans/ANS-HACCP.html>) plan (Appendix B). The HACCP plan is intended to prevent the spread of invasive species through a structured thought process assessing a specific natural resource management activity, identifying possible risks, and facilitating the removal or reduction of non-target species during activities. In order for plans to be effective all participants engaged in the activity must be informed about the steps taken to minimize the risk of inadvertent organism transfer. Questions regarding the composition, implementation of, or approval(s) for and concurrence with HACCP plan should be communicated to and confirmed with the Regional ANS coordinator.

Partner notification.- Prior to specimen collection contact all relevant partners about the intended number of specimens subjected to take, the locations where take may occur, and date(s) of activities. Communication should be done via e-mail to provide a record. The Project Leader should be copied on this correspondence. If the project is for an independent study, reimbursable agreement(s) and contract(s), graduate student research, or trial and error pilot study disseminate the study plan as an attachment on the e-mail for concurrence. Promptly reply to any inquiries regarding concerns. A list of commonly contacted partners is listed below; those denoted with asterisks must be included regardless of the species being collected.

Southwestern Region

*Dexter SNARRC Fish Health Unit Leader (Dr. Teresa Lewis)	575-734-5910 ext. 24
*Region 2 ANS Coordinator (Dr. Dave Britton)	817-272-3714
*TXFWCO Project Leader (Mr. Mike Montagne)	512-353-0011 ext. 236
*Austin ES (Mr. Patrick Conner)	512-490-0057
*TPWD (Mr. Christopher Maldonado)	
Fish (Mr. Stephan Magnelia)	512-754-6844
Invertebrates (Dr. Ben Hutchins)	(ben.hutchins@tpwd.texas)
Salamanders (Dr. Andy Gluesenkamp)	(andy.glusenka@tpwd.texas)
Plants (Ms. Jackie Poole)	(Jackie.poole@tpwd.texas)
TPWD Law Enforcement	512-389-4845
Comal	
(Nicole Leonard)	830-214-3110
(Michael Mccall)	830-660-9447
(Brent Satsky)	830-221-8529
San Marcos	
(James Michael)	512-497-5186
(Jake Scott)	512-497-5652
USFWS Law Enforcement	210-681-8419
Spring Lake (Dr. Thom Hardy)	512-245-6729
Schlitterbahn	830-708-3665
(Terri Adams)	830-625-2351 ext. 714
Landa Park (Stacey Dicke)	830-221-4350

Please note that this list is likely to change and is not exhaustive.

ACTIONS DURING COLLECTION AND TRANSPORT

Collection, handling and transport protocols.- All organisms will be handled with the best animal and plant husbandry practices available. Specimen collection and monitoring activities should be planned and coordinated to minimize undue stress. As an example, collection dates should occur during the spring and fall to avoid extreme air temperatures. Handling should be as efficient as possible. This can be accomplished by using appropriate gear and properly trained staff. Collection and identification should be made by professional experienced staff so that collection, net stress, and air exposure is minimized. Air exposure and mechanical damage due to nets and handling are significant stressors that commonly result in not only initial but delayed mortality. Collected specimens should be inspected visually for general condition. Specimens must be healthy and in good condition. Poor quality specimens increase avoidable losses. Specimens should be enumerated and transferred immediately.

Transport of all fish, salamanders, invertebrates, and plants including their seeds or eggs will follow guidelines herein:

- The specimens to be transported must be healthy and in good condition. Poor quality specimens increase avoidable losses during transport.
- Consideration should also be given to specimen densities. Not all containers will work for all species.
- The container should only contain the specimens and water. Through visual inspection of transport water, collection gear, and specimens should occur prior to placement into transport containers.
- Vehicle and clothing including footwear should be visually inspected for foreign materials. If needed the vehicle, collection gear, and clothing should be power washed and disinfected as close to the collection sites as possible. Collection gear and field clothing can be bagged and cleaned at a later time if warranted. See species specific SOPs for details.
- When multiple out-of basin sites or drainages are visited during a day or sampling trip all gear and the vehicle must be disinfected. Alternatively, different gear designated for specific sites, basins, or drainages can be employed. Proactive removal of foreign unwanted species and debris at the collection site will further reduce unwanted transfer of organisms back to the facility.
- Other objects placed in transport containers (e.g. frozen bottles of water, structure used to reduce water movements during transport etc.) can cause mechanical injury during transport and should not be used.
- Sedation of specimens during transport can reduce stress and injury but species specific doses and duration should be thoroughly investigated and vetted prior to use.
- Water quality during transport plays a significant role during transport. When specimens are placed in transport containers, they usually exert a large amount of muscular activity this coupled with the additive stressors of capture and handling can culminate into significant physiological stress. More oxygen is consumed within the first 15 minutes of transport than during any subsequent 15-min period for fish. For this reason, additional oxygen should be provided during loading and hauling for animals. Oxygen concentrations should be monitored every hour during transport and recorded.

- Water temperature in hauling container(s) should not deviate by more than ± 2 to 5 degrees Fahrenheit from the collection site water temperature. Water temperatures should also be monitored every hour and recorded.
- Specimens should be acclimated to the receiving water. Temperature differences between hauling and receiving waters should be equalized in increments of 2 degrees per 30 minutes. Acclimation to receiving waters can be accomplished in the shipping tank by adding receiving water to the tank at given intervals.
- Disinfection SOPs must be appropriate for individual species. Confirm with the Fish Health Unit.

QUARANTINE PROCEDURES

Once organisms arrive at the Center they must be enumerated and stocked into isolated quarantine systems. Quarantine is one of the most important animal and plant management and biosecurity measures. Quarantine is the procedure by which an individual or population is isolated, acclimated, observed and, if necessary, treated for specific diseases, unwanted hitchhiker organism and parasites. The principles of quarantine apply for new organisms coming into a facility, organisms moving from one area or system to another within the facility, and resident organisms that become diseased. Well-designed quarantine systems physically separate incoming organisms from the rest of the facility. Water in quarantine systems also should be separate from the main facility, and discharges should be handled and treated appropriately to ensure no inadvertent escapement. Proper quarantine not only protects established populations from potential exposure to pathogens but also gives the new animals time to acclimate to water, feeds and management and to recover from handling and transport. Handling and transport have been shown to reduce disease resistance and recovery may take weeks. Organisms in the general population that become sick should be isolated in tanks away from their healthy counterparts; signs or other methods (i.e. all staff meetings and e-mail notification) should be used to alert employees that the population is diseased. Major components of quarantine include all-in-all-out stocking, isolation or separation, observation and diet adjustment, and sampling and treatment.

All-in-all-out stocking.- This involves bringing animals or plants in as a group from only one original source population (e.g. San Marcos or Comal River) and maintaining them as a group throughout the quarantine period. It prevents exposure to other pathogens not currently in that population. No new animals or plants should be added to a group currently in quarantine. All-in-all-out quarantine may involve an entire facility, room, or tank system.

Isolation or separation.- A group of animals or plants in quarantine should be physically isolated from other quarantined populations and from the resident populations. Methods of isolation should be built into future SMARC culture buildings. Current logistics prevent complete isolation; therefore, populations should be separated by tank and system when possible. Regardless of the level of isolation, appropriate sanitation and disinfection measures must be used to reduce cross-contamination between quarantined and established populations and between separate populations in quarantine.

Observation and diet or growing condition adjustment.- Animals and plants should be observed for normal and abnormal appearance and behaviors throughout the quarantine period so disease problems can be detected early. For example, loss of appetite in animals and leaf necrosis in plants are very common, early signs of disease. Good nutrition and growing conditions will increase disease resistance and careful adjustment from the diet of origin or growing conditions to the standard facility diet or growing conditions will reduce problems from sudden changes. Records of abnormal appearance or behavior must be documented when observed. Photographs are strongly suggested when documenting issues.

Sampling and treatment.- Animals and plants in quarantine should be sampled for specific diseases of concern at the beginning of the quarantine period and at any time that disease signs develop (see species SOP's for specific treatments and options). Although complete necropsy evaluation of a number of specimens is best, limited sampling (i.e. 5%) of more valuable or population limited species is an option with consultation with the Fish Health Unit. Animals and plants remaining in quarantine until Fish Health Unit diagnostics are completed (approximately 30 days) will be subjected to prophylactic treatments for the control and management of internal and external bacteria, parasites and aquatic invasive species (see

species SOP for specific treatments). Staff will monitor the organism's response during the treatment and no treatment will be left unattended. If organisms begin to exhibit stress from the treatment, flush the chemical from the system with fresh water. Consultation and review with the Fish Health Unit and Aquatic Animal Drug Approval Partnership website should occur prior to any drug or treatment administration. No new or novel drug or treatment will be used unless covered under an Aquatic Animal Drug Approval Partnership's (AADP) investigational new animal drug (INAD) and approved by the Fish Health Unit. Veterinary drug treatments must be done in consultation with an accredited Veterinarian Fish Health Unit, consult with Regional Fish Health Unit to coordinate these types of treatments. Animal and plant condition will be monitored daily and mortality will be recorded while in quarantine. Unusual observations will be conveyed to the Fish Health Unit immediately.

New health treatment.- If the species has never been held on station or physiological response to the treatment chemicals is unknown, staff will consult with the Fish Health Unit and may require bioassays to determine appropriate doses prior to treatment.

Sequence of animal husbandry.- Organisms already being held in refugia and those cleared by the Fish Health Unit should be maintained (i.e. fed, tank cleaning, water quality) first, then diseased organisms that have a diagnosis and treatment, and finally specimens in quarantine and those thought to be diseased.

NEW EMPLOYEE OVERSIGHT

All new workers including new researchers, volunteers, and employees, will be monitored daily by lead biologists and primary care takers for two weeks or longer until new workers demonstrate that they are fully capable of following SMARC HACCP, SOPs, and biosecurity plans. They must demonstrate that they are reliable and capable of conducting daily routine refugia activities, including but not limited to recording applicable information into appropriate log books; acid washing, disinfecting, setting up holding systems, siphoning, and disassembling tanks systems, collecting invertebrates from ponds and raceways, preserving mortalities, feeding, and safely transferring plants, seedlings, eggs, larvae, and adults from tank

to tank. All species transfers must be pre-approved by the lead biologist before transfers occur. After approval, individuals will first record the transfer data into the appropriate log book and then perform the actual transfer.

DAILY ROUTINES

Pathogens, parasites, and unwanted organisms can survive and thrive in “reservoirs” within a facility. Non-living reservoirs include water and its delivery system, system components, ancillary equipment, floors and walls, and feed. Living reservoirs include the cultured animals and plants themselves, other animals (such as frogs and birds), plants, and live (or frozen) food. Understanding the biology of pathogens is important, including the factors that permit them to survive in reservoirs and how easily they can be killed by common disinfectants.

High animal or plant densities, biofilms, and sediments can help concentrate microorganisms. Uneaten feeds and other organic matter in the system favor pathogen survival and spread. *Aeromonas* and *Vibrio* species are bacteria that prefer these highly organic environments. They can also infect and live within aquatic species, sometimes without causing disease. Another group of bacteria, the *Streptococcus* genus, are not as common as *Aeromonas* and *Vibrio* but are difficult to eliminate once established within a system. The bacterial genera *Salmonella* are naturally found in the gastrointestinal tracts of amphibians and thus may also be present within tank systems housing salamanders. Mycobacteria also live within biofilms that coat the tanks, filters, and pipes. Some parasites, including microsporidia and trichodinids, sessile ciliates, *Tetrahymena* and *Uronema*, thrive in organic wastes and on dead or dying fish and salamanders. Viruses vary in their ability to survive, but some viruses may persist for weeks or months. Likewise, fungal spores can survive for a long time in detritus. Pathogens can enter facilities in aerosol (fine water droplets), on equipment and vehicles, and on people’s hands, arms and feet. Once inside, they may survive in pockets of moisture on the ground, walls, or ceiling. Pathogens can also be spread by other animals that live in or near ponds or tanks (birds, rodents, reptiles, insects, aquatic invertebrates). For example, midge fly egg masses (which develop into larvae sold as bloodworms) have been shown to harbor some *Vibrio* species. In addition to directly transmitting some pathogens, animals and plants may also serve to complete

parasite life cycles (e.g., white or yellow grub digeneans and myxozoans such as those that cause hamburger gill disease in catfish).

Sanitation, disinfection, and sterilization are different in regards to what kind and how many organisms they kill. Sanitation uses an antimicrobial agent on objects, surfaces or living tissue to reduce the number of disease-causing organisms to non-threatening levels. Sanitizing does not affect some spores and viruses. Disinfection uses antimicrobial agents on non-living objects or surfaces to destroy or inactivate microorganisms. Disinfectants may not kill all bacteria, viruses, fungi and spores. Most disinfectants are weakened or inactivated by organic matter such as dirt and feces. Sterilization is using chemicals, temperature, gas and/or pressure to kill or inactivate all disease-causing bacteria, spores, fungi and viruses. Cleaning is the first step; it involves removing all foreign material (soil, organic material, biofilm) from objects by scrubbing them thoroughly. Disinfection is the second step; it eliminates many or all pathogenic microorganisms. Disinfection is effective only if organisms on all surfaces are exposed to an appropriate disinfectant at the recommended concentration for the recommended length of time. There are a number of sanitation, disinfection, and sterilization methods that can be used in aquaculture. Logistics, residue toxicity, and cost will determine which methods will work best for a given situation (see species SOP for specifics). In order to combat the transfer of these organisms during daily care the following protocols should be followed.

Disinfection stations for people and equipment.- To help prevent the introduction of diseases, each building should be equipped with disinfectant footbaths (where applicable), hand-washing stations (dedicated sink) or alcohol (isopropyl) spray bottles. These should be placed in strategic locations (including at entrances and between systems) to make them easy to use and to maximize their effectiveness.

Hand washing.- Hand washing alone with soap and water will remove a significant number of pathogens. Using an antiseptic will further reduce risks. One option is a product with 60 to 90% alcohol, either straight rubbing alcohol (isopropyl), which can be applied with a spray bottle, or a gel product. Everyone entering or exiting a building should wash their hands.

Net disinfection stations (net dips).- Nets should be disinfected after use to prevent the spread of pathogens from one group of organisms to another. Net dips should be sized and filled to allow complete submersion of the net and, if possible, the portion of the handle that may be submerged during use. Stations should be strategically located in each building to simplify use.

Disinfectants used in net dips must be rinsed off after dipping and dried to lengthen the life of the nets. Disinfection stations must be filled with an appropriate amount of active ingredient and maintained per individual system SOP. Not all disinfectants work for all applications.

Physical disinfection.- Physical methods of disinfecting equipment include heat, sunlight and drying (desiccation). Exposure to temperatures of 80 to 100 °C for 10 minutes may kill active microorganisms, but some life stages such as spores may require much longer time periods or higher temperatures. Sunlight can be effective, but exposure time will vary depending upon intensity, temperature and other factors, and little research has been done on effective exposure times. Drying equipment can also help reduce pathogen numbers, although spores, cysts or eggs may survive treatment. In areas with high humidity (such as closed aquaculture buildings or outdoors during rainy or wet seasons), complete drying may not be possible. This method is applicable for sampling gear (e.g. salamander collection nets) and equipment (e.g. plant potting equipment).

Chemical disinfectants.- Chemical disinfectants are very useful, but vary in their effectiveness against specific disease organisms. Standard doses will kill many pathogens, but some may require more specific doses or contact times. The commonly used chemical disinfectants are inorganic chlorine products (bleach, as sodium hypochlorite or calcium hypochlorite), Virkon® Aquatic, quaternary ammonium compounds (QACs), chlorhexidines, alcohols (such as isopropyl alcohol), iodophors, hydrogen peroxide, phenol derivatives, and formaldehyde. It is important to consider EPA and TCEQ regulations and the appropriateness for each species.

- Chlorine disinfectants are effective against many common bacteria, viruses, parasites and fungi. A number of different commercial products are registered by the EPA for use in aquaculture as algicides and piscicides. Inorganic chlorine compounds include sodium hypochlorite and calcium hypochlorite. Sodium hypochlorite is available as a liquid, and

calcium hypochlorite is available as HTH powder. HTH chlorine can form precipitates in the presence of some organics. Chlorine compounds are very corrosive to metal and harsh to human skin and mucous membranes. Their effectiveness depends on pH (optimal ~6 to 8) since much of the activity is attributed to the hypochlorous acid (HOCl) product. Very low concentrations can kill aquatic organisms, so the accidental exposure of nearby tanks is always a potential problem. Chlorine can be inactivated with sodium thiosulfate. One suggested ratio for inactivation is 7 mg of sodium thiosulfate to 1 mg of chlorine. Chlorine test kits should be used to make sure there are no residues left. In recirculating aquaculture systems, biofilms within pipework may protect some pathogens even when high concentrations of chlorine are used; nevertheless, chlorine will still kill or inactivate a high percentage of organisms.

- Virkon® Aquatic is a disinfectant with potassium peroxymonosulfate (21.41%) and sodium chloride (1.5%) as active ingredients. Virkon® Aquatic is EPA registered in all states except California and kills many common aquatic pathogens. However, Virkon® Aquatic may not be effective against some mycobacteria at doses recommended for other pathogens. The recommended dilution varies, but a 1% dilution will be effective for most uses. Virkon® Aquatic has minimal environmental impact and is very safe. Virkon® Aquatic test strips are available and can be used to determine whether effective concentrations are still present within a given solution.
- Quaternary ammonium compounds (QACs) that include benzalkonium chlorides and benzethonium chlorides (e.g. Roccal), are cationic surfactant disinfectants. QACs are good cleaning agents that also kill many fungi, bacteria, and viruses but are not effective against *Pseudomonas*, mycobacteria and some viruses. Roccal is commonly used at the SMARC at a rate of 1L (50% benzalkonium chloride) to 873L of water. These compounds leave a residue that can be toxic to fish and must be thoroughly rinsed off nets and other equipment prior to use.
- Chlorhexidine is a cationic biguanide. The most widely available chlorhexidine products are chlorhexidine gluconate (e.g., ViroSan™) and diacetate (e.g., Nolvasan®-S), both of

which usually contain 2% chlorhexidine. Chlorhexidine kills bacteria, enveloped viruses, and most fungal organisms with a contact time of at least 5 minutes, but it has little activity against bacterial and fungal spores. Mycobacteria is inhibited but not killed. Organic material can inactivate chlorhexidine so surfaces must be cleaned first. However, chlorhexidine is not as affected by organic soil as chlorine and iodophors. For this reason, chlorhexidine is often used for footbaths. Compounds commonly found in hard water (sulfate, phosphate, carbonate, nitrate and chloride) can reduce chlorhexidine activity. Activity is also reduced when pH is very low or very high and in the presence of moisturizers and detergents.

- Alcohols (isopropyl alcohol or ethanol at concentrations of 60 to 95%) kill many common pathogens (bacteria, fungi, parasites, viruses) and are one of the few readily available disinfectants that are effective against mycobacteria. Alcohols are highly effective once surfaces have been cleaned of organic matter and soil. However, some life stages such as spores are more resistant. Isopropyl alcohol is less flammable than ethanol and is commonly used at a 70% concentration for disinfection.
- Iodophors (iodine stabilized within a solubilizing carrier complex, such as povidone-iodine products) can be effective disinfectants and antiseptics, but each product must be used according to manufacturer's recommendations. Iodophors are effective for pathogen-specific egg disinfection and for disinfecting surfaces.
- Hydrogen peroxide is a strong oxidizing agent that can be effective as a surface disinfectant at 3% concentration. It kills bacteria and some other pathogens. Because hydrogen peroxide breaks down into oxygen and water, it is considered environmentally safe. 35% PEROX-AID® (a 35% hydrogen peroxide product) is FDA approved to kill only specific pathogens in salmonids, cool-water species, and catfish and their eggs.
- Phenol derivatives are a common ingredient in household cleaners such as Lysol®. These compounds are effective against bacteria, viruses and fungi, but not against spores. Concentrations of 2 to 5% are generally recommended for disinfection, with the lower

concentration requiring a longer exposure time. Surfaces must be rinsed afterward because residues can be toxic. These products can irritate and be absorbed through human skin; take proper precautions. Lysol® maintains activity in the presence of organic material. Virkon Aquatic is a preferred choice for footbaths due to concerns about toxic residue and environmental exposures.

- Another practical method used for disinfection is pH adjustment. Depending upon the pathogen, either decreasing pH to acidic levels (using acids such as muriatic and hydrochloric acid or sulfuric acid) or increasing pH to extremely basic levels (using quick lime), and maintaining this pH for a specified time period can be effective. Never use acids and chlorine disinfects in combination.
- Formaldehyde products are better known to culturists as formalin. One hundred percent (100%) formalin is a saturated solution of formaldehyde gas in water (equal to approximately 37 to 40% formaldehyde by weight). Formaldehyde is effective against bacteria, viruses, bacteria spores, and acid-fast bacteria such as mycobacteria. Although 1 to 8% formaldehyde (~ 3 to 20% formalin) is still sometimes used in aquaculture, there are currently much better and safer disinfectant options. Formalin is no longer recommended because of its irritating vapors and potential carcinogenicity at these higher concentrations. Other products are recommended in place of formalin and should not be used unless approved by the Project Leader. The SMARC does not use formalin as a disinfectant. It should only be used for specific species and treatments as defined by individual SOPs.

Pond Disinfection.- Regular pond cleaning will greatly reduce the numbers of disease-causing organisms. However, pond disinfection efforts may be necessary after cleaning (harvest, drainage, and removal of organics), especially after an infectious disease outbreak.

- Personnel must follow proper safety precautions and wear personal protective equipment.
- SMARC ponds are lined as a result they should be cleaned by removing sediments and organics manually.

- Disinfection can be accomplished by using quick lime (CaO) or hydrated (slaked) lime (Ca[OH]₂) spread evenly over a freshly cleaned pond bottom. Quick lime or hydrated lime is not the same as agricultural limestone and dolomite limestone, which are used for a different purpose. Quick or hydrated lime rapidly increases soil and water pH and, if held to a pH of 11 or above for at least one hour, can kill many disease-causing organisms including some bacteria and viruses. If quick lime is applied to a dried pond, residual organics will undergo desiccation/dehydration. Ideal application rates will vary depending upon initial water pH, but commonly recommended rates range from 500 to 1,300 pounds per acre. After application pH levels should be measured and additional lime added as necessary to maintain pH >11.

Commercial feeds.- Commercially processed diets (e.g. fish flakes) are not normally a source of infectious disease and should be relatively free of pathogens. However, if these feeds are stored improperly (for example, in unsealed containers in warm, moist conditions) nutrients can break down. This will cause nutritional deficiencies and increase an organism's susceptibility to disease. Poorly stored food also can be contaminated by pests (rats, mice, roaches) and be prone to the growth of bacteria and/or fungi. Some fungi produce mycotoxins that can cause disease.

Live and frozen foods.- Many of the SMARC's cultured species are fed live foods (e.g. amphipods, zooplankton, brine shrimp), but these may harbor disease-causing pathogens. Live food culture vessels should be cleaned and disinfected regularly. Various methods of reducing pathogen loads include disinfecting the cysts with sodium or calcium hypochlorite or organic iodines, rinsing newly hatched animals with clean water before feeding, ultraviolet radiation (this can cause degradation of nutrients), ozone, hydrogen peroxide, and probiotic bacteria. In pond systems, live foods are very common and, although valuable for nutrition, can be a source of disease. Work with a Fish Health Unit professional and aquaculture production specialist to determine the best option. Older life stages, including broodstock, may be fed live or frozen food or invertebrates. These foods may also harbor pathogens and should be tested before feeding them to fish, or, if necessary, during an outbreak investigation.

Water quality.- Good water quality is critical to the health of the animals, plants and to the control of pathogens. System water quality and water chemistry parameters should be compatible with the species in culture and should be monitored closely for any deviations from the ideal. Water sources have different degrees of risk. Deep wells and municipal water sources are much less likely to harbor significant levels of disease organisms than surface water and shallow wells. Nevertheless, well water used at the facility should be tested to make sure it is safe. Water used to ship fish should never be poured into the SMARC fill or effluent lines.

Escapement.- When aquaculture resources escape they may pose a significant environmental risk. From a risk management perspective the unplanned escapement of any organism (i.e. cultured or surrogate research species) from the SMARC into the wild is unacceptable. Containment or eradication of escaped stock in the facilities infrastructure or off-site in most circumstances is impossible. As a result the proactive means to significantly reduce escapement at the SMARC must be followed:

- Physical containment is the SMARC's first line of defense to minimize the risk of escapement. As such all effluent lines must be screened or filtered to prevent any inadvertent escapement. The invertebrate room should be equipped with sticky traps when surrogate species are used, particularly when they are known to have terrestrial life history stages. Screens and filters must be effective at all life history stages including seeds and eggs. Screens and filters should be inspected routinely and replaced or repaired if needed. Please see escapement monitoring SOP for specifics.
- Chemical containment at the SMARC is a backup line of defense and should not be relied upon as the sole mechanism to reduce escapement from the facility. The facilities chlorination system does not guard against cross contamination among the building or eliminate escapement into the facilities infrastructure. Please see Chlorination System SOP for specifics.

Transfer Off SMARC Grounds

- Advance notification of the Supervisor is required for all outgoing shipments.
- Prior to shipment of any live materials from the SMARC requires the receiver to demonstrate his/her permit is current and covers intended activities.
- Live material that leaves the SMARC should be confined in clean, sealed containers or vials that are properly labeled. Materials should be double bagged and containers must be leak proof.
- All outgoing shipments from the SMARC must be accompanied by a signed transfer permit.
- Materials prepared for shipment must be inspected by the Supervisor and approved prior to exit. Only the materials to be shipped and the proper documentation will be allowed to exit the SMARC.
- The shipper is responsible for ensuring that the recipient completes and returns the transfer form in a timely manner. The completed form must be provided to the Supervisor, who is responsible for filing the document.
- Live organisms transferred off station should be maintained or disposed of by the recipient.

RECORDS

General record production.- Records must be kept and incorporated into SOP's where applicable. This includes general statistics such as broodstock fecundity, growth rates for younger life stages, numbers of plants, seeds, animals collected and stocked, stocking weights, etc. are ways of tracking groups and can be used to assess the effectiveness of a biosecurity plan.

Biosecurity management.- Documentation (check lists associated with HACCP plan) will be used and will help ensure that employees fulfill assigned tasks (such as changing net dips and footbaths), carry out system and equipment checks, document feeding and observations, and remove dead organisms. Documentation will be used to ensure that visitors are tracked and are made aware of all biosecurity policies. An annual biosecurity audit (overall review of biosecurity at the facility) by the Fish Health Unit will be conducted to ensure that adequate precautions are being taken. During a disease outbreak, procedures and records should demonstrate to authorities that good biosecurity measures were in place, that affected groups were isolated, that no cross-contamination with other groups occurred, and that outbreaks were reported to the supervisor and Project Leader in a timely fashion. This can be accomplished by an Incident Report. The report should be conveyed to TPWD, Austin ES, and the RO shortly

after it is composed. The report needs to be saved on the local drive for inclusion into the station annual report.

Health and disease management.- Records should also document morbidity and mortality numbers, disease signs including photographic documentation of disease stages if possible, feeding response and other general health observations, and water quality/chemistry. For diagnostic sampling, records should include sampling done by group or lot, date sampled, numbers sampled, diagnostics run, findings, treatments and amounts of drugs used, veterinary prescriptions if required, and, finally, methods of collecting and disposing of diseased or dead animals. Any Health reports generated needs to be saved on the local drive for its inclusion into the Station's Annual Report.

INVERTEBRATES

Invertebrate Room Entry and Exit.

- Entry into the Invertebrate Room is restricted to the Lead Biologist and primary caretaker and access is at their discretion.
- The lead, primary caretaker, or cleared designee must escort all visitors, contractors, and other personnel. Prior to entry wash hands and exposed forearms thoroughly with water or alcohol disinfectant. Do not use soaps or surfactants since they may be harmful to invertebrates particularly if hands drip into or are placed into the tanks. Visually inspect clothing to minimize the risk of accidental introductions. Close the door securely and immediately after entry. The Lead or primary caretaker must authorize in advance all items entering the Invertebrate Room.
- Approved items and personnel will enter through the north side door. Tools and supplies are provided in the Invertebrate Room. If special tools or supplies are required, the primary caretakers must approve their entry.
- Food and smoking is prohibited in Invertebrate Room. Leave all personal effects (e.g. lunchboxes, briefcases and attaches, backpacks, etc.) in the Main Office Building.
- Culture and research containers must be sealed and closed with a well fitted lid prior to exiting.

- Wash hands and exposed forearms prior to exiting.
- Exiting the room requires a thorough visual inspection of all clothing and shoes to minimize the risk of accidental escape of contained organisms from the laboratory.
- Exit the building quickly and ensure that the exterior door is completely closed and locked after passage.
- Personnel of the project(s) assigned space in the Room will be responsible for cleaning the area used. If assigned areas are shared with other projects, the occupants are expected to share equally in cleaning responsibilities. These areas must be cleaned and disinfected with bleach solution immediately upon completion of work (within 24 to 48 hours) or in accordance with annual schedules (i.e. muriatic acid wash annually for the tanks, chillers, and pumps).

Specimen collection.- Specimen collections, while critical for refugia maintenance and scientific research, are a common route of undesirable species introductions. Therefore the following steps must be taken 1) complete HACCP plan, 2) species identification must be done by experienced staff, 3) visual inspections of all field collection gear must be done *in situ*, and 4) containers and collection gear should be thoroughly rinsed and with clean water prior to transport. Given that collection sites for invertebrates commonly requires that vehicles take unpaved roads and cross rivers and streams the vehicle(s) should be visually inspected and power washed as close to the collection site as possible. Upon returning from the field all equipment used (waders, nets, transport containers, etc.) should be disinfected immediately using bleach or Roccal. No field equipment should enter the invertebrate room until it has completed the disinfection process. Transport containers for organisms brought in for culture at the SMARC should be visually inspected for undesirable species before any organism is transferred. Transport water should not be poured into the drains. Transport containers must be sterilized with Roccal, rinsed thoroughly, air dried, and stored for future use.

Daily routine.- Each location lot should be kept in at least two isolated and independently operated aquatic systems. All culture containers must be clearly marked with the name of the species and collection location. Each aquatic systems is equipped with a full complement of

labeled equipment (e.g. dip nets, siphon tubes etc.) that is never transferred between aquatic systems. The effluent pipe from the retention tank(s), post sand filter/chlorinator must be equipped with a 150 micron mesh cap. This acts as a safety measure and allows monitoring of system for inadvertent escapement.

AQUATIC AND TERRESTRIAL PLANTS

Specimen collection.- Plants can be successfully collected from the wild either as whole plants or tillers. Collection of tillers is preferable because it does not disturb the wild plant population as a whole or the sediments where the plant is rooted. Additionally, collecting whole plants often result in unwanted hitchhikers (snails, fish eggs, crayfish etc.) that should not be brought back to the hatchery.

Greenhouse (refugia tanks) entry.-

- Access to the refugia tanks (western one-third of the greenhouse) is limited to the botanist and primary caretaker. Volunteers and CRS clients are allowed access in the eastern two-thirds of the greenhouse for general work (potting plants, cleaning tanks, etc.) through the second and third gates on the south side of the greenhouse.
- Anyone potting plants or doing general cleaning must coordinate plans through the botanist or primary caretaker. If additional pots, equipment, soil, etc. are needed from the storage shed at the western end of the greenhouse, this must be arranged through the botanist or primary care taker.
- The use of muriatic acid ($\leq 10\%$ in solution with water) to clean tanks must be performed outside of the greenhouse at a distance greater than 20 feet from any plant as hydrogen gas released from contact with calcium carbonate can injury or kill plants.
- All visitors, contractors, and other personnel entering the greenhouse must be accompanied by the botanist, primary caretaker or SMARC staff member.

Texas wild rice.-

- Complete HACCP plan.

- Plants or tillers will be collected in furthest upstream followed sequentially at sites downstream. Each stand will be mapped using geographical propositioning system (GPS), measured (length x width), and depth of water (m) recorded.
- Tillers will be collected for the culms of Texas wild rice by cutting the tiller off at the node with pruning shears.
- The shears will be cleaned with 95% ethanol after the cut is made.
- All parts of the tiller will be inspected for signs of disease, fungus, egg casings, snails, etc. and all unwanted materials will be removed from the plant.
- Tillers will be individually bagged and a 6 to 8 cm section of the leaf will be excised for genetic analysis. Tissue collected for genetic analysis will be placed in a sealed labeled zip-lock bag. All collected plant tissue will be temporary placed in a cooler filled with river water.
- Water in cooler(s) will be replaced every 30 to 45 minutes to keep the samples cool.
- Tillers brought back to SMARC greenhouse will be immersed for 30 minutes in a 2% salt solution.
- Following treatment, the tillers will be potted in soil and placed in a quarantine tank(s) for at least one month. Water inflow will be 3 gallons/minute and outflow will be diverted into a sand/gravel bed outside the greenhouse. The water from the tank will percolate through the sand, gravel, and soil into a French drain below and around the greenhouse, into a sump, and then pumped into the hatchery waste water drain line.
- Potted tillers will be monitored for 30 days for signs of disease, fungus, egg casings, snails, etc., all of which will be removed. Any diseased plant part, fungus, egg casing, or snails that is removed from the plant will be placed into a container and treated with 5% hydrochloric acid for 24 hours, after which it will be disposed of in the trash.
- After 30 days of quarantine, and if no infections or insects are detected, the plants will be inspected by TXFWCO staff for clearance. Upon clearance, the plants will be placed in refugia tanks within the SMARC greenhouse.
- Genetic samples will be brought back to the SMARC East Lab in sealed zip-lock bags. Samples will be visually observed for signs of disease and other organisms. Any samples with observed signs of disease or insects will be disposed of as described above. The

tissue samples will be placed on ice and sent over night to the Southwestern Native Aquatic Resources and Recovery Center (SNARRC), Dexter, NM.

Other macrophytes, riparian and terrestrial plants .- All other macrophytes brought on station should be collected and transported as bare root stock. For aquatic plants, 1 to 3 stems should be collected at once and all soil and debris washed off in water at the time of collecting. Aquatic plants should never be collected in bulk by removing dozens of stems at once as this creates the likelihood of unwanted hitchhikers such as crayfish and hydrilla or hygrophila fragments. Prior to transport, plants should be rinsed free of sediment and debris and visually inspected for unwanted hitchhikers. Plants containing gelatinous material, egg casings, necrotic or chlorotic tissue, or otherwise look unhealthy should be discarded. Tolerant plants should be placed in a 2% salt solution for 30 minutes followed by quarantine in fresh water as per the Texas wild rice SOP.

Transfer to other locations.- Tolerant plants should be placed in a 0.5 % salt solution before leaving station. Intolerant plants should be shipped as bare root stock if possible and visually inspected prior to transfer. If transporting in a fish-hauling trailer or cooler(s) the water in the trailer can be the 0.5% salt solution.

FISH

Holding House Entry/Exit .-

- Entry into the Holding House is restricted to the Lead Biologist and primary caretaker and access is at their discretion.
- The lead, primary caretaker, or cleared designee must escort all visitors, contractors, and other personnel. Prior to entry wash hands and exposed forearms thoroughly. The Lead or primary caretaker must authorize in advance all items entering the Holding House.
- Approved items and personnel will enter through the north side door. Tools and supplies are provided in the Holding House. If special tools or supplies are required, the primary caretakers must approve their entry.

- Culture and research tanks are all operated with independent pumps and chillers and equipped with labeled equipment (e.g. siphon tubes, nets, buckets etc.) that are to be used for only that tank system.
- Upon exit ensure the door is secure and locked.

Specimen collection.-

- Complete HACCP plan.
- Clean and sterilize quarantine tanks before collecting any fish with NaCl and let sit for about an hour. Rinse well with fresh water. Place four clean and sterilized polyvinyl chloride (PVC) “houses” for fountain darters tanks or clean vegetation for Devils River minnows tanks. Add standpipe with mesh cover and fill tanks.
- Collection should involve at least two people, but if it is to be a large collection, more people are optimal. It is safer to collect in pairs and will expedite the process. One person should be designated to collect the specimens and the other should act as recorder.
- There are four large dip nets available for refugia and fish health collections. The gray-handled dip net is for use in the Comal River and Landa Lake, and the black handled dip net is for use in the San Marcos River. There is a small red-handled dip net that can be used in addition to the other nets if more than two collectors are necessary. Additional Comal and San Marcos River nets are available for alternative collection efforts such as research projects and monitoring efforts.
- There are also two canoes designated for use. The red canoe and black oars are for use in the Comal River and Landa Lake, and the yellow/green canoe and yellow oars are for use in the San Marcos River.
- Fish should be visually examined by an experienced staff member during collection and only healthy individuals should be retained. Water should be exchanged at regular intervals to maintain dissolved oxygen levels, the proper pH, and temperature. All debris should be removed from transport tanks (coolers) at the collection location. Each small transport tank can hold approximately 15 to 25 darters.
- Incoming fish are treated with a formalin bath at a rate of 170ppm of formaldehyde in 4L of water. This is a static treatment for one hour where fish are immersed. During the treatment fish must be monitored. If fish exhibit abnormal behavior (e.g. flashing, piping

for air) or sudden mortality occurs, flush out the system immediately with water or quickly transfer the fish to an empty tank. Flush out the system immediately at the end of the hour. It is important to note that this suggested dosage is for adult fish only. Smaller fish (less than 20 mm in total length) should be treated with a lower dose (e.g. 120ppm/4L) and confirmed with the lead biologist or fish health unit prior to treatment.

- Treated fish will be placed in the quarantine holding area within the pre-cleaned tanks. Tanks will be labeled by collection site and date of collection. Incoming fish will be treated with a formalin bath for one hour. Notes will also be made regarding the number, condition, treatment, origin of source and date within the laboratory record books.
- All nets coolers and oars must be sterilized in Roccal solution for at least 1 hour. Rinse off the vehicle floor mats and thoroughly dry in the sunlight. Rinse all personal gear (i.e. waders, boots etc.) with water and thoroughly dry in the sunlight.
- All fish must be quarantined until cleared by the Fish Health Unit prior to incorporation with older stock(s). During this time, fish will remain in isolation from the rest of the refugia.

Daily routine.-

- A dilute solution of bleach ($\frac{1}{3}$ bleach, $\frac{2}{3}$ water) serves as a disinfectant for all plasticware, rubber tubing, siphon tubes, miscellaneous parts, PVC pieces, and most other equipment (hydrolab, satumeter, etc.) monthly. Equipment should be soaked in bleach solution for only 10 to 15 minutes. All equipment is to be thoroughly rinsed after dipping in bleach and dried for 24 hours. Soaking nets for longer will cause erosion of the netting.
- Muriatic acid used for disinfecting purposes should only be used in a dilute form ($\frac{1}{4}$ muriatic acid, $\frac{3}{4}$ water). Caution should be taken in its preparation and use. Always use gloves, eye protection, and an apron when handling muriatic acid. If using large amounts in an unventilated area, it is best to protect oneself with the oxygen suit found in the laboratory as inhalation of the acid can cause breathing difficulties and is potentially very dangerous.

SALAMANDERS

Building Entry and Exit .-

- Entry into the PAD and Barton Springs Buildings are restricted to the Lead Biologist and primary caretaker and access is at their discretion.
- The lead, primary caretaker, or cleared designee must escort all visitors, contractors, and other personnel. Prior to entry wash hands and exposed forearms thoroughly. The Lead or primary caretaker must authorize in advance all items entering the buildings.
- Tools and supplies are provided in the buildings. If special tools or supplies are required, the primary caretakers must approve their entry.
- Culture and research tanks are all operated with independent pumps and chillers and equipped with labeled equipment (e.g. siphon tubes, nets, buckets etc.) that are to be used for only that tank system.
- Upon exit ensure the door is secure and locked.

Specimen collection.-

- Complete HACCP plan.
- Clean and sterilize quarantine tanks before collecting any salamanders with bleach ($\frac{1}{3}$ bleach, $\frac{2}{3}$ water) and let sit for about an hour. Rinse well with fresh water. Place substrate and/or clean vegetation into the tank. Add a standpipe with a mesh cover and fill tanks. Water level in each tank is not to exceed two-thirds of the tank height.
- Collection efforts require at least two people, but if it is to be a large collection, more people are optimal. Collection efforts must be communicated with the supervisor.

Daily routine.-

- Containers with salamanders generally are cleaned only when necessary to avoid disturbance. This means no scheduled cleaning. However, whenever the container must be disturbed (e.g., for collecting eggs, counting salamanders, and removing salamanders), siphon debris into a bucket (to capture accidentally siphoned salamanders).
- If there are hydra present in the salamander culture tanks, treat the entire system at 1% NaCl for 1 hour static. Start the treatment early in the morning so you can observe the salamanders all day long, during and after the treatment. Calculate amount of salt needed

for the entire volume of the system (include reservoir tank, aquaria, etc.), put this amount in a 5-gallon bucket, and add well water to the bucket to dissolve some of the salt. If there are excessive amounts of worms, remove them prior to treatment (salt usually kills them and it will result in water quality problems after treatment is over). Turn off the well water input to the system so that it will be a static treatment. Add the solution to the reservoir tank, slowly. Do not add undissolved salt directly to the system. If the reservoir tank has salamanders in it, be especially slow and careful adding the salt solution; avoid dumping the solution near salamanders. Repeat this process until most of the salt has been put in the system. There usually is a dark residue that doesn't dissolve easily - do not put this residue into the system. Begin timing the treatment (1 hour). Check on the salamanders several times during treatment and if they seem agitated, stop the treatment and add well water (using a hose to get the salt flushed out quickly). If the salamanders look fine after 1 hour, turn the well water supply back on to the system. The salt should be flushed out by the end of the day (make sure to check on the salamanders at the end of the day). If the salt treatment fails to kill all hydra, do not repeat treatment until at least 2 days later. Siphoning can be effective at removing hydra and can be used on consecutive days if needed.

- Sick salamanders are to be isolated from other individuals. Sometimes salamanders will stop eating, will become bloated (presumed not related to supersaturation), will swim in circles and spirals, will develop curved bodies or bumps on their heads, and females may have eggs erupt from their abdominal walls or cloaca in a large mass. These salamanders must be isolated, in quarantine or separated from the refugia or study salamanders. Make sure that there is plenty of cover since the salamanders can recover from many maladies on their own if left alone (still must be fed). The bumps on the head may be related to diet, as may be the case for many other problems, and it is possible that an iodide-rich flake might help and should be added to the worms used for feed and to the container with salamanders with the head bumps.
- Dead salamanders are to be removed immediately from tank systems and preserved to reduce the spread of disease. Snails present in tanks will feed on dead salamanders and

may serve as a vector. Sometimes the tails continue to move even after the heart has stopped beating.

- Aquatic systems need to be acid-washed annually to remove calcium carbonate deposits which affect efficiency of the temperature-conditioning equipment, valve functionality, flow, degassing, etc. Salamanders must be removed and transferred to a disinfected system during washing. However, if the salamanders cannot be moved to other systems (e.g., none available, not enough time, not enough labor available), the temperature conditioning loop can be acid-washed. This requires disconnecting the plumbing from the culture tanks and running the water through a “loop” - basically a trashcan replacing the culture system. A backup heater/chiller unit should be used to maintain water temperature in the culture system with salamanders. Add about a quarter of a gallon of HCL, slowly, to the heat exchanger and then pump water through the “loop.” After a few days, flush the “loop” with well water. Take reading of pH with pH-indicating paper (do not use pH meter, ever, for this purpose). If pH equals that of well water, then rinse a few more hours, drain, reconnect the plumbing, and begin using the system as before. Make absolutely sure there is no acid left in the system - it will kill salamanders.
- Aquarium certified silicone is commonly used to seal gaps between screen dividers and tank walls. This prevents salamanders from entering into an undesired section of the tank (e.g., bio-filter section with suction end of the recycling pipe system). Worms may find small gaps between the silicone and the tank wall and make them larger. Eventually salamanders may begin to hide in the gaps further increasing the size of the gaps until they are large enough to allow salamanders to pass through. Seal inspections should be done during daily walk-throughs. Should a gap be found where worms or salamanders are hiding under the silicone or if salamanders are found in an undesignated tank section, the seal must be repaired. The repair should be done following the acid-washing procedure. Once the tank system is clean, remove the old silicone with a scraper, clean the tank surfaces (where the silicone will be applied) with alcohol, and reapply the silicone along the gap as a uniform bead. Slide your finger (wearing a surgical glove) along the bead to compress the seal. Allow silicone to completely dry before refilling

with water. After filling with water, add bio-media, snails, vegetation, tank furniture, allow water to recirculate in tanks for about two weeks or when water quality is stable and safe for salamander reintroduction.

APPENDIX A

Study Plan Standard Operating Procedure

Study Plan Standard Operating Procedure

When preparing a study plan for a project it should contain the following components:

1. **Background and Justification** of project. This section of the study plan presents why it is important to conduct such research.
2. **Objectives**, this portion of the study plan clearly states the intended goals of project.
3. **Materials and Methods**, this section of the study plan includes detailed descriptions of methods and procedures intended to be performed to accomplish objectives.
4. **Investigator Responsibilities**, defines the tasks of the project and who will perform such tasks.
5. **Expected Products**, this section of the study pan states what the intended outcome of the project is to be. It includes to whom the results will be presented and most applicable.
6. Provide a **Schedule** of the purposed study. This section defines the expected timeline of the project, it states when the annual and final reports can be expected.
7. **Budget**, provides a detailed description of all allocated monies predicted to conduct such study.
8. **Intended Method of Dissemination**, states the anticipated method in which research results will be conveying.
9. **Management Implications**, defines how the results of this study will be applied for management implications.

GROWTH AND CONDITION OF JUVENILE CHINOOK SALMON IN DIFFERENT
FRESHWATER TIDAL HABITATS

Submitted by:

(INSERT AUTHORS)

U.S. Fish & Wildlife Service
San Marcos Aquatic Resources Center
500 East McCarty Lane
San Marcos Texas, 78666

(INSERT DATE)

Background and Justification

FOR EXAMPLE:

Tidally influenced habitats are used by anadromous salmonids to various extents during ocean emigration (Reimers 1971; Volk et al. 2010). These habitats presumably provide opportunities for growth, shelter from predators, and physiological preparation for saltwater entry (Healey 1982; Levy and Northcote 1982; Simenstad et al. 1982; Bottom et al. 2005; Semmens 2008). As a result salmon recovery efforts in the Columbia River estuary have focused on restoring available lands to once abundant tidally-influenced habitats.

Chinook salmon are often the most abundant juvenile salmonid in tidally-influenced wetland habitats of the Columbia River estuary (Bottom et al. 2008), but what benefits different habitats impart to emigrating Chinook salmon is unknown. Growth is often used as an indicator of habitat quality, and the growth of subyearling Chinook salmon during their sometimes long residency in freshwater and saltwater portions of the Columbia River estuary has been measured indirectly by examining otoliths (Bottom et al. 2009; Campbell 2010). Linking growth to specific habitats is difficult using otoliths due to the emigration behavior of juvenile Chinook salmon.

It is reasonable to expect that different tidally-influenced habitats may have different potentials for juvenile salmonid growth. Freshwater tidal habitat use by salmonids have been investigated in the Columbia River estuary at tributary junctions, backwater areas of island complexes, and shallow shoreline areas (Bottom et al. 2009; Lott 2004). Dietary inputs and feeding intensity have differed in habitat types within these areas (Lott 2004). Furthermore, the diets of emigrating juvenile salmon contain a disproportionately high amount of invertebrate prey from tidal wetland areas (Maier and Simenstad 2009). Multiple studies also show that Chinook salmon smolts traveling through temporarily available riverine habitats grow more than those traveling through mainstem habitats (Sommer et al. 2001; Jeffres et al. 2008).

The growth potential for fish in particular habitats may not be reflected solely by measures of prey abundance and consumption because many other abiotic and biotic factors influence fish growth (i.e. temperature, fish density). Without direct measures of growth it is unknown if some tidal wetland habitats confer more advantages to salmon than others, which may precludes strategic habitat restoration (as called for by Bottom et al. 2008). Moreover, growth can be a poor indicator of habitat quality when density dependent factors and selective mortality are not quantified (Searcy et al. 2007). Thus, caging experiments that measure the growth of individual fish restricted to locations with measurable fish densities and environmental conditions could be a important compliment to studies that quantify growth in free ranging juvenile salmon smolts (Sommer et al. 2001; Henning et al. 2006; Jeffres et al. 2008; López-Rasgado and Herzka 2009).

Objective

FOR EXAMPLE:

Our goal is to use multiple growth indices (somatic growth, condition factor, inductance) obtained from caged subyearling Chinook salmon to quantify the growth potential of fish exposed to 2 tidal freshwater habitat types (island backwaters, and shallow shorelines). Differences in Chinook salmon growth potential will be a relative measure of habitat quality for different tidal freshwater habitat types over time.

Materials and Methods

FOR EXAMPLE:

Subyearling Chinook salmon use of tidal freshwater habitats in the Columbia River estuary peaks between March and June. Residence time of Chinook salmon in the tidal freshwater portion of the estuary lasts for at least 33 days on average (Bottom et al. 2009). We therefore plan to conduct a 63-day caging experiment (April-June) to evaluate differences in growth and condition indices between Chinook salmon using two freshwater tidal habitats (island backwaters and shallow shorelines) using morphological and physiological traits.

We will deploy four cages at each of the two habitat types (8 enclosures total) on the shore of Cottonwood Island, WA. Cages will be constructed with 1.6mm PVC reinforced mesh with 5.1 cm diameter PVC tubing. Cages will be 1.2m X 1.2m and 1.2m in height (4ft X 4ft X 4ft). Cages will be placed at low tide prior to increases in water levels that occur from March to June. This will ensure that a portion of the cage will remain submerged during the entire experiment. Cages will be anchored to the substrate with cement blocks. Water temperature will be measured at hourly intervals during the 63 day study period by a data logger attached to the cages. Dissolved oxygen will be measured hourly during the lowest tidal cycle of each three week period.

Subyearling Chinook salmon will be obtained from Kalama Falls Hatchery in April. Thirty Chinook salmon will be sacrificed for blood and tissue collection immediately prior to stocking the cages with fish in order to characterize the physiological condition of fish prior to the treatments. Initially 60 Chinook salmon will be weighted, measured, and added to each cage (2 treatments $\times$ 4 cages $\times$ 60 fish = 480 Chinook salmon). Three cages per treatment site will be sampled. Sampling cages will each have five fish removed at three, six, and nine weeks after stocking (2 treatments $\times$ 3 cages $\times$ 5 fish = 30 Chinook salmon per 3 week period). These fish will be measured, weighed, and sacrificed for blood and tissue collection. The fourth cage at each treatment site will be used as a holding pen for extra fish. Biomass will be held constant in the sampling cages by replacing Chinook salmon removed for growth and physiology measurements with marked, size-matched fish from the fourth cage. We will only monitor the growth and physiology of unmarked fish that have spent the entire study period in the particular cage we sampled from. Protein and triglyceride content of the blood from sacrificed fish will be quantified and evaluated as a physiological indicator of condition. RNA/DNA ratios will be quantified from liver tissue as a physiological indicator of short term growth. Gill tissue will be collected as an indicator of smoltification.

Growth and physiological indicators will be compared between treatments and sample periods using a Two-way Analysis of Variance. The mean physiological indicator of each treatment will be compared to the physiology of pre-treatment fish using a Dunnett's test.

Investigator Responsibilities

FOR EXAMPLE:

Oversee cage construction-(INSERT NAME)

Oversee cage placement-(INSERT NAME)

Oversee fish collection-(INSERT NAME)

Oversee fish allocation to cages-(INSERT NAME)

Oversee fish measurement-(INSERT NAME)

Expected Products

FOR EXAMPLE:

A final report will be produced summarizing response variables and their relationship with habitat type. The final report will present the data (table and text) and will include summary statistics and the associated errors. Statistical analyses will be conducted on all appropriate comparisons.

Schedule

FOR EXAMPLE:

2011 February: Enclosure construction

2011 March: Deploy enclosures.

2011 April: Collect fish, add fish to enclosures.

2011 May: Sample and replace fish.

2011 June: Finalize sampling. Remove enclosures.

2011 July: Laboratory analyses of physiological variables. Enter and analyze fish measurements.

2011 December: Final report/manuscript prepared for internal review.

Budget

FOR EXAMPLE:

<u>Personnel</u>	Cost (dollars)
Supervisory Fisheries Biologist – physiology (PI, GS-13/5)	
Hourly rate \$44.43; Approximately 40 h	\$1,777
Fisheries Biologist (GS-12/1)	
Hourly rate \$34.07; Approximately 40 h	\$1,363
Fisheries Biologist (GS-11/4)	
Hourly rate \$30.26; Approximately 40 h	\$1,210
Fisheries Biologist (GS-9/6)	
Hourly rate \$26.52; Approximately 88 h	\$2,334
Fisheries Biologist (GS-9/5)	
Hourly rate \$25.77; Approximately 160 h	\$4,123
Fisheries Technician (GS-5/2)	
Hourly rate \$15.51; Approximately 160 h	\$2,482
Subtotal salaries	\$13,289
Fringe benefits (35%)	\$4,651
<i>Total, personnel</i>	<i>\$17,940</i>

Equipment & Supplies

Enclosures	\$800
Manuscript fees	\$900
Triglyceride(N = 120 fish,8.00 per fish)	\$960
Protein(N = 120 fish, 7.00 per fish)	\$840
RNA:DNA(N = 120 fish, 28.00 per fish)	\$3,360
ATPase activity (N=120, 8.00 per fish)	\$960

Misc. Equipment/supplies	\$200
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<i>Total Equipment & Supplies</i>	<i>\$8,020</i>
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Travel

Gas (@ \$4.00 per gallon)	\$400
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<i>Total Travel</i>	<i>\$400</i>
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Total:	\$26,360
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Intended Method of Dissemination

FOR EXAMPLE:

The study results will be presented at a scientific meeting and in a manuscript submitted to a scientific journal.

Management Implications

FOR EXAMPLE:

If differences in growth are found it might lead to prioritization for protection or restoration of particular tidal wetland habitats.

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APPENDIX B

Species-Specific Hazard Analysis and Critical Control Point (HACCP) Plan: Example for Devils River Minnow

(see SMARC Local Drive HACCP for all Complete Species Plans and Forms)

Methods to Prevent Transfer of Aquatic Nuisance Species

National Fish Hatchery and Technology Center
U. S. Fish and Wildlife Service
500 East McCarty Lane
San Marcos, Texas 78666

Aquatic species that are not native to an area and pose a threat to native species are considered aquatic nuisance species (ANS). These species cause problems with native species through displacement, predation, lethal defense mechanisms, toxins, disease introduction, and habitat alteration. They can also severely affect hatchery production and operations through fish kills, blockage of hatchery systems, and disease infection requiring quarantine or destruction of stock. Because of this, prevention of ANS transfer is important for field workers and hatchery personnel. Several procedures are implemented by employees of the San Marcos NFHTC.

Within the Hatchery – to prevent transfer of organisms (e.g., eggs, fry, algae, fungus, snails, flatworms, scuds, feed, diseases) between culture systems, staff wash equipment such as siphons, nets, water quality meters, and buckets in containers of bleach or Roccal at concentrations to provide a quick decontamination (kills most organism or induces release into the container resulting in eventual mortality).

Field Collection at Single Site – All equipment (coolers, nets, waders, footwear, water quality meters, dive gear, etc.) is washed, disinfected (e.g., Roccal, bleach, alcohol, extreme temperatures), and inspected before and after collection.

Species collected are quarantined for at least 2 weeks and holding systems are inspected for ANS at least every 3 days. Species brought in from other watersheds are permanently quarantined, and all water leaving these holding systems is screened, chlorinated, and filtered through sand before draining to effluent pond.

Fish are treated for external parasites with formalin and plants are treated for ANS with a salt bath before removal from quarantine.

Water brought in from field is spread on pavement to evaporate.

Field sites where work often takes place (San Marcos and Comal rivers) each have separate equipment (e.g., canoes, kayaks, large nets). These sites are not sampled on the same day as an extra safety precaution.

Field Collection at Multiple Sites – Equipment that is easily washed, inspected and disinfected on site is used for multiple daily collections such as smooth neoprene footwear without deep tread or spaces for ANS to retreat.

After collection at site, equipment is washed and inspected for ANS and then disinfected by spraying with alcohol or dipping in bleach solution away from water or moist ground.

Equipment is allowed to dry and inspected again before use at next site.

Any site with known ANS present is sampled at the end of the day after those sites that are presumed to be free of ANS.

All equipment is thoroughly washed, decontaminated, and dried in safe area at the hatchery after final collection.

Awareness and Monitoring – These are important components to the prevention of ANS transfer. Personnel on station or in the field, should keep in mind the threat of ANS, and realize when to practice procedures to prevent transfer. Also, when returning to field sites, keeping an eye out for newly introduced ANS is the best way to prevent a serious invasion through early detection.

EXAMPLE: HACCP (Hazard Analysis and Critical Control Point) Plan

Devils River minnow *Dionda diaboli*

Modified on 16-Dec-11

1. Product Description
2. Flow Diagram
3. Potential Hazards
4. Hazard Analysis Worksheet
5. HACCP Plan Form

1. Product Description

Firm Name:	San Marcos National Fish Hatchery and Technology Center
Firm Address:	500 East McCarty Lane San Marcos, Texas 78666
Species:	Devils River minnow (<i>Dionda diaboli</i>)
Cultured, wild-harvested, or both:	wild-harvested
Harvest method:	seine or dip net from spring-fed creeks
Method of storage:	flow-through units supplied with water directly from the Edward's Aquifer
Intended use and consumer:	refugia

2. Flow Diagram

Step 1	Devils River minnows are collected as needed from spring-fed creeks feeding the Devils River and the Rio Grande, vehicles are inspected before and after collection event for non-natives
Step 2	Devils River minnows are transported in coolers, filled with water from the collection site, to the Holding House at NFHTC. All water from this room is chlorinated and sand-filtered (primarily to prevent off-watershed escapement)
Step 3	Devils River minnows are placed in quarantine systems, are fed commercial flaked food, and are examined for ANS at least every 3 days

Step 4	Minnows remain in the refugia systems for at least 2 weeks
Step 5	
Step 6	

3. Potential Aquatic Nuisance Species (ANS) Hazards

List aquatic species here that are found in hatchery water supply or local waters that could potentially hitchhike to receiving waters and cause ecological harm.

Vertebrates	armored catfishes, mosquitofish, various aquarium-trade fishes, tadpoles
Invertebrates	<i>Melanoides tuberculata</i> , <i>Thiara granifera</i> , crayfish, various parasites
Plants	<i>Hydrilla</i> , <i>Hygrophila</i> , water hyacinth

4. Hazard Analysis Worksheet

(1) Harvest or Aquaculture Step	(2) Identify potential ANS hazards introduced or controlled		(3) Are any hazards significant?	(4) Justify your decisions for column 3	(5) What control measures can be applied to prevent the hazards?	(6) Is this step a critical control point?
Devils River minnows are collected as needed from spring-fed creeks feeding the Devils River and the Rio Grande, vehicles are inspected before and after collection event for non-natives	vertebrates	armored catfishes, mosquitofish, various aquarium-trade fishes, tadpoles	yes	ANS are present in the Comal River and Devils River	manually remove ANS from collection at site	yes
	invertebrates	<i>Melanoides tuberculata</i> , <i>Thiara granifera</i> , crayfish, various parasites	yes	ANS are present in the Comal River and Devils River	manually remove ANS from collection at site	yes
	plants	<i>Hydrilla</i> , <i>Hygrophila</i> , water hyacinth	yes	ANS are present in the Devils River	manually remove ANS from collection at site	yes
Devils River minnows are transported in	vertebrates	armored catfishes, mosquitofish, various aquarium-	no	ANS not present	not needed	no

coolers, filled with water from the collection site, to the Holding House at NFHTC. All water from this room is chlorinated and sand-filtered (primarily to prevent off-watershed escapement)		trade fishes, tadpoles				
	invertebrates	Melanoides tuberculata, Thiara granifera, crayfish, various parasites	yes	ANS may be transferred from net into quarantine tank	frequent observation during quarantine and removal of ANS	yes
	plants	Hydrilla, Hygrophila, water hyacinth	yes	ANS may be transferred from net into quarantine tank	frequent observation during quarantine and removal of ANS	yes
Devils River minnows are placed in quarantine systems, are fed commercial flaked food, and are examined for ANS at least every 3 days	vertebrates	armored catfishes, mosquitofish, various aquarium-trade fishes, tadpoles	no	ANS not present	ANS becomes more noticeable as develop and are removed during quarantine process	no
	invertebrates	Melanoides tuberculata, Thiara granifera, crayfish, various parasites	yes	ANS may be transferred from net into quarantine tank	ANS becomes more noticeable as develop and are removed during quarantine process	yes
	plants	Hydrilla, Hygrophila, water hyacinth	yes	ANS may be transferred from net into quarantine tank	ANS becomes more noticeable as develop and are removed during quarantine process	yes

4. Hazard Analysis Worksheet

(1) Harvest or Aquaculture Step	(2) Identify potential ANS hazards introduced or controlled		(3) Are any hazards significant?	(4) Justify your decisions for column 3	(5) What control measures can be applied to prevent the hazards?	(6) Is this step a critical control point?
Minnows remain in the refugium systems for at least 2 weeks	vertebrates	armored catfishes, mosquitofish, various aquarium-trade fishes, tadpoles	no	Control of ANS hazard completed	Not needed	no
	invertebrates	Melanoides tuberculata, Thiara granifera, crayfish, various parasites	no	Control of ANS hazard completed	Not needed	no
	plants	Hydrilla, Hygrophila, water hyacinth	no	Control of ANS hazard completed	Not needed	no
	vertebrates					

	invertebrates					
	plants					
	vertebrates					
	invertebrates					
	plants					

5. ANS-HACCP Plan Form

(1) Critical Control Point (CCP)	(2) Significant Hazard(s)	(3) Control Measures	Monitoring				(8) Corrective Measures	(9) Records	(10) Verification
			(4) What	(5) How	(6) Frequency	(7) Who			
dip nets, ice chests, and other equipment are removed from the river	vertebrates invertebrates plants	pick out ANS hazards by hand and dip all equipment in 10% bleach solution	presence of ANS hazards	visual inspection of equipment	before, during, and after use of equipment	hatchery and field personnel	remove ANS by hand	record day of inspection, observations, and actions	records to be reviewed by hatchery manager to verify that ANS control measures are being done
Devils River minnows are transported in coolers, filled with water from the collection site, to the Holding House at NFHTC. All water from this room is chlorinated and sand-filtered (primarily to prevent off-watershed escapement)	vertebrates invertebrates plants	pick out ANS hazards by hand and dip all equipment in 10% bleach solution, inspect transport vehicle and remove incidental debris	presence of ANS hazards	visual inspection of equipment	before, during, and after use of equipment	hatchery employee	remove ANS by hand	record day of inspection, observations, and actions	records to be reviewed by hatchery manager to verify that ANS control measures are being done
quarantine tank(s) is/are checked every 3 days for presence of ANS for at least 2 weeks	vertebrates invertebrates plants	pick out ANS hazards	presence of ANS hazards	visual inspection of quarantine tank	every 3 days	hatchery employee	if ANS are frequently noticed, then extend quarantine until ANS completely removed	record presence of ANS and final day of quarantine	records to be reviewed by hatchery manager to verify that ANS control measures are being done

