Texas Comptroller Final Report

Environmental tolerances and habitat association of three *Procambarus* crayfish in Texas

Joshua Banta (PI), Ryan Shartau (co-PI), Jared Dickson, and Andrew Walker

July 30th 2026

Background

The State of Texas contains a large number of native crayfish species (about 52). Of these, the Bastrop Crayfish, *Procambarus texanus*, and the Nueces Crayfish, *P. nueces*, are endemic, critically imperiled crayfish identified as high priority for conservation action by the Texas Parks and Wildlife Department (TPWD), while a newly described Texas endemic species, *P. fayettei*, whose species range abuts that of the widespread (non-endemic) species *P. acutus*. The general ranges of these species are known, indicating there is a degree of overlap, it is unknown what their physiological capacities are to tolerate abiotic environmental changes within their habitat range. The ability of crayfishes to tolerate environmental changes can indicate habitat quality from the perspective of the individual animal, and reveal environmental stressors before their negative consequences begin to manifest at a population level. Understanding this tolerance is critical for determining habitat suitability for these species, assessing their response to future environmental changes, and informing conservation responses to ensure the success of these endemics.

The goal of this Comptroller funded project was to assess the physiological tolerances of these endemic species and the widespread adjacent congener (*P. acutus*) to current environmental conditions and future environmental changes. The three environmental variables of interest in this study are thermal tolerance, hypoxia tolerance, and pH (acid) tolerance; this was performed as outlined by the three research tasks listed below.

Task 1: Survey for species of interest across their known occupied range.
Task 2: Conduct physiological tolerance bioassays using a minimum of six (6) individuals of each species for each abiotic variable tested.
Task 3: Data distribution.

Preparation for project

Following contract signing, a graduate student was recruited. We were fortunate to attract a student (Andrew (Andy) Walker) who has extensive field experience and worked with crayfish in Wyoming. Between June and October 2024, Andy conducted a literature review of what was known about crayfish physiological tolerances – this comprised the majority of Andy's MS proposal which he defended in the Fall of 2024.

In the summer of 2024, prior to acquisition of animals, the holding facility at UT Tyler was set up and various supplies and equipment were purchased. This includes heavy duty metal shelving to hold aquariums, nine 55-gallon aquariums along with aquarium filters, lids, substrate (rocks and PVC pipe), air pumps, aquarium heaters, and aquarium tubing. Figure 1 shows many of the tanks and equipment purchased. Various chemicals were also purchased to maintain water quality. Plastic containers were purchased to house individual crayfish due to their semi-aggressive nature towards conspecific as housing them separately prevents cannibalism and other undesirable aggressive behaviors (Fig. 2). We were also able to utilize an existing set-up at UT Tyler which allowed for further separation (Fig. 3).



Figure 1. 55-gallon tanks to hold crayfish at UT Tyler.



Figure 2. Plastic containers fitted with mesh screen or ventilation holes to separate crayfish in aquariums.

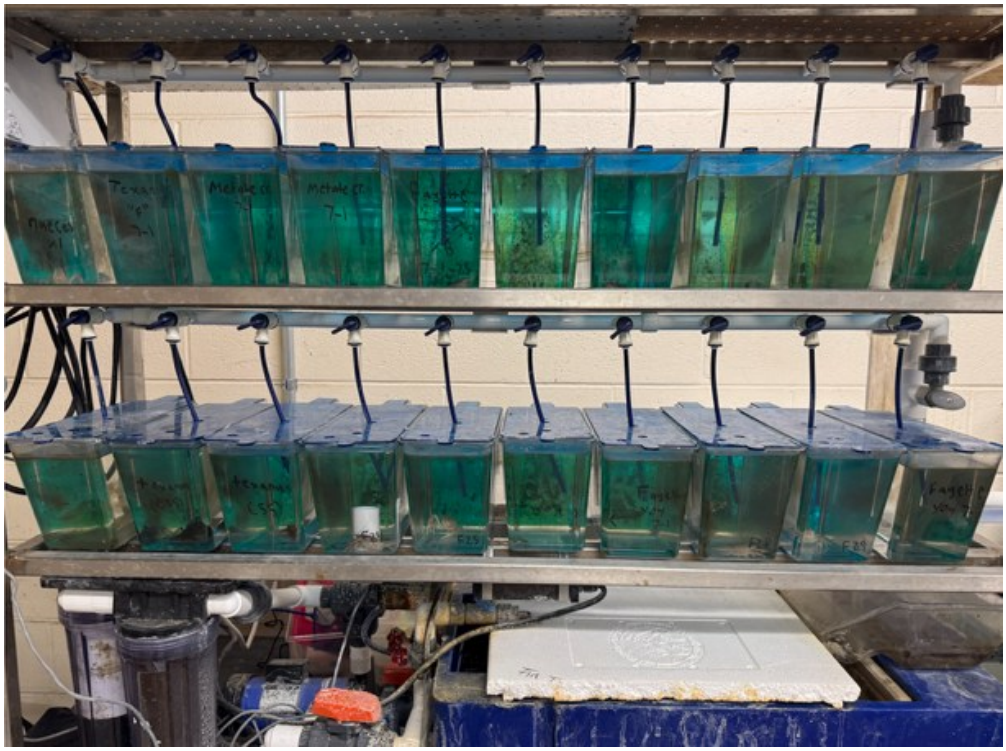


Figure 3. Smaller individual tanks used for individual crayfish.

For crayfish collecting, rubber boots and waders were purchased for project members and 30 crayfish traps and seine net for collections. Other items purchased included consumables for the oxygen sensor, plexi-glass sheeting to build a set-up for the tolerance exposures, chemicals for the pH tolerance work, plastic beakers, and small submersible pumps for water circulation. The largest purchase was for a heating immersion circulator ($1667), which was needed for the thermal trials.

Task Deliverables

There were three tasks specified in the contract. Outlined below are summaries of the completed elements of each task along with relevant findings.

Task 1: Survey for species of interest across their known occupied range.

1A. Collect a minimum of eighteen (18) individuals of each species of interest.
1B. Document abiotic and biotic habitat parameters once per sampling season at each collection site.
1C. Model habitat associations based on abiotic and biotic parameters collected.

Task 1A

We have visited 108 sites across six counties (Atascosa, Bastrop, Fayette, Lee, McMullen, Wilson) (Table 1). Some of these sites were those where species of interest had been previously collected but many were new sites. The majority of animals have been caught at public access points adjacent county roads or highways; most of the *P. acutus* were caught on or near the UT Tyler campus. Using baited crayfish and minnow traps, along with kick-net seining, a total of 223 crayfish were caught that were species of interest (*P. nueces* – 102, *P. fayettei* – 37, P. *texanus* – 49, *P. acutus* – 35). Figure 4 shows the range of site visits for each of the three endemic species. Other species were caught as well, including *P. kensleyii*, *P. simulans*, *P. clarkii*, and others that were unable to be positively identified (typically these were juveniles). Only 25-35 individuals of each species were kept and brought back to UT Tyler as we wanted to minimize the impact of collections on the local populations. Quarterly visits largely focused on sites where crayfish have been caught, and searching for new sites has been minimal. Figures 5-8 are from various field visits.

Task 1B

We documented habitat parameters at each collection site, as well as at most sites where traps were deployed but no animals were collected. These parameters include water pH, dissolved oxygen, conductivity, resistivity, total dissolved solids, salinity, turbidity, water and air temperature. Starting April 11, 2025, we began measuring sediment temperature beneath the water to assess if burrowing offers crayfish thermal refuge. Photos are taken to document each site and to assist with future analyses.

Table 1. Summary of crayfish field surveys conducted across seven Texas counties from February 2024 through December 2025. For each field trip, the table reports the sampling dates, counties visited, number of site visits, crayfish species recorded, and numerically documented captures. Field trips consisted of single-day or consecutive-day sampling events, and each site visit represents sampling at one location on one date. Blank cells indicate that no target species was recorded or that a numerical capture count was unavailable. The total of 245 target captures includes only individuals for which explicit counts were recorded and excludes two unidentified crayfish.

Field trips 21	Field dates 24	Site-visit records 187	Capture records 95	Counties 7	Numeric target captures 245

Visit dates	Counties	Site visits	Species recorded	Numeric catches recorded
2024-02-15	Fayette, Lee	2		
2024-05-15 to 2024-05-16	Bastrop, Fayette, Lee	24		
2024-06-24	Atascosa, Wilson	4		
2024-12-11 to 2024-12-12	Bastrop, Fayette, Lee	16		
2025-01-05 to 2025-01-06	Atascosa, McMullen	14	*P. nueces*	*P. nueces:* 58
2025-01-11	Angelina	5	*P. acutus*	
2025-02-07	Bastrop, Fayette, Lee	21		
2025-02-15	Angelina	5	*P. acutus*	
2025-03-14	Angelina	5	*P. acutus*	
2025-04-11	Atascosa	6	*P. nueces*	*P. nueces*: 44
2025-04-18	Angelina, Bastrop, Fayette	18	*P. acutus, P. fayettei, P. texanus*	*P. fayettei:* 37; *P. texanus*: 50
2025-05-15	Angelina	5	*P. acutus*	
2025-06-26	Angelina	5	*P. acutus*	
2025-07-01	Atascosa, Bastrop, Fayette	10	*P. fayettei, P. nueces, P. texanus*	*P. fayettei*: 26; *P. nueces*: 14; *P. texanus*: 16
2025-07-17	Angelina	5	*P. acutus*	
2025-08-07	Angelina	5	*P. acutus*	
2025-09-19	Angelina	5	*P. acutus*	
2025-10-10	Atascosa, Bastrop, Fayette	10	unknown, unknown.	unknown: 1; unknown.: 1
2025-10-18	Angelina	5	*P. acutus*	
2025-11-15	Angelina	5	*P. acutus*	
2025-12-12	Angelina	5	*P. acutus*	

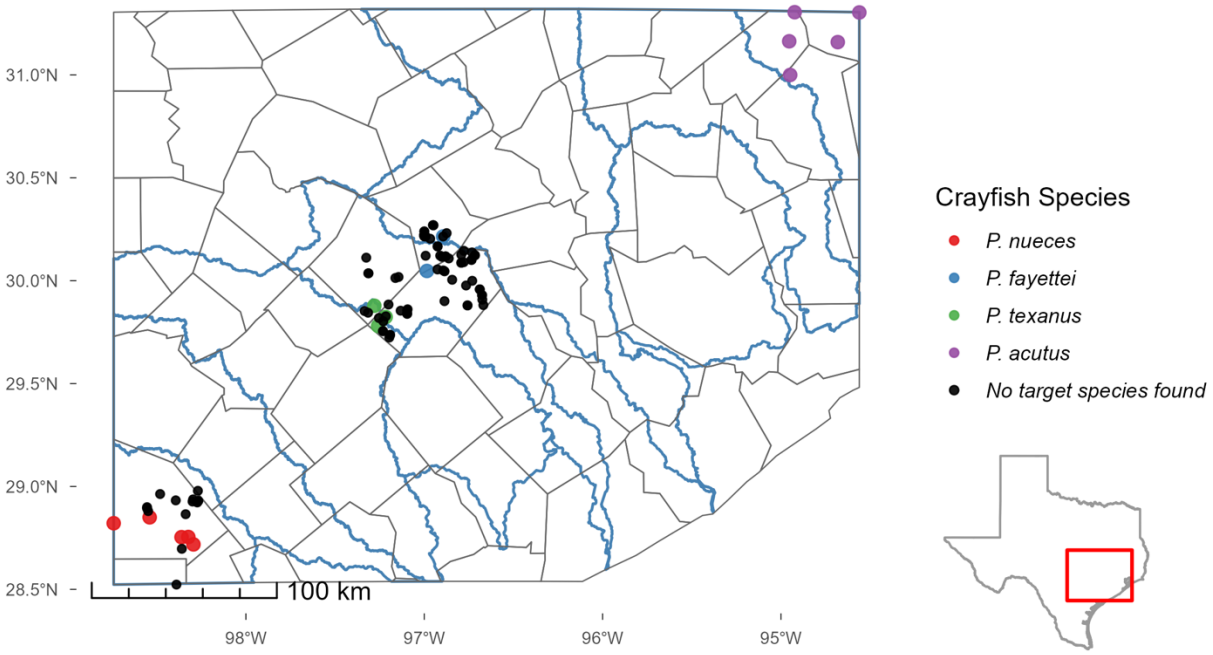


Figure 4. Capture sites of target crayfish species and survey locations where no target species were found. Colored points indicate sites where target species were captured: red points represent *P. nueces*, blue points represent *P. fayettei*, green points represent *P. texanus*, purple points represent *P. acutus*, and black points indicate surveyed sites where no target species were detected.



Figure 5. deploying traps



Figure 6. Seining



Figure 7. Kick-net seining



Figure 8. Water quality measurement

Task 1C

Habitat associations were evaluated using species-specific logistic regression models fit with the stats package in base R, relating crayfish presence–absence to physiochemical habitat variables (Hosmer et al., 2013). We conducted all statistical analyses in R (R Core Team, 2025). Data was imported using the readxl package (Wickham et al., 2023) and cleaned and transformed using dplyr (Wickham et al., 2023). For each species, we evaluated all possible additive combinations of standardized water temperature (°C), turbidity (FNU), salinity (PSU), specific conductivity ($\mu S \cdot cm^{-1}$), total dissolved solids (TDS), and pH, including the null model. Models were fit using a binomial error distribution with a logit link function and compared using Akaike's Information Criterion (AIC;(Akaike, 1974) corrected for small sample size (AICc; (Hurvich & Tsai, 1989), calculated using the AICcmodavg package. We identified the best-supported model for each species as the model with the lowest AICc, following standard information-theoretic model selection practices (Burnham & Anderson, 2002). Model uncertainty was evaluated by examining differences in AICc values and Akaike weights across the full candidate set. We interpreted predictor effects using regression coefficients from the best-supported model for each species, consistent with likelihood-based inference following information-theoretic model selection (Burnham & Anderson, 2002). Effect direction and magnitude were summarized using coefficient estimates and odds ratios for standardized predictors, with statistical support for individual predictors assessed using Wald tests implemented within the generalized linear modeling framework (Agresti, 2015). Figures were generated using ggplot2 (Wickham, 2016).

We found that habitat associations differed markedly among species, with no single environmental variable consistently predicting occurrence across all taxa (Table 2; Figure 9). Turbidity (FNU) was positively associated with the presence of *P. nueces* (β = 0.55, OR = 1.73, p = 0.005) but negatively associated with *P. acutus* (β = −1.36, OR = 0.26, p = 0.026). Salinity (PSU) also had strong but opposing effects: *P. acutus* exhibited a pronounced negative association with salinity (β = −2.14, OR = 0.12, p < 0.001), whereas *P. fayettei* exhibited a significant positive association (β = 0.97, OR = 2.63, p = 0.015). Temperature was retained in the best-supported models for *P. nueces*, *P. fayettei*, and *P. texanus* and was positively associated with occurrence in all three species, although the effect was statistically significant only for *P. fayettei* (β = 2.04, OR = 7.68, p = 0.035). The positive temperature associations for *P. nueces* (β = 0.65, OR = 1.92, p = 0.093) and *P. texanus* (β = 1.05, OR = 2.85, p = 0.092) were not statistically significant. *Procambarus texanus* also showed a positive but nonsignificant association with specific conductivity (β = 0.28, OR = 1.33, p = 0.097). Temperature was not retained in the best-supported model for *P. acutus*, and pH was not retained in the best-supported model for any species.

Table 2. Species-specific relationships between environmental predictors and occurrence probability derived from logistic regression models. Shown are model coefficients (Estimate), corresponding odds ratios, significance levels, and direction of effect for each crayfish species to communicate which predictors were most significant.

Species	Predictor	Estimate	Odds Ratio	P value	Direction
P. nueces	Turbidity	0.547	1.727	**0.01**	+
P. nueces	Temperature	0.654	1.923	0.09	+
P. acutus	Salinity	-2.135	0.118	**0.00**	-
P. acutus	Turbidity	-1.360	0.257	**0.03**	-
P. fayettei	Salinity	0.967	2.630	**0.02**	+
P. fayettei	Temperature	2.038	7.677	**0.03**	+
P. texanus	Conductivity	0.282	1.325	0.10	+
P. texanus	Temperature	1.049	2.854	0.09	+

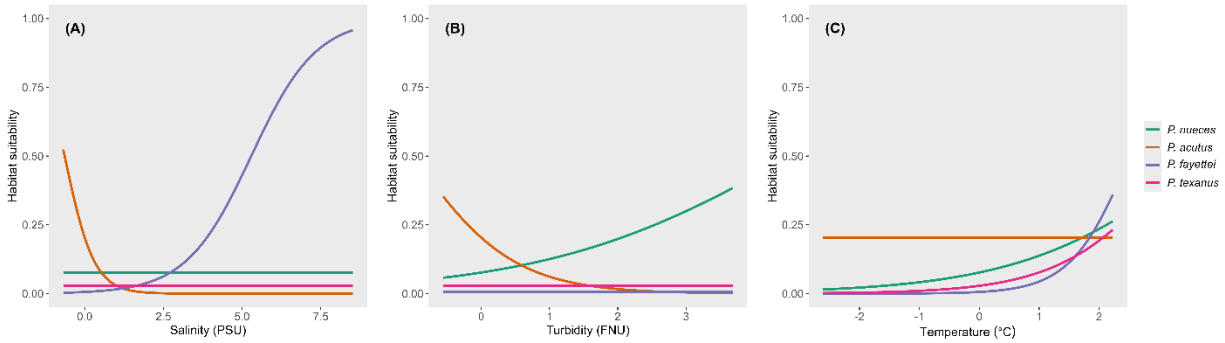


Figure 9. Habitat suitability responses of four Procambarus species, *P. nueces*, *P. acutus*, *P. fayettei*, and *P. texanus*, across three environmental gradients. (A) Salinity (PSU), (B) turbidity (FNU), and (C) temperature (°C) are shown on the x-axes, with modeled habitat suitability (0–1) on the y-axes. Species exhibit distinct, nonlinear suitability curves for each variable, highlighting differential environmental tolerances and niche separation within the genus.

Task 2: Conduct physiological tolerance bioassays using a minimum of six (6) individuals of each species for each abiotic variable tested.

2A. Determine thermal tolerance by measuring the critical thermal maximum and agitation temperature for each species of interest.

2B. Determine hypoxia tolerance by determining time to loss of equilibrium for each species of interest.

2C. Determine pH tolerance by determining time to loss of equilibrium for each species of interest.

Experimental design

Task 2A, the thermal tolerance for all four species of interest were completed by fall of 2025. Thermal tolerance was assessed by determining loss of equilibrium following increasing the temperature by 1°C per hour for 10-12 individuals. Crayfish were placed in custom-made chambers that kept individuals separated (Fig. 10). The immersion heater was used to control the rate of temperature increase (Fig. 11). Crayfish of each species were acclimated to either 24, 27, or 30 °C for at least two weeks prior to determining thermal tolerance, and trials were repeated three times at each acclimation temperature.



Figure 10. Plexi-glass chambers for holding crayfish during loss of equilibrium experiment.



Figure 11. Thermal tolerance set-up showing the plexi-glass chamber containing crayfish on right side of aquaria, with the immersion heater on the right.

Task 2B, hypoxia tolerance was determined by two methods: Pcrit and LOE. We employed two different methodologies for hypoxia tolerance as Pcrit represents the lowest partial pressure of oxygen (PO_2) at which an animal can maintain a stable rate of oxygen consumption, while LOE signifies a critical point of physiological distress and indicates the animal is struggling to cope with the lack of oxygen. Together these two methods provided a comprehensive understanding of crayfish hypoxia tolerance.

To assess oxygen consumption rates and hypoxia tolerance in crayfish, we used intermittent flow respirometry to measure Standard Metabolic Rate (SMR), Maximum Metabolic Rate (MMR), and Aerobic Scope (AS) for each species (Figure 12). These measurements were collected using a four-chamber fiber optic respirometry system with AutoResp™ 3.0 software (Loligo Systems, Inc.). Using this system, we tested four individuals at a time, with three trials for each species. Individual sex and mass was recorded prior to each trial. Crayfish were placed in suitable sized acrylic respirometry chambers (74–600 mL) that were submersed in a 160 L tank (Svendsen et al. 2016). Constant aeration of the large tank ensured near 100% saturation with dissolved oxygen and temperature was maintained within ± 0.5°C using aquarium heaters. Each chamber was attached to two water pumps, a recirculation pump that circulated water through the chamber and past an optical dissolved oxygen sensor during the closed-circuit cycle while respiration rates were being measured and a flush pump that brought fresh, oxygenated water from the outer tank into the chamber between cycles. Crayfish were acclimated in respirometry chambers for 24 hours before their SMR was measured. The MMR of crayfish was measured by first exposing individuals to air for 10 minutes to simulate stress then placing them in a respirometry chamber where their MMR was recorded.

P_{crit} was conducted immediately following the SMR and MMR trials. Crayfish remained in the respirometry chamber with the flush pump on for at least 0.5 h before beginning the acclimation/loop period and P_{crit} trial. During acclimation, the system was set to function on a loop setting: closed to allow oxygen saturation to drop to ~80%, then flushed until levels return to 95–100%. This acclimation period lasted for a minimum of 2 h, with longer periods if MO_2 rates indicated agitation. Once metabolic rate stabilizes to show <10%

difference in oxygen consumption between loops (as determined by real-time MO2 calculations performed by AutoResp software during acclimation and throughout the trial), the chamber was closed and oxygen was allowed to drop to near 0% levels until crayfish reach critical tension (P_{crit}). Once P_{crit} was reached, or crayfish showed visible loss of righting response, oxygenated water was returned to the chambers and crayfish were allowed to recover. Once trials were completed, analyses were conducted in winter 2025.

Hypoxia tolerance as determined by LOE was conducted in Fall 2025. Using a similar experimental set up as our CTmax trials, We used nitrogen to create hypoxic conditions in the testing chamber to determine the oxygen tension when LOE is reached. Bubbling rates of nitrogen and air were controlled by a Sierra Instruments mass flow controller to achieve oxygen tensions of 15, 10, and 5% saturation within the experimental tank. These O_2 tensions were monitored every half hour to maintain consistency. Starting at concentrations of 5% oxygen, crayfish were observed at 30-minute intervals to check for LOE as O_2 tensions approached near zero. When a loss of righting response was documented, crayfish recovered in oxygen-rich tanks.

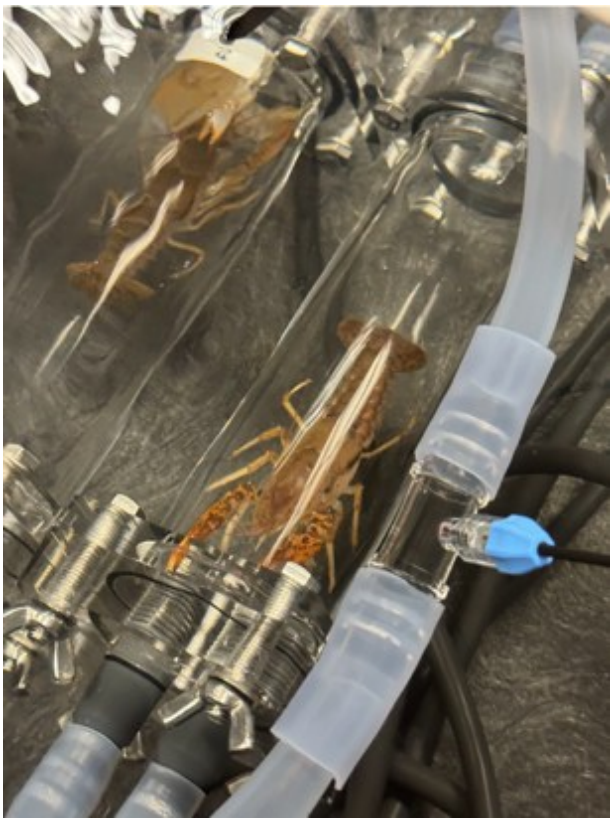


Figure 12. Crayfish in respirometry chamber.

Task 2C, pH (acidity) tolerance was determined by using a similar experimental design as the thermal tolerance study (Figure 13). Beginning with a pH of 7, individual crayfish were

exposed to increasing H+ concentration at a rate of 1 unit per 48 hours. Increased H+ concentration was achieved by gradually dripping 0.37% HCl dilute into the testing chamber. Water pH was monitored two times daily using a pH probe to ensure steady acidification. Submersible pumps were utilized to maintain flow and circulation. The righting response of crayfish was monitored two times a day for the duration of the trial period, typically at 9 am and 5 pm. Testing concluded at the observed Loss of Equilibrium (LOE) that exceeded a 30 second window. Upon completion of the study, individual crayfish were placed in recovery tanks and monitored for at least 24 hours to observe delayed acidity side effects.

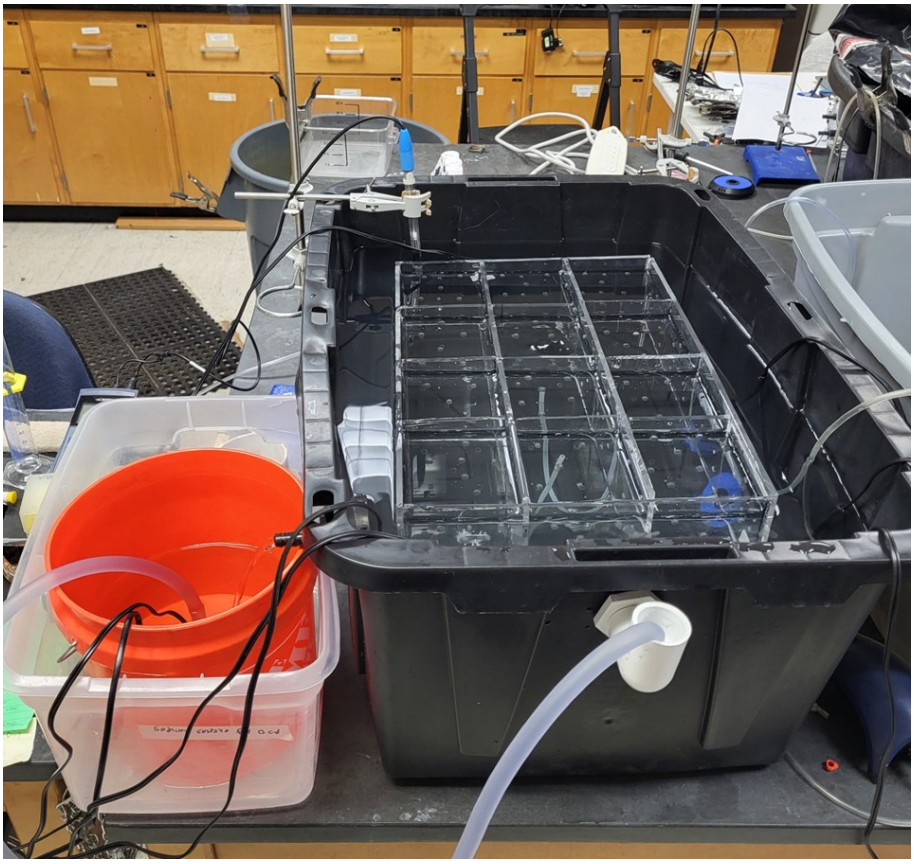


Figure 13. testing chamber used to assess acidity tolerance

Statistical Analyses

We conducted all analyses in R v4.3.1 (R Core Team, 2025). Linear and mixed-effects models were implemented using the packages *lme4* (D. Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), *car* (Fox & Weisberg, 2019), and *emmeans* (Lenth, 2024).

To evaluate interspecific differences in physiological performance, separate analyses were conducted for critical thermal maximum, hypoxia tolerance, acidity tolerance, standard

metabolic rate, maximum metabolic rate, aerobic scope, and critical oxygen tension. Body mass was included as a continuous covariate in all models, and sex was included as a fixed effect. To evaluate whether the effects of sex and body size differed among species, all ANCOVA models included Species × Sex and Species × Body Mass interaction terms. Type III sums of squares were used to evaluate main and interaction effects. Statistical significance was assessed at α = 0.05. For all response variables, Type III ANCOVA tables reporting sums of squares (SS), degrees of freedom (df), F-statistics, and associated P-values were generated to facilitate direct comparison of effect sizes and significance across physiological traits. The results from these analyses can be found below in Table 3.

Table 3. ANCOVA results for critical thermal maximum (CTmax), oxygen concentration at loss of equilibrium (O_2 LOE), pH at loss of equilibrium (pH LOE), standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS), and critical oxygen tension (Pcrit) in four crayfish species. Effects of species, acclimation temperature, sex, body mass, and their interactions are reported as type III sums of squares (SS), degrees of freedom (df), F-statistics, and P-values. Significant effects (P < 0.05) are shown in bold.

	Thermal Tolerance (CT_{max})				**Hypoxia Tolerance (O_2 LOE)**				**Standard Metabolic Rate (SMR)**				**Maximum Metabolic Rate (MMR)**			
Effect	SS	df	*F*	*P*	SS	df	*F*	*P*	SS	df	*F*	*P*	SS	df	*F*	*P*
Species	4.27	3	0.88	0.454	1.04	3	0.21	0.892	7241.75	3	2.59	0.069	14124.55	3	2.57	0.071
Acclimation Temperature	10.93	2	3.39	**0.037**	-	-	-	-	-	-	-	-	-	-	-	-
Sex	3.50	1	2.17	0.145	0.32	1	0.19	0.666	585.47	1	0.63	0.433	113.99	1	0.06	0.805
Body Mass	33.58	1	20.80	**<0.001**	0.56	1	0.34	0.567	20840.18	1	22.40	**<0.001**	10245.19	1	5.59	**0.024**
Species × Acclimation Temperature	41.57	6	4.29	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-
Species × Sex	4.80	3	0.99	0.401	1.54	3	0.30	0.822	12315.11	3	4.41	**0.010**	1600.76	3	0.29	0.832
Species × Body Mass	0.44	3	0.09	0.965	0.82	3	0.16	0.921	24504.79	3	8.78	**<0.001**	5030.85	3	0.91	0.445
Residuals	548.43	377	-	-	57.31	34	-	-	30707.89	33	-	-	60516.12	33	-	-

	Aerobic Scope (AS)				**Critical Oxygen Tension (P_{crit})**				**Acidity Tolerance (pH LOE)**			
Effect	SS	df	*F*	*P*	SS	df	*F*	*P*	SS	df	*F*	*P*
Species	26261.65	3	4.10	**0.014**	670.97	3	0.96	0.424	3.46	3	1.51	0.220
Acclimation Temperature	-	-	-	-	-	-	-	-	-	-	-	-
Sex	1216.14	1	0.57	0.456	698.50	1	3.00	0.094	0.34	1	0.45	0.504
Body Mass	1861.30	1	0.87	0.357	354.76	1	1.53	0.227	3.45	1	4.50	**0.037**
Species × Acclimation Temperature	-	-	-	-	-	-	-	-	-	-	-	-
Species × Sex	13005.45	3	2.03	0.129	1465.84	3	2.10	0.123	2.35	3	1.02	0.387
Species × Body Mass	22659.14	3	3.54	**0.025**	2279.57	3	3.27	**0.036**	6.92	3	3.02	**0.035**
Residuals	70447.85	33	-	-	6509.13	26	-	-	58.14	76	-	-

Results

Task 2A

After conducting our analyses, we found that thermal tolerance (CTmax) varied significantly with body mass (figure 14) and acclimation temperature (figure 15), with a strong species x acclimation temperature interaction. Acclimation temperature had a significant main effect ($\chi^2_2 = 3.39$, $p = 0.037$). Body mass was highly significant ($\chi^2_1 = 20.80$, $p < 0.001$). The species x acclimation temperature interaction remained significant ($\chi^2_6 = 4.29$, $p = 0.001$), indicating species-specific thermal plasticity.

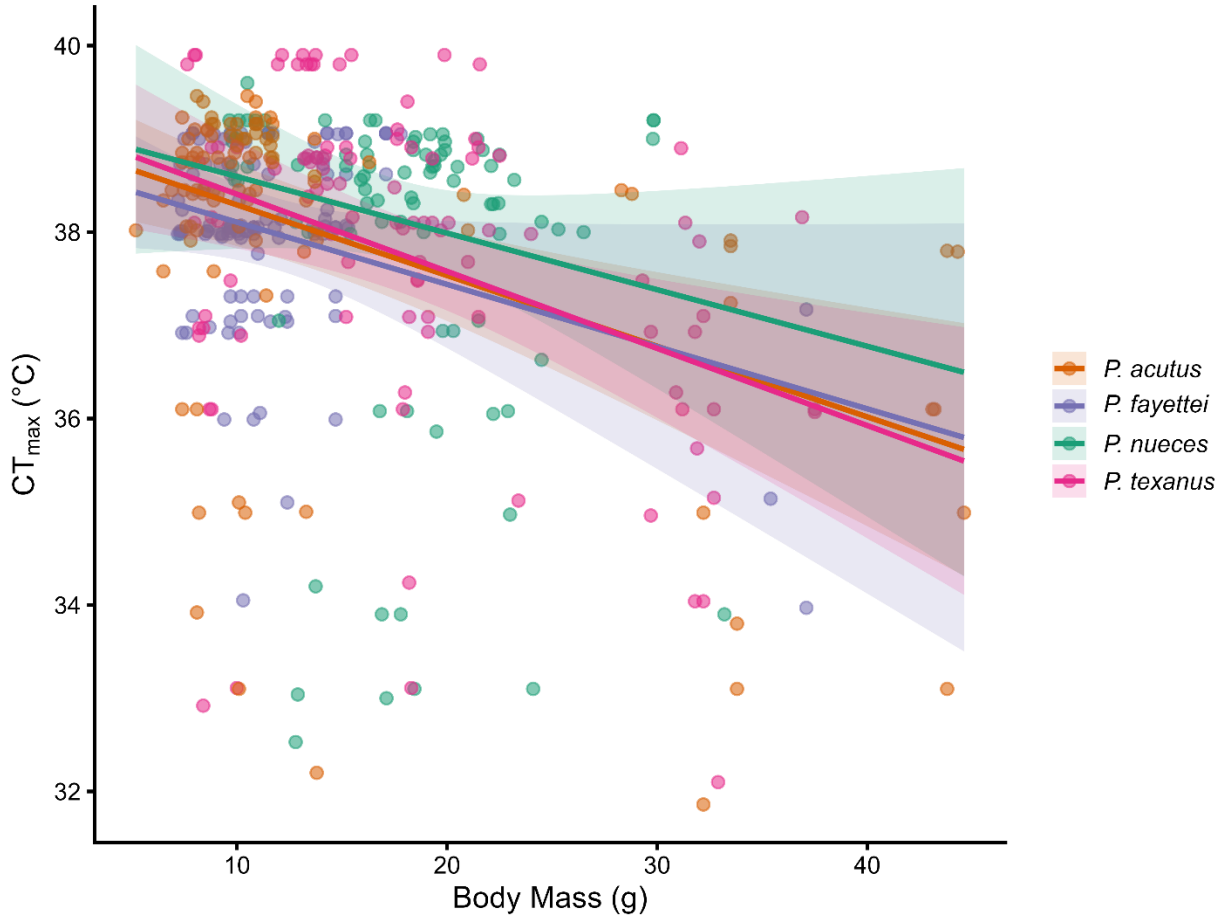


Figure 14. Relationship between body mass and mean CTmax across crayfish species. Points represent individual observations, with colors indicating species: *P. acutus*, *P. fayettei*, P. *nueces*, and *P. texanus*. Colored lines show species-specific linear relationships between body mass and CTmax, and shaded regions represent confidence intervals around each fitted relationship. Across species, CTmax generally decreased as body mass increased, although the strength of this relationship varied among species.

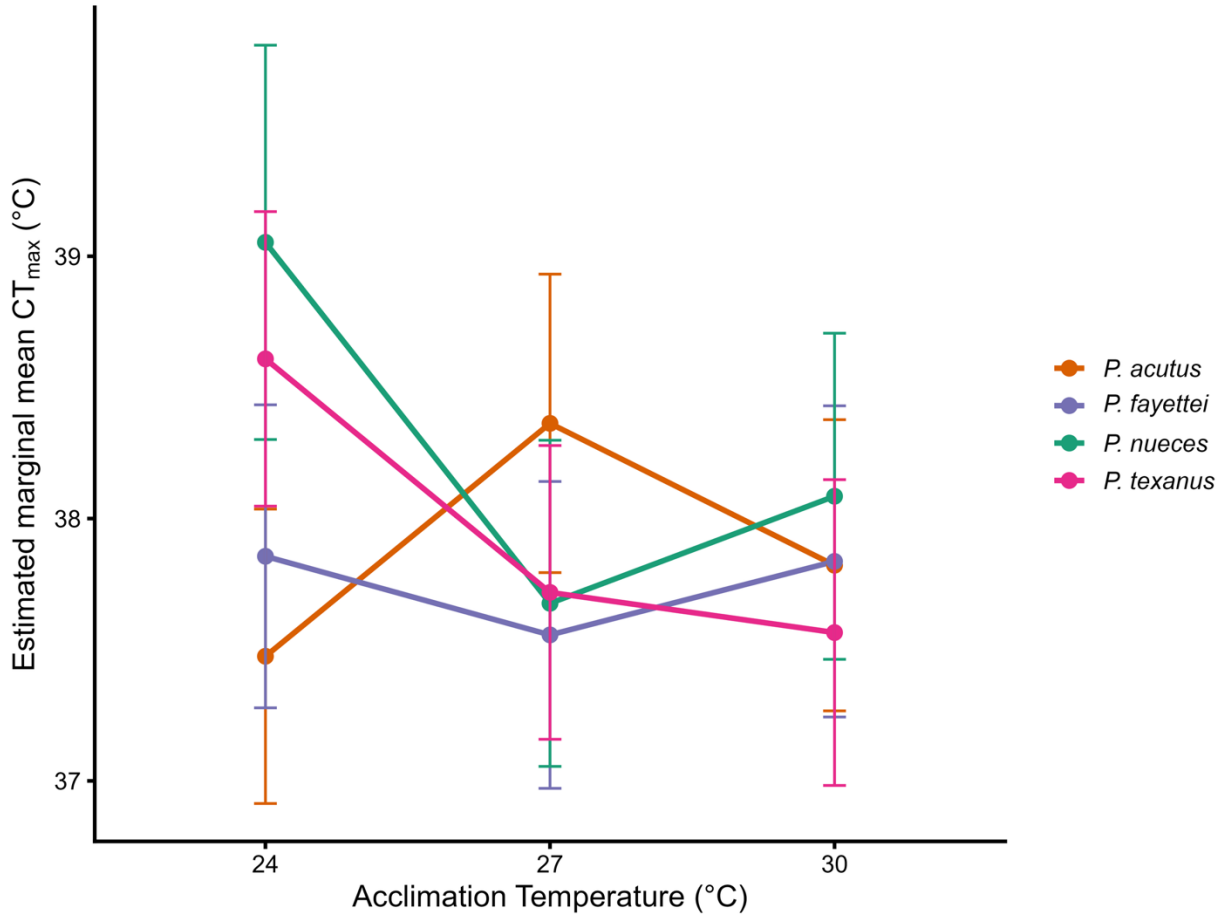


Figure 15. The estimated marginal means (± SE) of critical thermal maximum (CTmax, °C) was calculated for four crayfish species (*P. acutus*, *P. fayettei*, *P. nueces*, and *P. texanus*) across three acclimation temperatures (24°C, 27°C, and 30°C). The values are derived from a linear mixed-effects model including species, acclimation temperature, sex, and body mass as fixed effects and individual identity as a random effect. Points represent model-adjusted means, and error bars denote ±1 standard error. CTmax responses to acclimation temperature varied among species, indicating a species-by-temperature interaction.

Task 2B

Nitrogen Displacement

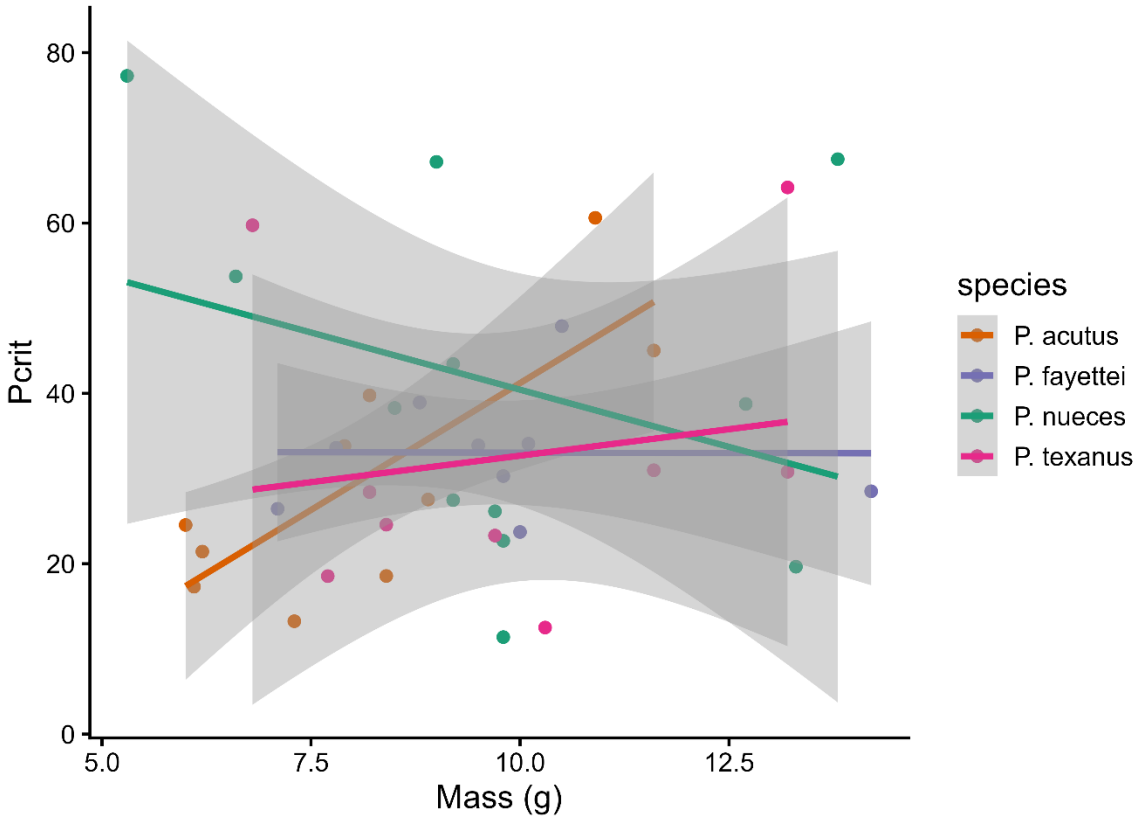
We found no significant effects of species identity, sex, body mass, or their interactions on hypoxia tolerance among the four crayfish species examined. Specifically, hypoxia tolerance did not differ among species ($F_{3,33}$ = 0.21, P = 0.89), nor was it influenced by sex ($F_{1,33}$ = 0.19, P = 0.66) or body mass ($F_{1,33}$ = 0.35, P = 0.56). Additionally, no interaction terms—including species x sex, species x mass, or sex x mass—were statistically significant.

Metabolic rate analysis

We found that aerobic scope (AS) differed significantly among species ($F_{3,26}$ = 4.10, *P* = 0.014). SMR exhibited no significant species effect ($F_{3,33}$ = 0.21, *P* = 0.892). Body mass was a significant predictor of SMR ($F_{1,33}$ = 5.59, *P* = 0.024), but not MMR ($F_{1,33}$ = 0.87, *P* = 0.357). AS also differed significantly among species ($F_{3,26}$ = 4.10, *P* = 0.014), with a significant Species × Body Mass interaction ($F_{3,26}$ = 3.54, *P* = 0.025) (Figure 17), indicating that the relationship between body mass and aerobic scope varied among species.

P_{crit}

Pcrit varied among species, though the main effect of species was not significant ($F_{3,76}$ =0.96, p = 0.424). There was a significant species x body mass interaction ($F_{3,76}$ = 3.27, p = 0.036) (Figure 16). Sex and species x sex interactions were not significant.



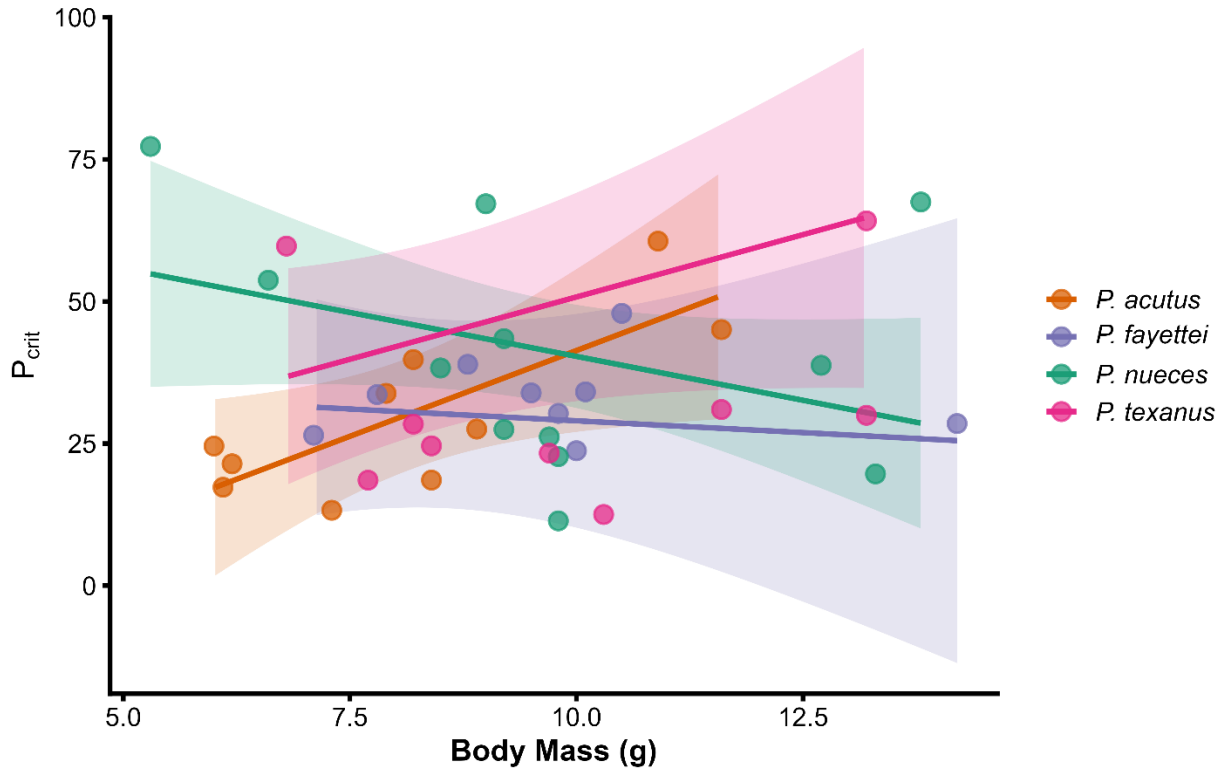


Figure 16. The relationship between critical oxygen tension (P_{crit}) and body mass varied among species and showed no consistent overall trend. When examined within species, *P. acutus* exhibited a positive relationship between body mass and P_{crit}, with larger individuals tending to have higher P_{crit} values. In contrast, *P. nueces* showed a negative relationship, with P_{crit} decreasing as body mass increased. *P. texanus* displayed a slight positive trend, although the slope was relatively shallow, while *P. fayettei* exhibited little to no relationship between body mass and P_{crit}, as indicated by a nearly flat regression line. Collectively, these results indicate that the effect of body mass on hypoxia tolerance is not consistent across species and suggests that body size alone is not a strong or universal predictor of P_{crit} in these crayfish.

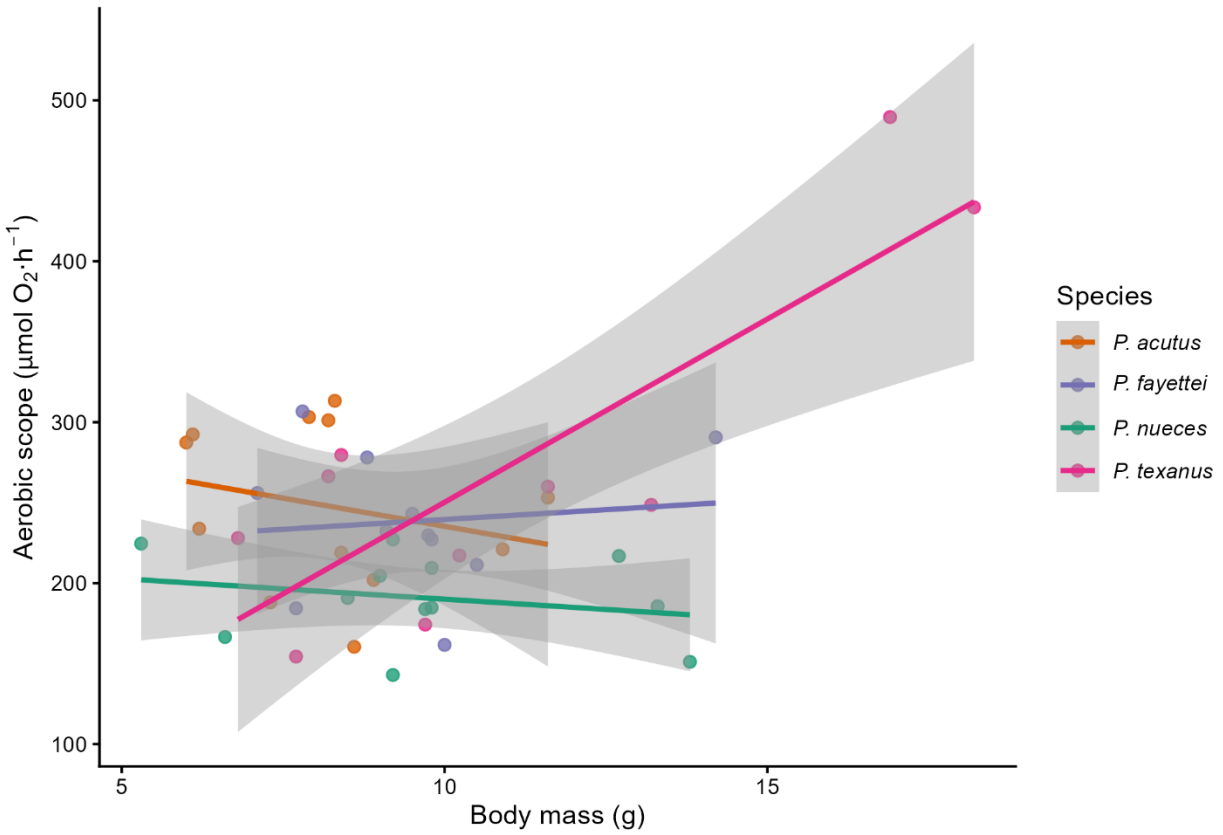


Figure 17. Relationship between body mass and aerobic scope (AS) among four Procambarus species. Points represent individual crayfish, and colored regression lines depict species-specific least-squares linear relationships between body mass and aerobic scope. Shaded regions indicate 95% confidence intervals around the fitted regression lines. Aerobic scope increased with body mass in P. texanus, exhibited little change across the observed mass range in P. fayettei, and tended to decrease slightly with increasing body mass in P. acutus and P. nueces. The differing slopes among species are consistent with the significant Species × Body Mass interaction detected by ANCOVA ($F_{3,26}$ = 3.54, P = 0.025), indicating that the relationship between body mass and aerobic scope varied among species.

Task 2C

Our pH tolerance assays found that body mass significantly influenced pH LOE ($F_{1,76}$ = 4.50, *P* = 0.037), indicating size-dependent variation in acid tolerance (Figure 18). Species alone was not significant ($F_{3,76}$ = 1.51, *P* = 0.220). However, the Species × Body Mass interaction was significant ($F_{3,76}$ = 3.02, *P* = 0.035), demonstrating that the relationship between body mass and acid tolerance differed among species. Neither sex ($F_{1,76}$ = 0.45, *P* = 0.504) nor the Species × Sex interaction ($F_{3,76}$ = 1.02, *P* = 0.387) significantly influenced pH LOE.

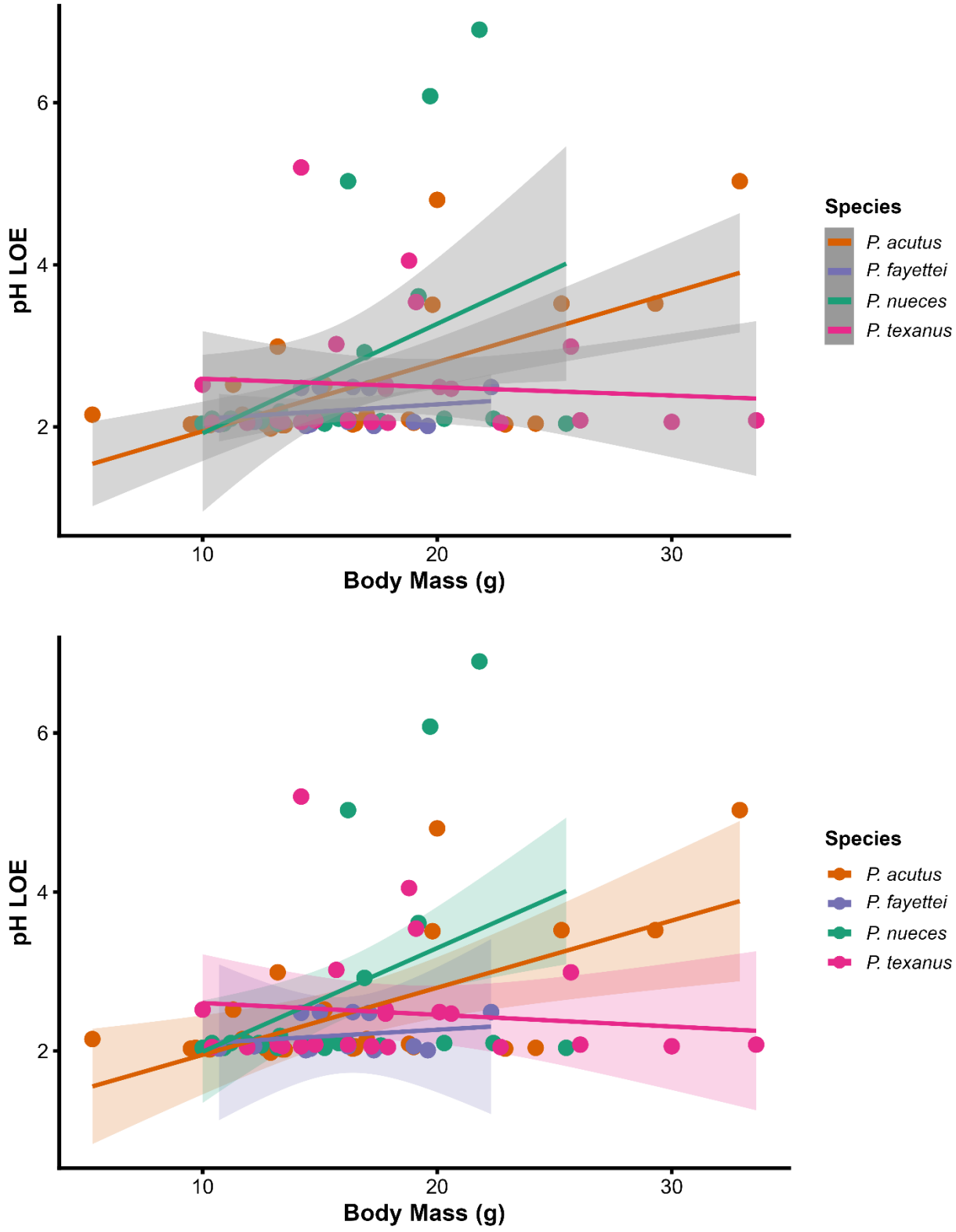

pH LOE
6
4
2
10
20
30
Body Mass (g)
Species
P. acutus
P. fayettei
P. nueces
P. texanus
pH LOE
6
4
2
10
20
30
Body Mass (g)
Species
P. acutus
P. fayettei
P. nueces
P. texanus

Figure 18. Species-specific relationships between body mass and acidity tolerance, measured as pH at loss of equilibrium. Points represent individual crayfish, with colors indicating species: orange represents *P. acutus*, purple represents *P. fayettei*, green represents *P. nueces*, and pink represents *P. texanus*. Lines show species-specific fitted relationships between body mass and pH loss of equilibrium. The differing slopes indicate a species by body mass interaction, with acidity tolerance changing with body mass in different ways among species.

Discussion

This study demonstrates that the widespread *Procambarus acutus* and the three Texas-endemic species differed in their physiological responses when body mass was taken into account. Body mass was an important predictor of several responses, but its effect frequently depended on species. The four species therefore cannot be arranged along a single continuum from broadly tolerant to broadly sensitive. Instead, each exhibited a distinct combination of responses to high temperature, declining oxygen, and declining pH, and the relative performance of the species often changed with body size. The principal physiological distinction was thus not simply between endemic and widespread species, but among species-specific relationships between growth and environmental tolerance.

The habitat-association models reinforce the conclusion that the four species interact differently with their environments. No environmental variable consistently predicted the occurrence of all four species. *Procambarus nueces* was significantly associated with greater turbidity. Temperature also had a positive, although marginally nonsignificant, association with *P. nueces* occurrence. *Procambarus acutus* showed the opposite general pattern: its occurrence was strongly and negatively associated with salinity, negatively associated with turbidity, and not associated with temperature. *Procambarus fayettei* was positively associated with both salinity and temperature, with temperature producing the largest estimated odds ratio in the habitat models. *Procambarus texanus* showed positive but statistically weak associations with temperature and conductivity. These results indicate that the species differ not only in their geographic ranges but also in the local water conditions under which they were detected.

These local associations complement earlier ecological niche modeling. *Procambarus texanus* and *P. fayettei*, which occur primarily on opposite sides of the Colorado River, occupy largely indistinguishable broad-scale environmental niches. In contrast, *P. nueces* occupies a more distinctive western niche associated with drier conditions, larger catchments, steeper slopes, and more erodible soils (Burgett et al. 2026). *Procambarus acutus* differs from the three endemics in being a widespread generalist whose range extends much farther north. The species therefore represent a contrast among two geographically separated endemics occupying similar broad-scale environments, a western endemic specialized to more arid and hydrologically variable conditions, and a widespread species occurring across a much broader climatic region (Hobbs, 1989; Johnson, 2018; Burgett et al., 2026).

The physiological and logistic-regression findings correspond most clearly for *P. nueces*. This species tolerated high temperatures slightly better than the others across the

range of body sizes represented in the experiment, and its probability of occurrence tended to increase with water temperature in the field, although the field relationship did not meet the conventional threshold for statistical significance. Its positive association with turbidity further distinguishes its occupied habitats from those of *P. acutus*. Together with the earlier niche models, these results support the interpretation that *P. nueces* is specialized to relatively warm, sediment-laden, and hydrologically variable western waters. The correspondence is not exact, but both laboratory and field results point in the same general direction.

Body size is central to understanding that specialization. Across species, critical thermal maximum generally declined as body mass increased, meaning that smaller crayfish retained higher acute thermal limits. Because *P. nueces* also had slightly greater thermal tolerance than the other species, small *P. nueces* combined the species-level thermal advantage with the general advantage associated with smaller body size. In habitats where shallow or contracting waters become especially warm, selection may therefore favor individuals that mature at a smaller size or allocate less energy to continued somatic growth. Crayfish thermal performance varies among species, populations, acclimation histories, and experimental methods, and upper thermal limits do not by themselves define the temperatures at which animals normally occur. Nevertheless, the body-size effect provides a plausible mechanism through which remaining small could improve performance in the warm environments occupied by *P. nueces* (Westhoff & Rosenberger, 2016).

The species-by-acclimation-temperature interaction further indicates that thermal plasticity differed among species. This is important because habitat use may depend not only on the maximum temperature an animal can survive but also on its capacity to adjust after prolonged exposure. The strong positive association between temperature and *P. fayettei* occurrence, despite the absence of clearly superior acute thermal tolerance in that species, illustrates this distinction. *Procambarus fayettei* may be able to occupy relatively warm sites through acclimation, behavioral thermoregulation, use of thermal refuges, or other ecological traits without possessing the highest acute CTmax. Field occurrence models describe the conditions under which species were detected, whereas laboratory tolerance assays isolate particular components of physiological capacity. Agreement between the two is therefore informative, but complete correspondence should not be expected because realized distributions also reflect behavior, dispersal, biotic interactions, habitat structure, and historical geography (Kearney & Porter, 2009).

The two measures of hypoxia response also yielded different conclusions. In the nitrogen-displacement trials, oxygen concentration at loss of equilibrium did not differ significantly among species and was not significantly affected by body mass. Thus, the four species were broadly similar in the oxygen concentration at which severe whole-animal distress became apparent. Pcrit, however, showed a significant species-by-body-mass interaction. A lower Pcrit generally indicates that an animal can maintain a stable rate of oxygen uptake to a lower ambient oxygen level, although Pcrit does not capture all behavioral, metabolic, and anaerobic mechanisms that can contribute to survival during severe or prolonged hypoxia (Rogers et al., 2016).

Pcrit increased strongly with body mass in *P. acutus*, increased more weakly in *P. texanus*, changed little in *P. fayettei*, and declined with body mass in *P. nueces*. Larger *P. acutus* therefore became oxygen limited at higher oxygen levels than smaller individuals, and *P. texanus* showed the same tendency to a lesser extent. In contrast, larger *P. nueces* maintained oxygen uptake to lower oxygen levels than smaller conspecifics, while low-oxygen performance in *P. fayettei* was largely independent of body mass. These relationships demonstrate that body size alone is not a universal predictor of low-oxygen performance. Increasing size produced a benefit in *P. nueces*, a cost in *P. acutus* and possibly *P. texanus*, and little change in *P. fayettei*.

The Pcrit result qualifies, rather than overturns, the small-body hypothesis for *P. nueces*. Remaining small did not improve every component of its physiology. Indeed, larger *P. nueces* performed better according to Pcrit. The more plausible interpretation is that the species experiences opposing physiological consequences of growth. Small individuals retain higher thermal tolerance, whereas larger individuals gain an advantage in maintaining aerobic oxygen uptake as oxygen declines. Because oxygen concentration at loss of equilibrium showed no corresponding body-size effect, the Pcrit pattern may represent improved oxygen regulation without necessarily translating into substantially greater resistance to the most severe hypoxia. Selection in the wild may consequently favor an intermediate or relatively small adult size that balances thermal performance, oxygen regulation, reproduction, competition, and other ecological demands.

Aerobic scope revealed a further species-specific body-size pattern. Aerobic scope increased substantially with body mass in *P. texanus*, changed little in *P. fayettei*, and tended to decline slightly in *P. acutus* and *P. nueces*. The *P. texanus* result suggests that larger individuals may possess greater metabolic capacity above maintenance requirements. However, the fitted relationship was influenced by a small number of unusually large *P. texanus*, and it should therefore be regarded as provisional. More large individuals are needed to determine whether increasing aerobic scope is a consistent feature of the species or an artifact of the sampled body-size distribution. The slight decline in aerobic scope among larger *P. nueces* provides another possible cost of increasing size, even though their Pcrit improved.

The acidity experiment produced the strongest evidence that increasing body size may be disadvantageous for *P. nueces*. Because the response variable was pH at loss of equilibrium during progressive acidification, a higher value indicates lower tolerance: an individual losing equilibrium at pH 5 was less tolerant than one remaining upright until pH 3. The pH at loss of equilibrium increased steeply with body mass in *P. nueces*. Larger individuals therefore lost equilibrium under substantially less acidic conditions than smaller individuals. *Procambarus acutus* showed the same general relationship, although the increase was less steep. *Procambarus fayettei* showed only a weak increase, while *P. texanus* showed a slight decline, suggesting that acid tolerance changed little or improved modestly as that species became larger.

Low pH can disrupt ion regulation, acid–base balance, oxygen uptake, and metabolic function in crayfish, and these effects can vary with calcium availability and exposure duration (Wood & Rogano, 1986; Ellis & Morris, 1995). The particularly steep body-size effect in *P. nueces* identifies a substantial physiological cost of becoming large. A

small individual retains greater tolerance of both high temperature and acidification, whereas a large individual loses much of that advantage. Although the western habitats of *P. nueces* should not be assumed to be chronically acidic, abrupt changes in runoff, groundwater contribution, concentration of solutes, or other aspects of water chemistry could still produce periods of physiological stress.

Taken together, the thermal, aerobic-scope, and pH results support a stronger working hypothesis that relatively small body size is part of the ecological specialization of *P. nueces*. The hypothesis is not that small individuals outperform large ones under every environmental condition; the Pcrit result clearly shows otherwise. Rather, remaining small may preserve the traits most important for occupying hot and variable habitats: a higher upper thermal limit, greater tolerance of acidification, and avoidance of the slight decline in aerobic scope observed with increasing mass. The cost is that smaller individuals have a higher Pcrit and therefore become oxygen limited sooner. The physiology of *P. nueces* may thus be organized around a body-size trade-off rather than uniformly superior stress tolerance.

This trade-off provides a coherent explanation for why *P. nueces* may remain smaller than its congeners. In its hot, drought-prone western habitats, selection may place a premium on avoiding the especially strong thermal and acid-related costs associated with becoming large. The benefits of larger size, namely, potentially including greater fecundity, competitive ability, predator resistance, and improved oxygen regulation, may eventually be outweighed by declining heat and acid tolerance. Under this interpretation, reduced adult size is not merely a consequence of poor environmental conditions. It may itself be an adaptation that helps *P. nueces* persist under conditions that would place greater physiological demands on larger crayfish. Confirming this hypothesis will require comparisons of wild size distributions, age at maturity, growth rates, and survival among size classes.

The habitat models are consistent with this hypothesis but do not prove it. The positive association of *P. nueces* with temperature agrees directionally with its relatively high thermal tolerance, and the strong positive association with turbidity confirms that the species occupies locally distinctive aquatic conditions. However, pH was not retained as an important occurrence predictor for *P. nueces*, and dissolved oxygen was not among the variables evaluated in the reported logistic-regression candidate set. The field analysis therefore does not directly confirm that acid or hypoxia tolerance limits its distribution. Instead, it identifies the environmental setting in which the physiological trade-offs may operate.

The results for *P. acutus* should be interpreted in the context of its generalist ecology. Because its geographic range extends much farther north, there is no reason to expect a Texas population of *P. acutus* to outperform a locally specialized western endemic under the hottest conditions encountered within the range of *P. nueces*. A widespread generalist succeeds by maintaining adequate performance across many environments, not by possessing the greatest tolerance of every local extreme. The negative temperature association in the logistic regression is therefore directionally consistent with its geographic distribution and with the absence of a strong thermal advantage in the laboratory. Its strong negative associations with salinity and turbidity further indicate that,

within this study, *P. acutus* was most likely to occur in relatively fresh, clear water rather than in the more turbid conditions associated with *P. nueces*.

The body-size physiology of *P. acutus* is also consistent with a generalist rather than a western-specialist strategy. Increasing body mass was associated with higher Pcrit, lower CTmax, declining aerobic scope, and loss of equilibrium at a higher pH. Larger Texas *P. acutus* therefore incurred physiological costs under each of the acute stress gradients examined. This does not undermine its characterization as a widespread generalist. Instead, it indicates that the broad range of the species probably arises from ecological breadth, population-level variation, behavioral flexibility, dispersal ability, and the availability of diverse habitats across its range rather than exceptional tolerance of every stressor by every individual. The sampled Texas population represents only a small and relatively southern portion of the species' geographic and potentially physiological variation.

The comparison of *P. texanus* and *P. fayettei* is informative for a different reason. Their broad-scale modeled niches are largely indistinguishable, yet their body-size relationships were not identical. Increasing body mass was associated with a modest increase in Pcrit but a substantial increase in aerobic scope in *P. texanus*. In *P. fayettei*, both responses were comparatively independent of body mass. Acid tolerance may have improved slightly with size in *P. texanus* but declined weakly in *P. fayettei*. The habitat models likewise separated the species to some degree: *P. fayettei* was significantly associated with warmer and more saline sites, whereas *P. texanus* showed only weak positive associations with temperature and conductivity. Similar broad-scale niches can therefore be occupied through somewhat different physiological and local habitat relationships (Burgett et al., 2026).

Environmental stressors in natural waterways are unlikely to occur independently. Flow reduction can produce hypoxia or anoxia, while storm events, drying, and rewetting can alter dissolved oxygen and other aspects of water quality. Temperature, discharge, runoff, and changing hydrologic connectivity can therefore expose organisms to multiple simultaneous changes rather than to the isolated temperature, oxygen, or pH gradients used in laboratory experiments (Diamond et al., 2023). This is particularly important for *P. nueces*, because the body size that performs best under one stressor may not perform best under another. Small individuals may be favored when temperature rises or pH declines, whereas larger individuals may regulate oxygen uptake more effectively as dissolved oxygen falls.

The relationship between the logistic-regression and physiological findings is therefore best described as partial correspondence. The temperature results for *P. nueces* and *P. acutus* agree directionally between the laboratory and field, but *P. fayettei* demonstrates that occurrence in warmer sites does not require the highest acute thermal limit. The significant turbidity and salinity associations cannot be matched directly to the physiology because neither variable was experimentally manipulated. The pH experiments revealed strong size-dependent differences, but pH was not an important predictor in the occurrence models. Finally, the absence of dissolved oxygen from the reported logistic-regression candidate set prevents a direct field test of the Pcrit and hypoxia-LOE findings. The two approaches are complementary rather than redundant: the logistic regressions

identify where the species occur, while the laboratory assays help identify physiological mechanisms and trade-offs that could contribute to those patterns.

The interpretation of the habitat models should also account for the sampling design. The species were collected in geographically separated portions of Texas, and most *P. acutus* were obtained on or near the UT Tyler campus. Consequently, some apparent habitat associations may reflect regional differences among sampling areas as well as causal responses by the crayfish. The limited number of accessible sites, concentration of sampling at public access points, and separation of species' ranges also constrain the ability of logistic regression to distinguish local habitat selection from larger geographic gradients. The models should therefore be treated as strong baseline evidence identifying candidate environmental relationships rather than definitive estimates of the full habitat niche of each species.

Overall, the four species exhibited distinct physiological and ecological strategies rather than a simple division between tolerant widespread species and narrowly tolerant endemics. *Procambarus nueces* was associated with warmer and more turbid habitats and exhibited slightly greater high-temperature tolerance, particularly at smaller body sizes. Increasing size improved its Pcrit but reduced its aerobic scope slightly and caused a pronounced loss of thermal and acid tolerance. *Procambarus acutus* was associated with cooler, clearer, and less saline waters within the sampled region and incurred increasing physiological costs with body size across several assays. *Procambarus fayettei* was strongly associated with warmer and more saline sites but exhibited comparatively little body-size dependence in oxygen and pH responses. *Procambarus texanus* showed weak positive associations with temperature and conductivity and a distinctive increase in aerobic scope among larger individuals.

The major take-home message is that relatively small size may be a key component of *P. nueces* specialization to its unusual western environment. Small individuals retain the species' thermal advantage and avoid the particularly severe decline in acid tolerance that accompanies growth. Although larger individuals gain improved aerobic oxygen regulation, that benefit may not offset the thermal, acid-related, and metabolic costs of becoming large in hot and hydrologically variable habitats. The results therefore generate a clear hypothesis: natural selection may favor a smaller adult phenotype in *P. nueces* because small body size provides the most advantageous overall balance among the interacting environmental stresses characteristic of its range.

These findings have direct conservation implications. Physiological vulnerability depends on both species identity and population size structure. Environmental assessments based solely on species-wide average tolerances could overlook vulnerable size classes or fail to recognize that the effects of growth differ among taxa. Future work should combine continuous field measurements of temperature, dissolved oxygen, pH, conductivity, turbidity, and flow with repeated measurements of crayfish body-size distributions. Such data would provide a stronger test of whether the small-body strategy proposed for *P. nueces* operates under natural conditions and would help identify the environmental changes most likely to threaten each endemic species.

Task 3: Data distribution.

3A. Agency will share georeferenced species assemblage data with Texas Parks and Wildlife as part of annual sampling permit reporting.
3B. Agency will submit data to the Texas Natural Diversity Database annually.
3C. Agency will combine historical and project data and post through a publicly available online repository such as Dryad or FigShare.

Archived data

The data and R scripts supporting the analyses presented in this report have been deposited in the Dryad Digital Repository. The archived materials include field-survey and habitat data, distribution records, physiological-tolerance measurements, analysis scripts, supporting documentation, and metadata. It is permanently available at:

https://datadryad.org/dataset/doi:10.5061/dryad.q2bvq841t

Challenges and changes

We did not have any significant challenges impacting the progress of this project. We had to push animal collections back a few months in Fall 2024 due to the dry weather but ultimately were successful in collecting enough of our target species.

Ryan Shartau transitioned to a co-PI role effective August 1, 2025 as he moved from UT Tyler to Washington State University (Pullman Washington) and Dr. Joshua Banta joined the project as the UT Tyler PI. Banta has extensive experience with crayfish and provided key project oversight and helped Ryan manage the project. Ryan oversaw the completion of this project and the experiments. These personnel changes had no impact on the progression of this project.

Summary of task status

The progress made towards this project's completion is summarized in the below table (Table 4) that are updated from the Research Plan submitted in the Summer of 2024.

Table 4: Tasks completed

COMPLETED			
Obj.	**Task**	**Date**	**Notes**
All	Recruited and hired a graduate student	Year 1 (Jun. 1 – Aug. 31)	Start date was August 19th, 2024
2	Permits and Permissions	Year 1 (Jun. 1 – Aug. 31)	Scientific collection permit from TPWD
2	Purchased field collection supplies	Year 1 (Jun. 1 – Aug. 31)	This includes waders, traps, nets
2	Purchased and set up crayfish holding tanks	Year 1 (Jun. 1 – Aug. 31)	Tanks set up (2 per species + 1 extra for sorting)
1	Literature review	Year 1 (Jun 1 – Nov 30)	Review of Texas crayfish literature with focus on target species
2	Laboratory set-up	Years 1 & 2 (Jun 1, 2024 – Jul 31, 2025)	Preparing for tolerance experiments, testing equipment, and ordering necessary supplies
2	Crayfish collections	Years 1 & 2 (Jun 1, 2024 – Apr 11, 2025)	Obtained individuals from each of the target species (*Procambarus texanus*, *P. nueces*, *P. fayettei*, and *P. acutus*)
2	Thermal tolerance	Year 2 (Feb 1, 2025 – Aug 31, 2025)	Completed in August 2025
2	Hypoxia tolerance	Year 2 Mar 1, 2025 – Nov 30, 2025	Both Tolerance assays were completed in the summer and spring of 2025
2	pH tolerance	Year 2 Jul 1, 2025 – Nov 30, 2025	pH trials were completed fall of 2025
2	Physiology data analysis	Year 2 Mar 1, 2025 – Mar 1, 2026	Data analysis occured as data was collected and was thoroughly analyzed across physiological factors in Winter 2026
3	Habitat association	Year 2 Aug 1, 2025 – Jan 31, 2026	Physiological and ecological data collected were used to determine species-habitat associations in Winter-Spring 2026.
All	Manuscript and final report	Year 2 Jan 1 2026- Jul 31, 2026	Completion of the final report, along with the manuscript, took place in Summer 2026.

Literature cited

Agresti, A. (2015). *Foundations of linear and generalized linear models*. Wiley.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control, 19*, 716–723.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*(1), 1–48.

Burgett, S. A. (2024). *Ecological niche modeling of endemic Texas crayfish species under current conditions* (Biology Theses, Paper 79).

Burgett, S. A., Schubert, N., Dickson, J., & Banta, J. A. (2026). Competitive exclusion theory predicts the ecological niches of three congeneric endemic crayfish species. *Hydrobiologia, 853*, 1187–1202.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*. Springer.

Croft, E. J. (2026). *Assessing the conservation needs of native Texas crayfish species through distribution analysis and species distribution modeling* (Master's thesis, The University of Texas at Tyler). Scholar Works at UT Tyler.

Diamond, J. S., Moatar, F., Recoura-Massaquant, R., Chaumot, A., Zarnetske, J., Valette, L., & Pinay, G. (2023). Hypoxia is common in temperate headwaters and driven by hydrological extremes. *Ecological Indicators, 147*, 109987.

Ellis, B. A., & Morris, S. (1995). Effects of extreme pH on the physiology of the Australian "yabby" *Cherax destructor*: Acute and chronic changes in haemolymph oxygen levels, oxygen consumption and metabolite levels. *Journal of Experimental Biology, 198*, 409–418.

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage.

Hobbs, H. H., Jr. (1984). On the distribution of the crayfish genus *Procambarus* (Decapoda: Cambaridae). *Journal of Crustacean Biology, 4*(1), 12–24.

Hobbs, H. H., Jr. (1989). *An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae).* Smithsonian Contributions to Zoology No. 480. Smithsonian Institution Press.

Hosmer, D. W., Lemeshow, S., & Sturdivant, R. X. (2013). *Applied logistic regression* (3rd ed.). Wiley.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1596), 1665–1679.

Hurvich, C. M., & Tsai, C.-L. (1989). Regression and time series model selection in small samples. *Biometrika, 76*(2), 297–307.

Johnson, D. P. (2018). The subgenus *Ortmannicus* (Decapoda: Cambaridae) in Texas, with descriptions of new species. *Zootaxa, 4468*(1), 1–63.

Kearney, M., & Porter, W. P. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters, 12*, 334–350.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software, 82*(13), 1–26.

Lenth, R. V. (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means*.

Logarbo, J. R., & Bonvillain, C. P. (2020). Thermal stress responses of two sympatric crayfishes in Louisiana, *Procambarus clarkii* Girard, 1852 and *Procambarus zonangulus* Hobbs & Hobbs, 1990 (Decapoda: Astacoidea: Cambaridae). *Journal of Crustacean Biology, 40*(6), 734–738.

Long, S. A., Mango-Mutiti, C., Mutiti, S., & Weese, D. A. (2023). Investigating the effects of increasing water salinity on an endemic crayfish. *Journal of Coastal Research, 39*(5).

Mandic, M., Todgham, A. E., & Richards, J. G. (2009). Mechanisms and evolution of hypoxia tolerance in fish. *Proceedings of the Royal Society B: Biological Sciences, 276*, 735–744.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften, 88*, 137–146.

R Core Team. (2025). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rogers, N. J., Urbina, M. A., Reardon, E. E., McKenzie, D. J., & Wilson, R. W. (2016). A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit). *Conservation Physiology, 4*, cow012.

Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry systems for aquatic organisms. *Journal of Fish Biology, 88*, 26–50.

Todgham, A. E., & Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: Relevance in a changing world. *Integrative and Comparative Biology, 53*(4), 539–544.

Westhoff, J. T., & Rosenberger, A. E. (2016). A global review of freshwater crayfish temperature tolerance, preference, and optimal growth. *Reviews in Fish Biology and Fisheries, 26*(3), 329–349.

Wheatly, M. G., De Souza, S. C. R., & Hart, M. K. (1996). Related changes in hemolymph acid-base status, electrolytes, and ecdysone in intermolt crayfish (*Procambarus clarkii*) during extracellular acidosis induced by exposure to air, hyperoxia, or acid. *Journal of Crustacean Biology, 16*(2), 267–277.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* (2nd ed.). Springer.

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2023). *dplyr: A grammar of data manipulation*.

Wood, C. M., & Rogano, M. S. (1986). Physiological responses to acid stress in crayfish (*Orconectes*): Haemolymph ions, acid–base status, and exchanges with the environment. *Canadian Journal of Fisheries and Aquatic Sciences, 43*, 1017–1026.