



**CONTRACT BETWEEN THE CITY OF AUSTIN ("City")
and
UNIVERSITY OF TULSA ("Contractor")
for**

Description: Transcriptomic evaluation of chronic stress in Austin's endangered salamanders

Contract Number: MA 6300 NA210000209

The City accepts the Contractor's Offer for the above requirement and enters into the following Contract. This Contract is between University of Tulsa having offices at Tulsa, Oklahoma and the City, a home-rule municipality incorporated by the State of Texas. Capitalized terms used but not defined herein have the meanings given in the Solicitation.

1.1 This Contract is composed of the following documents in order of precedence:

- 1.1.1 This Document
- 1.1.2 Negotiated Terms and Conditions dated October 12 , 2021
- 1.1.3 Scope of Work along with Exhibit A

1.2 Compensation.

The Contractor shall be reimbursed a total Not-to-Exceed amount of \$ 40,534 for the initial Contract term and a Not-to-exceed amount of \$ 34,103 for the second contract term as indicated in the Offer.

1.3 Term of Contract.

This Contract shall remain in effect for an initial term from the date of execution of this Agreement through December 31, 2022 or the City terminates the Contract. This Contract may be extended automatically beyond the initial term for an additional 12 month period.

- 1.4 Designation of Key Personnel.** The City and the Contractor resolve to keep the same key personnel assigned to this engagement throughout its term. In the event that it becomes necessary for the Contractor to replace any key personnel, the replacement will be an individual having equivalent experience and competence in executing projects such as the one described herein. Additionally, the Contractor shall promptly notify the City and obtain approval for the replacement. Such approval shall not be unreasonably withheld. The Contractor's and City's key personnel are identified as follows:

	<u>Name</u>	<u>Phone Number</u>	<u>Email Address</u>
Contractor Technical Manager	<u>Ronald M. Bonett, Phd</u>	<u>734-872-5016</u>	Ron-bonett@utulsa.edu
Contractor Contract Manager	<u>Debbie Newton</u>	<u>918-631-2192</u>	deborah-newton@utulsa.edu
City Contract Manager	<u>Josie Archer</u>	<u>512-974-9734</u>	Josephine.archer@austintexas.gov
City Project Manager	<u>Nathan Bendik</u>	<u>512-974-2040</u>	Nathan.bendik@austintexas.gov
City Contract Administrator, Procurement Specialist	<u>Jim Howard</u> <u>Procurement</u> <u>Specialist IV</u>	<u>512-974-2031</u>	Jim.howard@austintexas.gov

1.5 **Invoices.** The City's preference is to have invoices emailed to WPDInvoices@austintexas.gov

For questions regarding your invoice/payment please contact the City Contract Manager.

This Contract (including any Exhibits and referenced Documents) constitutes the entire agreement of the parties regarding the subject matter of this Contract and supersedes all prior agreements and understandings, whether written or oral, relating to such subject matter. This Contract may be altered, amended, or modified only by a written instrument signed by the duly authorized representatives of both parties.

By signing below, Contractor hereby certifies the following are true and will ensure the following will remain true throughout the term of this Contract:

1. That its firm and its principals are not currently suspended or debarred from bidding on any Federal, State, or City of Austin Contracts.
2. That it has not in any way directly or indirectly:
 - a. Colluded, conspired, or agreed with any other person, firm, or corporation, as to the amount of this contract or the terms or conditions of this contract.
 - b. paid or agreed to pay any other person, firm, or corporation any money or anything of value in return for assistance in procuring or attempting to procure a contract or in return for establishing the prices in the contract.
3. That it has not received any compensation or a promise of compensation for participating in the preparation or development of the underlying Contract documents.
4. In accordance with Chapter 176 of the Texas Local Government Code, that the Offeror:
 - a. does not have an employment or other business relationship with any local government officer of the City or a family member of that officer that results in the officer or family member receiving taxable income;
 - b. has not given a local government officer of the City one or more gifts, other than gifts of food, lodging, transportation, or entertainment accepted as a guest, that have an aggregate value of more than \$100 in the twelve month period preceding the date the officer becomes aware of the execution of the Contract or that City is considering doing business with the Offeror; and
 - c. does not have a family relationship with a local government officer of the City in the third degree of consanguinity or the second degree of affinity.
5. Pursuant to City Council Resolution No. 20191114-056, that its firm and its principals are not currently and will not during the term of the Contract engage in practicing LGBTQ+ conversion therapy; referring persons to a healthcare provider or other person or organization for LGBTQ+ conversion therapy; or Contracting with another entity to conduct LGBTQ+ conversion therapy. If the City determines in its sole discretion that Contractor has during the

term of this Contract engaged in any such practices, the City may terminate this Contract without penalty to the City.

6. Pursuant to Texas Government Code §2271.002, the Contractor verifies that it does not boycott Israel and will not boycott Israel during the term of this contract.
7. Pursuant to Texas Government Code Chapter 2274, the Contractor verifies that if it will have remote or direct access to communication infrastructure systems, cybersecurity systems, the electric grid, hazardous waste treatment systems, or water treatment facilities as a result of this contract, that it is not owned by or the majority of stock or other ownership interest of the Contractor is not held or controlled by:
 - a. individuals who are citizens of China, Iran, North Korea, Russia, or a Governor-designated country; or
 - b. a company or other entity, including a governmental entity, that is owned or controlled by citizens of or is directly controlled by the government of China, Iran, North Korea, Russia, or a Governor-designated country; or
 - c. headquartered in China, Iran, North Korea, Russia, or a Governor-designated country.
8. Pursuant to Texas Government Code Chapter 2274, the Contractor verifies that if it has 10 or more full-time employees, unless the contract contains a written verification from the company that it: (1) does not have a practice, policy, guidance, or directive that discriminates against a firearm entity or firearm trade association; and (2) will not discriminate during the term of the contract against a firearm entity or firearm trade association.
9. Pursuant to Texas Government Code Chapter 2274, the Contractor certifies that, if they have 10 or more full-time employees: (1) they do not boycott energy companies; and (2) will not boycott energy companies during the term of the contract.

In witness whereof, the parties have caused a duly authorized representative to execute this Contract on the date set forth below.

UNIVERSITY OF TULSA, OKLAHOMA

Debbie Newton

Printed Name of Authorized Person

Debbie Newton

Digitally signed by Debbie
Newton
Date: 2021.10.12 11:20:11
-05'00'

Signature

Director of Research and Sponsored Programs

Title:

10/12/2021

Date:

CITY OF AUSTIN

JAMES T. HOWARD

Printed Name of Authorized Person

James T
Howard

Digitally signed by James T
Howard
Date: 2021.10.12 12:34:39
-05'00'

Signature

PROCUREMENT SPECIALIST IV

Title:

10/12/2021

Date:

**CITY OF AUSTIN
TERMS AND CONDITIONS**

The Contractor agrees that the Contract shall be governed by the following terms and conditions.

1 GENERAL

1.1 TERM OF CONTRACT:

- A. The Contract shall commence upon execution unless otherwise specified and shall remain in effect for an initial term through December 31, 2022. The Contract may be extended beyond the initial term for up to one (1) additional twelve (12) month period with the mutual agreement of the Parties. If the City exercises any extension option, all terms, conditions, and provisions of the Contract shall remain in effect for that extension period.
- B. Upon expiration of the initial term or any period of extension, the Contractor agrees to holdover under the terms and conditions of this Contract for such a period as is reasonably necessary for the City to re-solicit and/or complete the Deliverables due under this Contract. Any holdover period will not exceed 180 calendar days unless mutually agreed on by both parties in writing.

1.2 COMPENSATION:

The Contractor will be paid as indicated herein upon the successful completion of the Scope of Work. In consideration for the services to be performed under this Contract, the Contractor shall be reimbursed an amount not-to-exceed \$40,534 for all fees and expenses for the initial term and an amount not-to-exceed \$34,103 for the extension period for a total amount of \$74,637. Unspent funds from the first period may be carried forward and expended during the second period.

1.3 INVOICES:

- A. The Contractor shall submit separate Invoices no more often than monthly for reimbursable expenses.
- B. Invoices shall be submitted to WPDInvoices@austintexas.gov. Invoices shall contain a unique Invoice number, the purchase Order or delivery Order number, the master agreement number (if applicable), the Department's Name, and the name of the point of contact for the Department. Invoices shall be itemized by line item according to pricing structure in the Contract. The Contractor's name and, if applicable, the tax identification number on the Invoice must exactly match the information in the Vendor's registration with the City. Unless otherwise instructed in writing, the City may rely on the remittance address specified on the Contractor's Invoice. Invoices received without all required information cannot be processed and will be returned to the vendor within ten (10) days of receipt.

1.4 PAYMENT:

- A. All proper Invoices received by the City will be paid within 30 calendar days of the City's receipt of the Invoice.
- B. If payment is not timely made, (per Paragraph A), interest shall accrue on the unpaid balance at the lesser of the rate specified in Texas Government Code §2251.025 or the maximum lawful rate; except, if payment is not timely made for a reason for which the City may withhold payment hereunder, interest shall not accrue until 10 calendar days after the grounds for withholding payment have been resolved.
- C. The City may withhold or set off the entire payment or part of any payment otherwise due the Contractor to such extent as may be necessary on account of:
 - i. Failure of the Contractor to pay Subcontractors, or for labor, materials or equipment;
 - ii. Damage to the property of the City or the City's agents, employees or Contractors, which is not covered by insurance required to be provided by the Contractor;
 - iii. Failure of the Contractor to submit proper Invoices with all required attachments and supporting documentation; or
 - iv. Failure of the Contractor to comply with any material provision of the Contract Documents.

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- D. Notice is hereby given of Article VIII, §1 of the Austin City Charter which prohibits the payment of any money to any person, firm or corporation who is in arrears to the City for taxes, and of §2-8-3 of the Austin City Code concerning the right of the City to offset indebtedness owed the City.
- E. The Contractor agrees to accept payment by either credit card, check or Electronic Funds Transfer for all goods and/or services provided under the Contract. The Contractor shall factor the cost of processing credit card payments into the Offer. There shall be no additional charges, surcharges, or penalties to the City for payments made by credit card.
- F. The awarding or continuation of this Contract is dependent upon the availability of funding. The City's payment obligations are payable only and solely from funds appropriated and available for this Contract. The absence of appropriated or other lawfully available funds shall render the Contract null and void to the extent funds are not appropriated or available and any Deliverables delivered but unpaid shall be returned to the Contractor. The City shall provide the Contractor written notice of the failure of the City to make an adequate appropriation for any fiscal year to pay the amounts due under the Contract, or the reduction of any appropriation to an amount insufficient to permit the City to pay its obligations under the Contract. In the event of inadequate appropriation of funds, there will be no penalty nor removal fees charged to the City. The City shall pay the Contractor, to the extent of funds appropriated or otherwise legally available for such purposes, for all goods delivered and services performed, and obligations incurred prior to the date of notice of non-appropriation.

1.5 FINAL PAYMENT AND CLOSE OUT:

- A. The making and acceptance of final payment will constitute:
 - i. A waiver of all claims by the City against the Contractor, except claims (1) which have been previously asserted in writing and not yet settled, (2) arising from defective work appearing after final inspection, (3) arising from failure of the Contractor to comply with the Contract or the terms of any warranty specified herein, (4) arising from the Contractor's continuing obligations under the Contract, including but not limited to indemnity and warranty obligations, or (5) arising under the City's right to audit; and
 - ii. A waiver of all claims by the Contractor against the City other than those previously asserted in writing and not yet settled.

1.6 AUDITS AND RECORDS:

- A. The Contractor agrees that the representatives of the Office of the City Auditor or other authorized representatives of the City shall have access to, and the right to audit, examine, or reproduce, any and all records of the Contractor related to the performance under this Contract, at the City's expense. The Contractor agrees to refund to the City any overpayments disclosed by any such audit. Contractor acknowledges that City is required to comply with Chapter 552 of the Texas Government Code (Public Information Act or Act). Under the Act, this Contract, and documents related to this Contract, which are in City's possession, or to which City has access, are presumed to be public and the City may release these records to the public unless an exception described in the Act applies to a document.
- B. Records Retention:
 - i. The Contractor shall retain all records for a period of three years after final payment on this Contract or until all audit and litigation matters that the City has brought to the attention of the Contractor are resolved, whichever is longer.

1.7 FINANCIAL DISCLOSURES AND ASSURANCE:

The City may request and review financial information as the City requires to determine the credit worthiness of the Contractor, including but not limited to, annual reports, audited financial Statements and reports, bank letters of credit or other credit instruments. Failure of the Contractor to comply with this requirement shall be grounds for terminating the Contract.

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1.8 RIGHT TO ASSURANCE:

Whenever one party to the Contract in good faith has reason to question the other party's intent to perform, demand may be made to the other party for written assurance of the intent to perform. If no assurance is given within the time specified after demand is made, the demanding party may treat this failure as an anticipatory repudiation of the Contract.

1.9 STOP WORK NOTICE:

The City may issue an immediate Stop Work Notice in the event the Contractor is observed performing in a manner that is in violation of Federal, State, or local guidelines, or in a manner that is determined by the City to be unsafe to either life or property. Upon notification, the Contractor will cease all work until notified by the City that the violation or unsafe condition has been corrected.

1.10 DEFAULT:

The Contractor shall be in default under the Contract if the Contractor (a) fails to fully, timely and faithfully perform any of its material obligations under the Contract, (b) fails to provide adequate assurance of performance, (c) becomes insolvent or seeks relief under the bankruptcy laws of the United States or (d) makes a material misrepresentation in Contractor's Offer, or in any report or Deliverable required to be submitted by the Contractor to the City. The City shall be in default if it fails to make payment in accordance with the Payment terms of this Contract.

1.11 TERMINATION FOR CAUSE:

In the event of a default by either party, the non-defaulting party shall have the right to terminate the Contract for cause, by written notice effective ten 10 calendar days, unless otherwise specified, after the date of such notice, unless the defaulting party, within such 10 day period, cures such default, or provides evidence sufficient to prove to the non-defaulting party's reasonable satisfaction that such default does not, in fact, exist. Additionally, the City shall have the right to act in accordance with the terms defined by "City of Austin Purchasing Office Probation, Suspension and Debarment Rules for Vendors." In addition to any other remedy available under law or in equity, either party shall be entitled to recover all actual damages, costs, losses and expenses, incurred by the party as a result of the Contractor's default, including, without limitation, cost of cover, court costs, and prejudgment and post-judgment interest at the maximum lawful rate. All rights and remedies under the Contract are cumulative and not exclusive of any other right or remedy provided by law.

1.12 ATTORNEY'S FEES:

In consideration of the award and execution of this Contract and in consideration of the City's waiver of its right to attorney's fees, the Contractor knowingly and intentionally waives its right to attorney's fees under §271.153, Texas Local Government Code, in any administrative proceeding, alternative dispute resolution proceeding, or litigation arising out of or connected to this Contract.

1.13 TERMINATION WITHOUT CAUSE:

The City shall have the right to terminate the Contract, in whole or in part, without cause any time upon 30 calendar days' prior written notice. Upon receipt of a notice of termination, the Contractor shall promptly cease all further work pursuant to the Contract, with such exceptions, if any, specified in the notice of termination. The City shall pay the Contractor, to the extent of funds appropriated or otherwise legally available for such purposes, for all goods delivered and services performed, and obligations incurred prior to the date of termination in accordance with the terms hereof.

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1.14 FRAUD:

Fraudulent Statements by the Contractor on any Offer or in any report or Deliverable required to be submitted by the Contractor to the City shall be grounds for the termination of the Contract for cause by the City and may result in legal action.

1.15 DELAYS:

The City may delay scheduled delivery or other due dates by written notice to the Contractor if the City deems it is in its best interest. If such delay causes an increase in the cost of the work under the Contract, the City and the Contractor shall negotiate an equitable adjustment for costs incurred by the Contractor in the Contract price and execute an amendment to the Contract. The Contractor must assert its right to an adjustment within 30 calendar days from the date of receipt of the notice of delay. Failure to agree on any adjusted price shall be handled under the Dispute Resolution Clause. However, nothing in this provision shall excuse the Contractor from delaying the delivery as notified.

1.16 FORCE MAJEURE:

Contractor may be excused from performance under the Contract for any period when performance is prevented as the result of an act of God, strike, war, civil disturbance, epidemic, pandemic, sovereign conduct, or court order provided that the Contractor experiences the event of force majeure and prudently and promptly acts to take any and all steps that are within the Contractor's control to ensure performance and to shorten the duration of the event of force majeure. Contractor shall provide notice of the force majeure event to the City to establish a mutually agreeable period of time reasonably necessary to overcome the effect of such failure to perform. Subject to this provision, such non-performance shall not be deemed a default or a ground for termination. However, the City may terminate an order under the Contract if it is determined by the City that the Contractor will not be able to deliver goods or services in a timely manner to meet the business needs of the City.

1.17 INDEMNITY:

A. IN THIS SECTION, THE FOLLOWING TERMS HAVE THE MEANINGS ASSIGNED BELOW:

- i. "INDEMNIFIED PARTY" IS THE CITY AND THE CITY'S OFFICERS, ELECTED AND APPOINTED OFFICIALS, EMPLOYEES, AGENTS, REPRESENTATIVES, SUCCESSORS AND ASSIGNS.
 - (1) "INDEMNIFYING PARTY" IS THE CONTRACTOR, ITS OFFICERS, AGENTS, EMPLOYEES, SUBCONTRACTORS, SUCCESSORS AND ASSIGNS.
 - (2) THE INDEMNIFYING PARTY SHALL INDEMNIFY, HOLD HARMLESS, AND DEFEND THE INDEMNIFIED PARTY AGAINST ANY AND ALL LOSSES, DAMAGES, LIABILITIES, DEFICIENCIES, CLAIMS, CAUSES OF ACTION, JUDGMENTS, SETTLEMENTS, INTEREST, AWARDS, PENALTIES, FINES, COSTS OR EXPENSES, INCLUDING PROFESSIONAL FEES AND ATTORNEYS' FEES, THAT ARE INCURRED BY THE INDEMNIFIED PARTY ARISING OUT OF ANY DIRECT OR THIRD PARTY CLAIM OF:
- ii. BREACH OR NON-FULFILLMENT OF ANY PROVISION OF THIS CONTRACT BY THE INDEMNIFYING PARTY;
- iii. ANY FALSE REPRESENTATION OR WARRANTY MADE BY THE INDEMNIFYING PARTY IN THIS CONTRACT OR IN THE INDEMNIFYING PARTY'S PROPOSAL/RESPONSE LEADING TO THIS CONTRACT;
- iv. ANY NEGLIGENT OR MORE CULPABLE ACT OR OMISSION OF THE INDEMNIFYING PARTY, INCLUDING ANY RECKLESS OR WILLFUL MISCONDUCT, RELATED TO THE PERFORMANCE OF ITS OBLIGATIONS UNDER THIS CONTRACT;
- v. BODILY INJURY; DEATH OF ANY PERSON; OCCUPATIONAL ILLNESS OR DISEASE; LOSS OF SERVICES, WAGES, OR INCOME; OR DAMAGE TO REAL OR PERSONAL PROPERTY CAUSED BY THE NEGLIGENT

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- OR MORE CULPABLE ACTS OR OMISSIONS OF INDEMNIFYING PARTY, INCLUDING ANY RECKLESS OR WILLFUL MISCONDUCT; OR
- vi. ANY FAILURE OF THE INDEMNIFYING PARTY TO COMPLY WITH ANY APPLICABLE FEDERAL, STATE, OR LOCAL LAWS, REGULATIONS, OR CODES RELATED TO THE PERFORMANCE OF ITS OBLIGATIONS UNDER THIS CONTRACT.
 - B. THE INDEMNIFIED PARTY SHALL GIVE THE INDEMNIFYING PARTY WRITTEN NOTICE (A "CLAIM NOTICE") OF ANY CLAIM RECEIVED RELATED TO THIS CONTRACT. THE INDEMNIFYING PARTY'S DUTY TO DEFEND APPLIES IMMEDIATELY. THE INDEMNIFIED PARTY'S FAILURE TO PROVIDE A CLAIM NOTICE TO THE INDEMNIFYING PARTY DOES NOT RELIEVE THE INDEMNIFYING PARTY OF ITS DUTY TO INDEMNIFY, HOLD HARMLESS AND DEFEND THE INDEMNIFIED PARTY.
 - C. THE INDEMNIFIED PARTY MAY, AT ITS OWN COST, SELECT ITS OWN LEGAL COUNSEL TO REPRESENT ITS INTERESTS. THE INDEMNIFYING PARTY SHALL REMAIN RESPONSIBLE TO THE INDEMNIFIED PARTY FOR ANY LOSSES INDEMNIFIED UNDER THIS SECTION. THE INDEMNIFYING PARTY SHALL GIVE PROMPT, WRITTEN NOTICE TO THE INDEMNIFIED PARTY OF ANY PROPOSED SETTLEMENT OF A CLAIM THAT IS INDEMNIFIABLE UNDER THIS SECTION. THE INDEMNIFYING PARTY MAY NOT, WITHOUT THE INDEMNIFIED PARTY'S PRIOR, WRITTEN CONSENT, SETTLE OR COMPROMISE ANY CLAIM OR CONSENT TO THE ENTRY OF ANY JUDGMENT REGARDING WHICH INDEMNIFICATION IS BEING SOUGHT UNDER THIS SECTION.
 - D. MAINTENANCE OF THE INSURANCE REQUIRED BY THIS CONTRACT SHALL NOT LIMIT THE INDEMNIFYING PARTY'S OBLIGATIONS UNDER THIS SECTION. THE INDEMNIFYING PARTY SHALL REQUIRE ALL SUBCONTRACTORS TO INDEMNIFY THE CITY IN THE SAME MANNER AS PROVIDED IN THIS SECTION.

1.18 NOTICES:

Unless otherwise specified, all notices, requests, or other communications required or appropriate to be given under the Contract shall be in writing and shall be deemed delivered three business days after postmarked if sent by U.S. Postal Service Certified or Registered Mail, Return Receipt Requested. Notices delivered by other means shall be deemed delivered upon receipt by the addressee. Notices to the Contractor shall be sent to the address registered with the City. Notices to the City shall be addressed to the City at P.O. Box 1088, Austin, Texas 78767 and marked to the attention of the assigned Procurement Specialist.

1.19 CONFIDENTIALITY:

The Contractor may be granted access to certain of the City's or licensor's confidential information (including inventions, employee information, trade secrets, confidential know-how, confidential business information, and other information which City or its licensors consider confidential) (Confidential Information) to provide the Deliverables to the City. The Contractor acknowledges and agrees that the Confidential Information is the valuable property of the City and its licensors and any unauthorized use, disclosure, dissemination, or other release of the Confidential Information will substantially injure the City and its licensors. The Contractor (including its employees, Subcontractors, agents, or representatives) agrees it will maintain the Confidential Information in strict confidence and shall not disclose, disseminate, copy, divulge, recreate, or otherwise use the Confidential Information without prior written consent of City, or in a manner not expressly permitted under this Contract, unless the Confidential Information is required to be disclosed by law or an Order of a court or other governmental authority (including a Texas Attorney General opinion) with proper jurisdiction. In all cases, the Contractor agrees to promptly notify the City before disclosing Confidential Information to permit the City reasonable time to seek an appropriate protective Order. The Contractor agrees to use protective measures no less stringent than the Contractor uses in its business to protect its own most valuable information. In all circumstances, the Contractor's

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protective measures must be at least reasonable measures to ensure the continued confidentiality of the Confidential Information.

Information shall be deemed confidential if so marked in writing or so designated orally or in writing. In case of oral designation, disclosure must be followed by written documentation within thirty (30) days, confirming that the information is Confidential Information. Contractor agrees to hold and maintain all Confidential Information, whether oral or written, in confidence and not disclose to others, not make copies of, not use it, except as expressly agreed beforehand by the City. This restriction continues for three (3) years from the date of receiving information, and does not apply to any items of information which (a) are in the public domain at the time of disclosure; or (b) becomes part of the public domain after disclosure by publication otherwise, other than in violation of the commitment herein; or (c) was in possession of Contractor at the time of disclosure by the City and was not acquired or received, directly or indirectly, from the City; or (d) was received by Contractor after the time of disclosure from the City by a third party who did not require it to be held in confidence and who did not acquire it, directly or indirectly, from the City under an obligation of confidence; or (e) is subject to disclosure pursuant to the Texas Public Information Act, currently codified under Texas Government Code Chapter 552.

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1.20 OWNERSHIP AND USE OF DELIVERABLES:

- A. PATENTS. Title to all inventions and discoveries made solely by Contractor inventors resulting from the work shall reside with the Contractor; title to all inventions and discoveries made solely by City inventors resulting from the work shall reside with City; title to all inventions and discoveries made jointly by Contractor and City inventors resulting from the work shall reside jointly with Contractor and City.
- B. DATA AND COPYRIGHTS. Title to data (which is defined as including, but not limited to, software, writings, sound recordings, pictorial reproductions, drawing or other graphical representations, reports, blueprints and works of any similar nature, whether or not copyrighted or copyrightable) first produced or composed by Contractor employees in the performance of work under this Agreement shall be the sole and exclusive property of Contractor. Contractor shall have the sole right to determine the disposition of copyrights or other rights resulting from the performance of work; provided that Contractor hereby grants to City a royalty-free, perpetual, non-exclusive license to reproduce, modify and use all such data for its own purposes.
- C. PUBLICATION. Both parties shall be free to publish the results of work under this Agreement. Title to and right to determine disposition of any copyrights on publications relating to the performance of work hereunder shall remain with Contractor, who shall have the sole right to determine the disposition of those copyrights. However, prior to publication, Contractor shall provide City a thirty (30) day period which to review proposed publications, identify proprietary or confidential information, and submit comments. Contractor shall not publish or otherwise disclose proprietary or confidential information identified by City and will give full consideration to all comments before publication. Furthermore, upon request of the reviewing party, publication may be deferred for up to thirty (30) additional days for preparation and filing of any patent application which City has the right to file.

1.21 NO CONTINGENT FEES:

The Contractor warrants that no person or selling agency has been employed or retained to solicit or secure the Contract upon any agreement or understanding for commission, percentage, brokerage, or contingent fee, excepting bona fide employees of bona fide established commercial or selling agencies maintained by the Contractor for the purpose of securing business. For breach or violation of this warranty, the City shall have the right, in addition to any other remedy available, to cancel the Contract without liability and to deduct from any amounts owed to the Contractor, or otherwise recover, the full amount of such commission, percentage, brokerage or contingent fee.

1.22 GRATUITIES:

The City may, by written notice to the Contractor, cancel the Contract without liability if it is determined by the City that gratuities were Offered or given by the Contractor or any agent or representative of the Contractor to any officer or employee of the City of Austin with a view toward securing the Contract or securing favorable treatment with respect to the awarding or amending or the making of any determinations with respect to the performing of such Contract. In the event the Contract is canceled by the City pursuant to this provision, the City shall be entitled, in addition to any other rights and remedies, to recover or withhold the amount of the cost incurred by the Contractor in providing such gratuities.

1.23 PROHIBITION AGAINST PERSONAL INTEREST IN CONTRACTS:

No officer, employee, independent consultant, or elected official of the City who is involved in the development, evaluation, or decision-making process of the performance of any Solicitation shall have a

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financial interest, direct or indirect, in the Contract resulting from that Solicitation. Any willful violation of this Section shall constitute impropriety in office, and any officer or employee guilty thereof shall be subject to disciplinary action up to and including dismissal. Any violation of this provision, with the knowledge, expressed or implied, of the Contractor shall render the Contract voidable by the City.

1.24 INDEPENDENT CONTRACTOR:

The Contract shall not be construed as creating an employer/employee relationship, a partnership, or a joint venture. The Contractor's services shall be those of an independent Contractor. The Contractor agrees and understands that the Contract does not grant any rights or privileges established for employees of the City.

1.25 ASSIGNMENT DELEGATION:

The Contract shall be binding upon and ensure to the benefit of the City and the Contractor and their respective successors and assigns, provided however, that no right or interest in the Contract shall be assigned and no obligation shall be delegated by the Contractor without the prior written consent of the City. Any attempted assignment or delegation by the Contractor shall be void unless made in conformity with this Paragraph. The Contract is not intended to confer rights or benefits on any person, firm or entity not a party hereto; it being the intention of the parties that there be no third-party beneficiaries to the Contract.

1.26 WAIVER:

The claim or right arising out of a breach of the Contract can be discharged in whole or in part by a waiver or renunciation of the claim or right unless the waiver or renunciation is supported by consideration and is in writing signed by the aggrieved party. No waiver by either the Contractor or the City of any one or more events of default by the other party shall operate as, or be construed to be, a permanent waiver of any rights or obligations under the Contract, or an express or implied acceptance of any other existing or future default or defaults, whether of a similar or different character.

1.27 MODIFICATIONS:

The Contract can be modified or amended only in writing and signed by both parties. No pre-printed or similar terms on any Contractor Invoice, Order, clickwrap agreement or other document shall have any force or effect to change the terms, covenants, and conditions of the Contract.

1.28 INTERPRETATION:

The Contract is intended by the parties as a final, complete and exclusive Statement of the terms of their agreement. No course of prior dealing between the parties or course of performance or usage of the trade shall be relevant to supplement or explain any term used in the Contract. Although the Contract may have been substantially drafted by one party, it is the intent of the parties that all provisions be construed in a manner to be fair to both parties, reading no provisions more strictly against one party or the other. Whenever a term defined by the Uniform Commercial Code, as enacted by the State of Texas, is used in the Contract, the UCC definition shall control, unless otherwise defined in the Contract.

1.29 DISPUTE RESOLUTION:

- A. If a dispute arises out of or relates to the Contract, or the breach thereof, the parties agree to negotiate prior to prosecuting a suit for damages. However, this Section does not prohibit the filing of a lawsuit to toll the running of a statute of limitations or to seek injunctive relief. Either party may make a written request for a meeting between representatives of each party within 14 calendar days after receipt of the request or such later period as agreed by the parties. Each party shall include, at a minimum, one

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senior level individual with decision-making authority regarding the dispute. The purpose of this and any subsequent meeting is to attempt in good faith to negotiate a resolution of the dispute. If, within 30 calendar days after such meeting, the parties have not succeeded in negotiating a resolution of the dispute, they will proceed directly to mediation as described below. Negotiation may be waived by a written agreement signed by both parties, in which event the parties may proceed directly to mediation as described below.

- B. If the efforts to resolve the dispute through negotiation fail, or the parties waive the negotiation process, the parties may select, within 30 calendar days, a mediator trained in mediation skills to assist with resolution of the dispute. Should they choose this option, the City and the Contractor agree to act in good faith in the selection of the mediator and to consider qualified individuals nominated to act as mediator. Nothing in the Contract prevents the parties from relying on the skills of a person who is trained in the subject matter of the dispute or a Contract interpretation expert. If the parties fail to agree on a mediator within 30 calendar days of initiation of the mediation process, the mediator shall be selected by the Travis County Dispute Resolution Center. The parties agree to participate in mediation in good faith for up to 30 calendar days from the date of the first mediation session. The City and the Contractor will share the mediator's fees equally and the parties will bear their own costs of participation such as fees for any consultants or attorneys they may utilize to represent them or otherwise assist them in the mediation.

1.30 JURISDICTION AND VENUE:

The Contract is made under and shall be governed by the laws of the State of Texas. All issues arising from this Contract shall be resolved in the courts of Travis County, Texas and the parties agree to submit to the exclusive personal jurisdiction of such courts. The foregoing, however, shall not be construed or interpreted to limit or restrict the right or ability of either party to seek and secure injunctive relief from any competent authority as contemplated herein.

1.31 INVALIDITY:

The invalidity, illegality, or unenforceability of any provision of the Contract shall in no way affect the validity or enforceability of any other portion or provision of the Contract. Any void provision shall be deemed severed from the Contract and the balance of the Contract shall be construed and enforced as if the Contract did not contain the particular portion or provision held to be void. The parties further agree to reform the Contract to replace any stricken provision with a valid provision that comes as close as possible to the intent of the stricken provision. The provisions of this Section shall not prevent this entire Contract from being void should a provision which is the essence of the Contract be determined to be void.

1.32 HOLIDAYS:

The following holidays are observed by the City:

<u>Holiday</u>	<u>Date Observed</u>
New Year's Day	January 1
Martin Luther King, Jr.'s Birthday	Third Monday in January

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President's Day	Third Monday in February
Memorial Day	Last Monday in May
Juneteeth	June 19
Independence Day	July 4
Labor Day	First Monday in September
Veteran's Day	November 11
Thanksgiving Day	Fourth Thursday in November
Friday after Thanksgiving	Friday after Thanksgiving
Christmas Eve	December 24
Christmas Day	December 25

If a Legal Holiday falls on Saturday, it will be observed on the preceding Friday. If a Legal Holiday falls on Sunday, it will be observed on the following Monday.

1.33 SURVIVABILITY OF OBLIGATIONS:

All provisions of the Contract that impose continuing obligations on the parties, including but not limited to the warranty, indemnity, and confidentiality obligations of the parties, shall survive the expiration or termination of the Contract.

1.34 NON-DEBARMENT CERTIFICATION:

When using Federal funds, the City of Austin does not Contract with or make prime or sub-awards to parties that are debarred or whose principals are debarred from Federal Contracts. By accepting a Contract with the City, the Vendor certifies that its firm and its principals are not currently debarred from doing business with the Federal Government, as indicated by the General Services Administration List of Parties Excluded from Federal Procurement and Non-Procurement Programs. The Contractor shall notify the Procurement Specialist within five business days if they become debarred from doing business with the Federal Government during the term of the Contract.

1.35 EQUAL OPPORTUNITY:

- A. **Equal Employment Opportunity:** Contractor does and shall comply with Executive Order 11246, as amended.
- B. **Americans with Disabilities Act (ADA) Compliance:** No Contractor, or Contractor's agent, shall engage in any discriminatory practice against individuals with disabilities as defined in the ADA, including but not limited to: employment, accessibility to goods and services, reasonable accommodations, and effective communications.

1.36 PROHIBITION ON LGBTQ+ CONVERSION THERAPY:

The Contractor certifies that it is aware of City Council Resolution No. 20191114-056, which prohibits the City from Contracting with entities that engage in certain practices related to conversion therapy. By

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accepting this Contract, the Contractor agrees that: (1) its firm and its principals are not currently and will not during the term of the Contract engage in practicing LGBTQ+ conversion therapy; referring persons to a healthcare provider or other person or organization for LGBTQ+ conversion therapy; or Contracting with another entity to conduct LGBTQ+ conversion therapy; and (2) if the City determines in its sole discretion that Contractor has during the term of this Contract engaged in any such practices, the City may terminate this Contract without penalty to the City.

If the requested adjustment is not supported by the referenced index, the City, at its sole discretion, may consider approving an adjustment on fully documented market increases.

1.37 INSURANCE:

Insurance not required. All work is done on contractor premises.

1.38 Pursuant to Texas government code chapter 2274, the contractor certifies that if we have remote or direct access to communication infrastructure systems, cybersecurity systems, the electric grid, hazardous waste treatment systems, or water treatment facilities as a result of any city contract, that we are not owned by or the majority of stock or other ownership interest of our firm is not held or controlled by:

- A. individuals who are citizens of China, Iran, North Korea, Russia, or a Governor-designated country; or
- B. company or other entity, including a governmental entity, that is owned or controlled by citizens of or is directly controlled by the government of China, Iran, North Korea, Russia, or a Governor-designated country; or
- C. headquartered in China, Iran, North Korea, Russia, or a Governor-designated country.

1.39 PURSUANT TO TEXAS GOVERNMENT CODE CHAPTER 2274, THE CONTRACTOR CERTIFIES THAT, IF WE HAVE 10 OR MORE FULL-TIME EMPLOYEES: (1) WE DO NOT HAVE A PRACTICE, POLICY, GUIDANCE, OR DIRECTIVE THAT DISCRIMINATES AGAINST A FIREARM ENTITY OR FIREARM TRADE ASSOCIATION; AND (2) WILL NOT DISCRIMINATE DURING THE TERM OF THE RESULTING CONTRACT AGAINST A FIREARM ENTITY OR FIREARM TRADE ASSOCIATION. SERVICES

3.1 ACCEPTANCE OF INCOMPLETE OR NON-CONFORMING DELIVERABLES AND/OR SERVICES:

If, instead of requiring immediate correction or removal and replacement of defective or non-conforming Deliverables or Services, the City prefers to accept it, the City may do so.

3.2 WORKFORCE:

- A. The Contractor shall employ only orderly and competent workers, skilled in the performance of the services which they will perform under the Contract.
- B. The Contractor, its employees, Subcontractors, and Subcontractor's employees may not while engaged in participating or responding to a Solicitation or while in the course and scope of delivering goods or services under a City of Austin Contract or on the City's property:
 - i. Illegally use or possess a firearm, except as required by the terms of the Contract; or
 - ii. Use or possess alcoholic or other intoxicating beverages, illegal drugs or controlled substances, nor may such workers be intoxicated, or under the influence of alcohol or drugs, on the job.
- C. If the City or the City's representative notifies the Contractor that any worker is incompetent, disorderly or disobedient, has knowingly or repeatedly violated safety regulations, has illegally possessed any

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firearms, or has possessed or was under the influence of alcohol or drugs on the job, the Contractor shall immediately remove such worker from Contract services, and may not employ such worker again on Contract services without the City's prior written consent.

3.3 GUARANTEE – SERVICES:

The Contractor warrants and represents that all services to be provided to the City under the Contract will be fully and timely performed in a good and workmanlike manner in accordance with generally accepted standards and practices following the terms, conditions, and covenants of the Contract, and all applicable Federal, State and local laws, rules or regulations.

- A. The Contractor may not limit, exclude or disclaim the foregoing warranty or any warranty implied by law, and any attempt to do so shall be without force or effect.

3.4 COMPLIANCE WITH HEALTH, SAFETY, AND ENVIRONMENTAL REGULATIONS:

The Contractor, its Subcontractors, and their respective employees, shall comply fully with all applicable Federal, State, and local health, safety, and environmental laws, ordinances, rules and regulations in the performance of the services, including but not limited to those promulgated by the City and by the Occupational Safety and Health Administration (OSHA). In case of conflict, the most stringent safety requirement shall govern. The Contractor shall indemnify and hold the City harmless from and against all claims, demands, suits, actions, judgments, fines, penalties and liability of every kind arising from the breach of the Contractor's obligations under this Paragraph.

Scope of Work

PROFESSIONAL SERVICES AGREEMENT BETWEEN THE CITY OF AUSTIN AND THE UNIVERSITY OF TULSA

Description: Transcriptomic evaluation of chronic stress in Austin's endangered salamanders

1.0 Purpose

The City of Austin, Watershed Protection Department (hereinafter referred to as the "City"), intends to enter into a Professional Services Agreement with University of Tulsa (hereinafter referred to as the "Contractor"), working specifically with Ronald M. Bonett, Ph.D., Department of Biological Science, at University of Tulsa. The purpose of this contract is to study the transcriptomic evaluation of chronic stress in Austin's endangered salamanders.

The objective of the proposed research is to perform a comprehensive investigation of stress physiology and gene expression of Barton Springs and Austin Blind salamanders.

2.0 Background

This study was proposed to the Barton Springs Salamander Conservation Fund (BSSCF) and the proposal was accepted by a committee of Watershed Protection Department (WPD) staff and the U.S. Fish and Wildlife Service (USFWS). The fund was established in accordance with the provisions set forth in the Barton Springs Pool Habitat Conservation Plan (HCP) and incidental take permit (ITP) TE-839031-2, issued under the authority of 16 USC 1539(a)(1)(B) and regulation 50 CFR §§ 13 & 17.

The BSSCF was established as a conservation measure in the City of Austin's HCP and associated ITP from the USFWS that allows for the operation and management of Barton Springs Pool as a recreational facility. The objective of the BSSCF is to support educational, scientific, or management projects that promote the conservation of endangered Barton Springs and Austin Blind salamanders, and the groundwater ecosystem where they exist. Each year, a portion of the proceeds from entrance fees to Barton Springs Pool is allocated to the BSSCF.

In accordance with the HCP and ITP, the use of this fund is restricted to the study of salamander biology, captive breeding, refugium development, reintroduction, watershed related research, improved cleaning techniques for natural water bodies, education and/or land acquisition. The attached proposal addresses this requirement by providing novel and potentially actionable information on salamander stress and gene expression. Information generated by this study may positively impact efforts to captive breed and manage wild Barton Springs and Austin Blind salamanders as part of a comprehensive set of conservation strategies.

3.0 Tasks/Requirements

3.1 Contractor Responsibilities

In accordance with the attached proposal, the Contractor shall:

3.1.1 Conduct experiments in the laboratory at the University of Tulsa designed to:

- evaluate mechanisms and failures of the stress axis in central Texas *Eurycea*, and
- develop a panel of stress response genes to evaluate stress of endangered

Austin *Eurycea* in the field and in captive colonies.

- 3.1.2 Use a combination of Oxford Nanopore and Illumina RNA TruSeq preparation and sequencing to collect comprehensive transcriptome sequences of the brain (including hypothalamus and pituitary) and tail tips of *E. pterophila* and *E. latitans*, and the tail tips of *E. sosorum* and *E. waterlooensis*.
- 3.1.3 Assess Plasma ACTH and CORT levels using Liquid Chromatography Mass Spectroscopy (LC-MS) at the University of Tulsa. Specimens will be compared against five-point standard curves of purified hormone. Alternative methods could include Elisa Assays of water-borne or plasma samples.
- 3.1.4 Design quantitative PCR assays (TaqMan) for several stress response loci and a normalizing gene (ribosomal protein L8) based on laboratory experiments 1 and 2.
- 3.1.5 Collect a minimum of 20 tail tip samples of *E. sosorum* in the field at Eliza Spring during two sampling trips in year 2 to evaluate how expression levels of identified stress response genes from the field compare to lab experiments on surrogates.
- 3.1.6 Submit an interim report due at the end of Year 1 of the project schedule and a final report due at the end of the contract.

3.2 **City's Responsibilities**

The City will:

- 3.2.1 Assist in sample collection by providing access to study sites and capturing salamanders during visits.

4.0 **Designation of Key Personnel**

4.1 **University of Tulsa, Project SPOCs:**

Ronald M. Bonett, Ph.D.

Department of Biological Science, University of Tulsa

Phone number: Cell (734) 972-5016 | Office (918) 631-3328

Email Address: ron-bonett@utulsa.edu

Authorized Official for Signature:

Debbie Newton

Director of Research and Sponsored Programs

The University of Tulsa

800 S Tucker Drive

Tulsa, OK 74104

918-631-2192

deborah-newton@utulsa.edu

Pre-Award Services and Contract Negotiations:

Adrienne Blalack
Director of Pre-Award Services
The University of Tulsa
800 S Tucker Drive
Tulsa, OK 74104
918-631-2480
adrienne-blalack@utulsa.edu

Debbie Newton
Director of Research and Sponsored Programs
The University of Tulsa
800 S Tucker Drive
Tulsa, OK 74104
918-631-2192
deborah-newton@utulsa.edu

4.2 City of Austin, Project SPOC:

Nathan Bendik
Environmental Scientist Senior, Watershed Protection
512-974-2040
Nathan.Bendik@austintexas.gov

5.0 **Pricing and Invoicing**

- 5.1 Contractor shall perform the tasks identified above in section 3.1, also identified in Exhibit A, Transcriptomic evaluation of chronic stress in Austin's endangered salamanders' proposal, for a total cost not to exceed \$74,637 over a two year period. The project will begin on October 1, 2021 and will conclude on September 30, 2023, or upon completion of all deliverables, to be approved in writing by City staff.

	Fiscal Year 1 12 Months 10/1/2021 to 9/30/2022	Fiscal Year 2 12 Months 10/1/2022 to 9/30/2023	Total 24 Months 10/1/2021 to 9/30/2023
Graduate Research Assistant	\$21,000	\$21,000	\$42,000
Fringe (8% of GRA Salaries)	\$1,680	\$1,680	\$3,360
Undergraduate Research Assistant	\$3,285	\$3,285	\$6,570
Total Personnel			(\$51,930)
Travel	\$1,569	\$3,138	(\$4,707)
Materials and Supplies	\$13,000	\$5,000	(\$18,000)
Total Direct Costs	\$40,534	\$34,103	\$74,637

- 5.2 Payment will be made upon receipt of an acceptable invoice, as follows:
- 5.2.1 The City will pay a not to exceed amount of \$40,534 between October 1, 2021 and September 30, 2022.
- 5.2.2 The City will pay a not to exceed amount of \$24,103 between October 1, 2022 and September 30, 2023.
- 5.2.2.1 The City will pay the retainage of \$10,000 once the final report has been approved by City staff and upon completion of the project.

Transcriptomic evaluation of chronic stress in Austin's endangered salamanders

Ronald M. Bonett, Ph.D., *Department of Biological Science, University of Tulsa*

Project Goals: Monitoring the health of species is fundamental to their management and conservation. The goals of this project are: 1) to understand the stress axis pathway in Texas *Eurycea* in order to evaluate whether there is a deviation in the pathway when responding to chronic stressors; and 2) to develop gene expression (transcriptomic) assays to monitor for chronic stress in wild or captive populations of endangered Texas *Eurycea*.

Project Objectives: Climatic and anthropogenic alterations to the landscape can enforce a wide range of environmental and biological changes that potentially impact local communities. Effective management and conservation relies on understanding when such changes negatively impact the health of species. Especially compared to humans, very few parameters have been developed to assess the health of wildlife.

Over the last few decades, conservation biologists have used physiological metrics to evaluate the impact of stressors on wildlife. In vertebrates, levels of “stress” steroids have been most commonly used to interpret physiological stress. In amphibians, it is thought that stress can be internalized through the hypothalamic-pituitary-interrenal axis (HPI-axis; Denver 2009). Briefly, external stressors trigger the hypothalamus to release corticotropin releasing factor (CRF) or arginine vasotocin (AVT) that serve as signals to the pituitary to release adrenocorticotrophic hormone (ACTH; Tonon et al. 1986; Denver 2009). Corticosteroids (e.g. cortisol and corticosterone; CORT) are synthesized in the interrenal glands, and released by stimulation from ACTH (Denver 2009). Over a short term, corticosteroids can be important for tissue repair, regulating metabolism, and even learning. However, long-term (chronic) elevation of corticosteroids can result in suppression of the immune system, dysregulation of metabolism, and neurodegeneration (Sapolsky et al. 2000; McEwen 2007). Therefore, corticosteroids have been widely used to assess when individuals are under duress. However, our general understanding of this pathway is based on a few representative amphibian species, with very limited data for salamanders (Bonett 2016).

Corticosteroid levels of amphibians have been measured in blood plasma (Glennemeier & Denver 2002; Moore & Jessop 2003), fecal pellets (Nagel et al. 2020), and excretion (i.e. waterborne hormone assays; Gabor et al. 2013, 2016). A major challenge of this assessment is that single point monitoring of corticosteroids may not reflect chronic stress (Watts et al. 2004; Dickens & Romero 2013). This is because levels of circulating corticosteroids can elevate normally from activity and feeding, which can present false positives. But worse, chronically stressed individuals may lose their ability to mount a corticosteroid response due to the loss of sensitivity of the interrenal glands to ACTH (Gabor et al. 2016). Meaning that an individual could chronically perceive stress and suffer physiological consequence, but not release corticosteroids to ameliorate these effects. In such cases low corticosteroid levels would be false negatives that appear to be “unstressed” to the investigator, but may still be suffering physiological and developmental damage.

An organism's cells can also respond to environmental stressors as well as being the targets of endogenous signals such as corticosteroids (Clay et al. 2019). One of the most common cellular responses is altering expression of genes. Vertebrate genomes include

tens of thousands of genes, many of which can be independently regulated. This provides the potential for unique transcriptomic (gene expression) responses to be induced by different conditions. It also can provide a deeper graded response to corticosteroid based stress than instantaneous measurements, as well as signatures of stressors that are not mediated through the HPI axis (Clay et al. 2019). Recently, my lab showed that chronic corticosterone exposure and temperature stressors (elevated and fluctuating) resulted in unique gene expression responses in tail tissues biopsied from Oklahoma Salamanders (*Eurycea taylori*; Clay et al. 2019). Specifically, we found that several of the chronic corticosteroid response genes were associated with immune response, oxidative stress, and tissue repair, while diverse thermal inducible genes were impacted by temperature stress treatments. Our study demonstrated that transcriptomic analyses provide a more nuanced assessment of physiological stressors than circulating hormones. It also revealed functional information on the cellular pathways that were impacted by the stressor.

This proposal offers to disentangle the upstream regulatory mechanisms of the stress axis in Central Texas *Eurycea*, evaluate chronic stress from chemical cues of predators, and develop a transcriptome assay that could be used to evaluate stress in wild and captive populations of *E. sosorum* and *E. waterlooensis*. Beyond waterborne and fecal corticosterone assays (Gabor et al. 2013, 2016; Nagel et al. 2020), which are single value assessments, there are currently no other non-invasive biochemical stress indication methods for endangered salamanders. Therefore, in the short term this research will enhance our understanding of the biology of these species by providing critical information of how their stress axis functions. It will also provide assessments of expression patterns in wild populations and captive colonies. In the long term, these assays can generate an additional line of potential physiological health metrics that can be evaluated in the context of current data such as tail condition, body condition, and reproductive status (Bendik & Gluesenkamp 2013; Nissen & Bendik 2020). This could be an enlightening line of multimetric data for monitoring stressors in wild and captive populations into the future.

Scope of Work

Methodology: Experiments will be conducted in my laboratory at the University of Tulsa and are designed to 1) evaluate mechanisms and failures of the stress axis in Central Texas *Eurycea*, and 2) develop a panel of stress response genes to evaluate stress of endangered Austin *Eurycea* in the field and in captive colonies.

Lab Experiments: We will utilize offspring from captive bred *E. pterophila* (formerly *neotenes*; surface phenotype) and *E. latitans* (subsurface phenotype) as surrogates for *E. sosorum* and *E. waterlooensis* respectively. All experiments will be carried out in BPA free containers housed inside of Percival incubators set to 12 h Light: 12 h dark and 21°C. Images, overall body metrics, tail tips and blood plasma will be taken at sampling periods.

The first experiment will evaluate gene expression in the tail tips of *E. pterophila* and *E. latitans* after simulation of chronic stress axis activation (15 days and 30 days). This will be performed by individually injecting three primary hormones of the HPI-axis (CRF, ACTH, and CORT). Doses and concentrations will follow prior and published experiments. Tail tips of hormone-injected salamanders will be compared to control saline injected, control uninjected, and exogenously CORT treated individuals. Circulating ACTH and CORT will be assessed in the blood plasma to evaluate the efficacy of CRF in instigating the release of

ACTH, and the effectiveness of ACTH at instigating the release of CORT (i.e. test whether chronic stress results in interrenal failure to mount a CORT response, which would be false negatives in the field). If CRF and ACTH still elicit CORT release at 30 days, we may conduct a follow up evaluation with a longer treatment (60 days) or AVT treatment.

The second lab experiment will test transcriptional response in tail tips of *E. pterophila* and *E. latitans* that were subjected to a chronic stressor (predator chemical cues) for 30 days. This experiment will use chronic treatment with chemical cues from largemouth bass (*Micropterus salmoides*) and Red Swamp Crayfish (*Procambarus clarkii*), based on prior research showing significant behavioral modification (reduced movement) by surface dwelling Texas *Eurycea* (*sosorum* and *nana*) to these potential predators (DeSantis et al. 2013; Davis & Gabor 2015; Davis et al. 2017). Crayfish are potential predators of surface and subsurface *Eurycea*. While subsurface *Eurycea* likely are not often the subject of predation by surface fishes, the presence of their chemicals may be a chronic stressor. Salamanders subject to predator cues will be compared to exogenous CORT (positive control) and control water without predator cues. Preparation of predatory cues (e.g. 230 ml water per 1 ml of stimulus animal) and experimental conditions will follow published studies (DeSantis et al. 2013; Davis et al. 2017).

2) *Transcriptomics*: We extract RNA using Trizol (Invitrogen) and quantify it on a Nanodrop or Qubit Fluorometer (Thermo Fisher Sci.). We will use a combination of Oxford Nanopore and Illumina RNA TruSeq preparation and sequencing to collect comprehensive transcriptome sequences of the brain (including hypothalamus and pituitary) and tail tips of *E. pterophila* and *E. latitans*, and the tail tips of *E. sosorum* and *E. waterlooensis*. Nanopore sequencing permits collection of longer reads that have high error, but help in transcript assembly. Illumina sequencing reads are much shorter, but have a lower error rate for assembling accurate transcripts. For each of these samples the transcriptome will be assembled in CLC Genomics Workbench and based on tens of thousands of Nanopore long reads and several million Illumina short reads.

Deep sequencing the brain transcriptomes permit the collection of gene sequences for neurohormones. Select neurohormones (e.g. CRF, ACTH, also alternative stimulators of ACTH such as AVT). Amphibian versions of these hormones can be 25% divergent or more in amino acid sequence and thus far it has been challenging to distinguish between loss of sensitivity from chronic stress or just using an inadequate commercially available mammalian analog. The proteins of these neurohormones will be synthesized and injected to test the function and limits of the stress axis (experiments described above). Deep sequencing the tail fins will allow us to annotate the transcriptomes for the tissues, and will serve as the references for experimental samples. Transcriptomes will also be sequenced for tail tips of experimental individuals (described above) using the same methods as above, but only sequencing with via Illumina for ~1 million reads per sample). Read counts will be mapped against annotated tail transcriptomes, and differences in expression among experimental groups will be compared using DeSeq2 with correction for false positives.

3) *Hormone levels*: Plasma ACTH and CORT levels will be assessed using Liquid Chromatography Mass Spectroscopy (LC-MS) at the University of Tulsa. Specimen will be compared against five point standard curves of purified hormone. Alternative methods could include Elisa Assays of water-borne or plasma samples. We are equipped to do both.

4) *Assay Development*: Quantitative PCR assays (TaqMan) will be designed for several stress response loci and a normalizing gene (ribosomal protein L8) based on laboratory experiments 1 and 2. Based on our prior work on Oklahoma Salamanders, I anticipate many genes will be differentially regulated based on CORT alone (Clay et al. 2019). The genes selected will be based on their known functional significance such as being associated with the immune system or tissue repair. The assays will be made as general as possible (fewest mutations against other TX *Eurycea* for the same genes), but when specificity is necessary, they will be designed to match *E. sosorum* and *E. waterlooensis*.

5) *Field and Colony Testing*: Tail tip samples of at least 20 *E. sosorum* will be collected in the field at Elisa Springs during two sampling trips in year two to evaluate how expression levels of identified stress response genes from the field compare to lab experiments on surrogates. This site was chosen due to its regular monitoring and highest abundance of *E. sosorum* (Bendik & Dries 2018). If permissible and feasible, it may be ideal to analyze samples from all four quarters.

Since only juvenile *E. waterlooensis* are rarely recovered in the field. I request to evaluate stress response genes in up to 20 individuals from captive colonies, based on availability. If possible, comparisons could include individuals that seem to be stressed (based on robustness or any apparent disease) to captive individuals that appear healthy. For both sample of *E. sosorum* from the field and *E. waterlooesis* from captive stock, gene expression values could be analyzed against body metrics, tail metric, and reproductive status data collected at the time of sampling.

Research/Management Implications: This study includes the first multi-level investigation of the HPI (stress) axis in this family of salamanders (Plethodontidae) and therefore has broad implication for biology and conservation. For the Austin endangered salamanders this project seeks to develop gene expression assays that can be used to evaluate stress in wild and captive populations.

Dissemination: The findings of this work will be disseminated through reports, presentations at national meetings, and publications in international journals.

Permits: The PI has current state permits to collect and retain the surrogate species *Eurycea pterophila* and *E. latitans*. Small tissue samples (tail tips) collected during surveys will be under the current US Fish and Wildlife Permits of City of Austin investigators.

Project Schedule: This is a 2-year proposal, ideally starting in October 1st 2021 and ending September 30th 2023. **Year 1** will entail primarily transcriptome sequencing and laboratory experiments on surrogate species. **Year 2** will involve assay development, and wild and captive population testing of endangered species.

Project Participants: This project will include the PI, a graduate research assistant, and undergraduate research assistants. The PI has been studying plethodontid salamanders for 23 years and particular expertise in the evolution, endocrinology, and development of the genus *Eurycea* (e.g. Aran et al. 2014; Bonett et al. 2014a,b; Bonett 2016; Martin et al. 2011; Clay et al. 2019). Some portions of the project will include collaborator Dr. Timothy Clay (former postdoc, currently Assistant Professor at Nicholls State, LA).

Partnerships: Field and captive populations sampling will be in coordination with the City of Austin's Salamander Team.

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Budget

	Fiscal Year 1 12 Months 10/1/2021 to 9/30/2022	Fiscal Year 2 12 Months 10/1/2022 to 9/30/2023	Total 24 Months 10/1/2021 to 9/30/2023
Graduate Research Assistant	\$21,000	\$21,000	\$42,000
Fringe (8% of GRA Salaries)	\$1,680	\$1,680	\$3,360
Undergraduate Research Assistant	\$3,285	\$3,285	\$6,570
Total Personnel			(\$51,930)
Travel	\$1,569	\$3,138	(\$4,707)
Materials and Supplies	\$13,000	\$5,000	(\$18,000)
Total Direct Costs	\$40,534	\$34,103	\$74,637

Budget Justification

Personnel (\$51,930)

Funding for one graduate research assistant (GRA) is requested in each year of the 2-year project; each GRA at the rate of \$1,750/month for 12 months, 0.5 FTE for a total of \$42,000 (\$21,000/year) for salaries. The GRA will be involved with conducting experiments, annotating and analyzing transcriptomes, developing stress assays, and analyzing field collected samples.

Fringe benefits (\$3,360; \$1,680/year) are calculated at TU's current rate of 8% for graduate research assistant salaries.

Additionally, \$6,570 (\$3,285/year) is requested to hire two undergraduate research assistants (URAs) each summer at the rate of \$10.95/hour for up to a total of 300 hours per year. The URA(s) will assist the graduate student and PI in conducting the project.

Travel (\$4,707)

Three field trips to collect samples of salamander tissue are budgeted at a total of \$4,707; one in the first year (\$1,569), and two in the second year (\$3,138). Expenses for each 4-day, 3-night trip for 3 people include round-trip mileage from Tulsa, OK to Austin, TX for \$504 (900 miles at the City of Austin's mileage rate of \$0.56/mile), lodging \$540 (\$90/night, 2 rooms, 3 nights) and per diem \$525 (\$175/person at TU's rate of \$50/full day and \$37.50/travel day, 4 days) for a total of \$1,569 per trip.

Equipment (\$0)

No funds are requested for equipment.

Contractual Services (\$0)

No funds are requested for contractual services.

Material and Supplies (\$18,000)

A total of \$18,000 (\$13,000 in the first year; \$5,000 in the second year) is requested for supplies for transcriptome sequencing (\$12,000), hormone assays (\$2,000), protein hormone synthesis (\$1,000), and assay development and field-testing (\$3,000).

Other Direct Costs (\$0)

No funds are requested for other direct costs.

Total Request: \$74,637