1. **Student Researcher**: Brittany Pulcini
   **Faculty Advisor**: Zachary Mitchell

2. **Project title**: Influence of Seasonality on the Relative Importance of Abiotic and Biotic Factors in Determining Fish Survival in Isolated Pools in New Mexico

3. **Description of research problem and research objectives**.

New Mexico and many other regions have been experiencing extreme drought conditions. These droughts can cause ecological, economic, and social impacts and negatively affect river ecosystems. The negative impacts of drought on river ecosystems are a growing concern because of the increasing drought frequency and intensity projected to occur in the coming decades due to global climate change (Cayan et al., 2010). Drought decreases water quality due to the loss of connectivity between aquatic habitats causing isolated pools. Aquatic organisms need to find refuge to survive these drying disturbances. Ideally, aquatic organisms would be able to relocate to perennial (i.e., flowing) river segments at the onset of stream drying. Organisms that become trapped in these isolated pools are subjected to an increase in abiotic stressors such as increased water temperature and decreases in dissolved oxygen. They can also be subjected to increased biotic factors such as predation due to the vulnerability these isolated pools cause (Magoulick, 2004). These stressors can cause an increase in mortality rates and substantial shifts in community changes (Dekar and Magoulick, 2013) which could lead to a loss in important ecosystem functions provided by these organisms (Dubose et al., 2019). The spring and fall trials could expect to see higher biotic mortality in comparison to summer and winter where abiotic stressors are the cause of mortality (Figure 1). The hypothesis in this research is based on previous studies such as Mundaha, 1990. Less research has examined the influence of seasonality on the relative importance of abiotic or biotic factors in structuring fish communities in isolated pools. Thus, it is important to better understand how seasonal stream drying will impact riverine biota to develop better-informed management and conservation decisions.

The goal of this study is to gain a better understanding of the relative importance of various abiotic (i.e., environmental filtering) and biotic (i.e., predation) factors in determining fish survival in isolated pools during periods of reduced stream flows. Additionally, this study will examine the influence of seasonality on the relative importance of abiotic and biotic control of fish communities in isolated pools. A seasonal mesocosm experiment will quantify the relationship between structuring forces (abiotic and biotic) and survival rates of a common species (Red shiner, *Cyprinella lutrensis*) found in the Pecos River, New Mexico.

While abiotic factors are hypothesized to be the dominant force affecting fish survival in isolated pools during the temperature extremes of summer and winter, biotic factors, particularly predation, are expected to intensify in the transitional seasons of spring and fall (Fig 1). This may be due to increased predators accruing energy reserves in preparation for winter or to replenish after the season's hardship. Accordingly, the prediction is that predation will play a more significant role in shaping fish survival rates in spring and fall, not only due to the reduced abiotic stress but also because of a potential spike in predator activity. Predators may be more active and have higher feeding rates during these seasons, leading to increased biotic interactions and potentially higher predation-related mortality in fish populations. This seasonal dynamic in predator-prey interactions could significantly influence the relative importance of biotic control

on fish communities in isolated pools. Ultimately, this study will yield a better understanding of how abiotic and biotic controls might influence riverine communities differently across seasons.

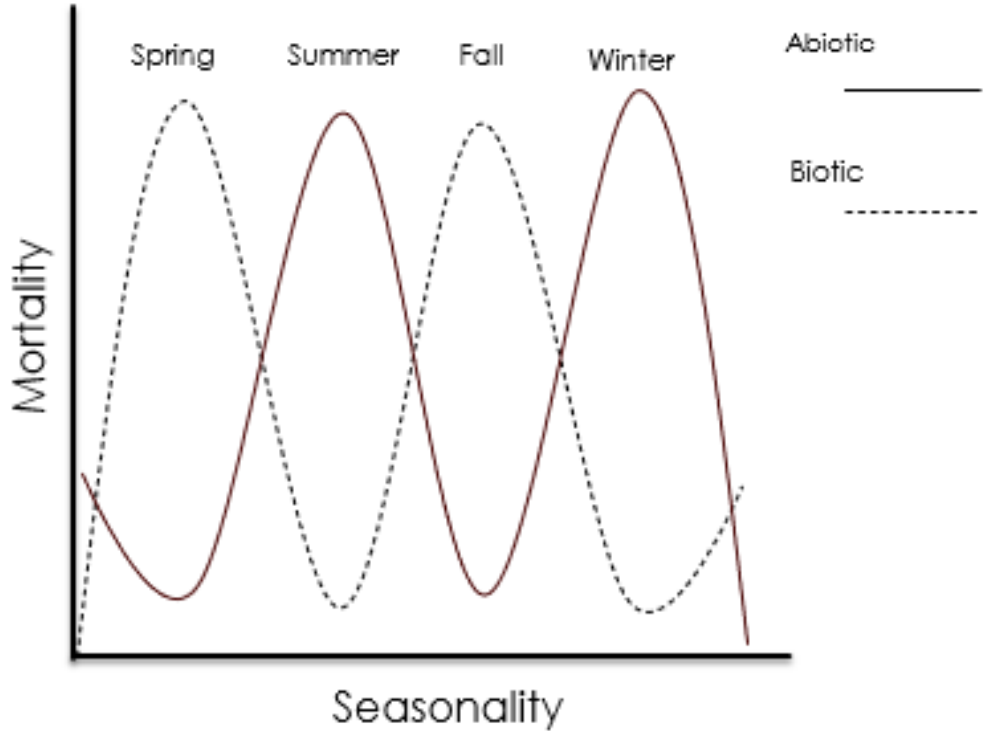


**Figure 1. Predicted seasonal variation in the relative importance of abiotic (solid line) and biotic (dotted line) factors on fish mortality in isolated pools.**

4. **Description of methodology employed.**

The mesocosm experiment took place in the Pecos River (-34.40305, -104.19864) near Fort Sumner, NM to assess the seasonal importance of environmental and biotic control on fish communities in isolated pools. The Pecos River provides several benefits (e.g., irrigation, recreation) for both New Mexico and Texas but has long suffered natural and anthropogenic river flow alterations and can experience substantial flow decrease and even stream drying, which provides a useful model system to address our research question.

The experiment consists of 10 small pools (91 cm in diameter) and 10 large pools (115 cm in diameter) representing different sizes of isolated pools that could be found during the year in this stream reach. The pools were buried on a sand bar adjacent to the main river channel. Pools were filled with about 25 cm and 3-5 rocks from the river to provide cover and nutrients for the fish. Furthermore, to explicitly assess the impact of predation on fish survival, half of the pools – specifically, five small and five large ones – were equipped with wire mesh covers to impede predator ingress ( see Figures 2 and 3). This strategic setup allows for a comparative analysis of the relative importance of predation versus environmental filtering in governing the survival rates of fish in isolated pools. Ten Red Shiners (*Cyprinella lutrensis*) were placed into each pool at the onset of the trials. Temperature was continuously measured in half of the pools using ONSET HOBO temperature loggers while dissolved oxygen was measured twice a day

using a YSI, once in the early morning (0800-0900) and once in the afternoon (1300-1500). Game cameras were set up to document predation events (Figures 2 and 3). These experiments were conducted on a seasonal basis (i.e., spring, summer, and fall) starting in the spring of 2023. Each trial ran for five days. In the next phase, a winter trial is scheduled for February 2024, followed by a repeated spring trial in the same year. The repeat of the spring trial is necessitated by the fact that the initial attempt was prematurely terminated after two days due to site flooding caused by a significant rainfall event.

A three-way Analysis of Variance (ANOVA) was employed to assess the main effects and interactions of season, pool size, and predation control on fish survival rates. This approach allowed for the examination of not only the individual impacts of each factor but also how these factors interacted with each other in different combinations. Subsequent to the ANOVA, Tukey's Honestly Significant Difference (HSD) post hoc test was conducted to explore the nuances of significant interactions. The analysis was conducted using R studio.

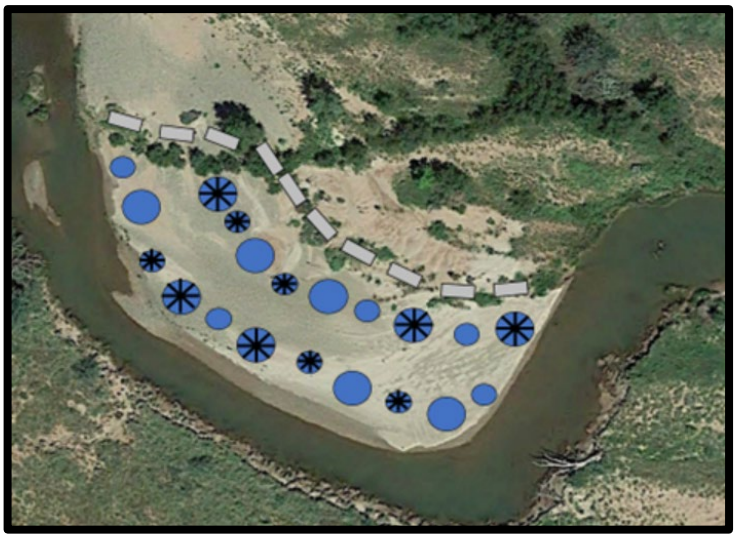

**Figure 2. Aerial view of the experimental setup for the study of Red shiner fish mortality in isolated pools. The rectangles represent strategically placed game cameras for monitoring, while the circles indicate the distribution of the pools varying in size – larger circles for larger pools and smaller circles for small pools. Pools with a black asterisk- like markings are equipped with wire mesh covers as a means of predation control, designed to prevent predator ingress.**

**Figure 3. This composite image showcases two critical aspects of the Red Shiner mortality study. On the left, the setup is displayed with multiple pools of varying sizes bred within a sandy bar, simulating isolated aquatic environments. On the right, a close-up view of one such pool is provided, featuring the predator control wire mesh cover in place., which is utilized to assess the impact of predation on fish survival rates within the experimental framework.**

## 5. Description of results; include findings, conclusions, and recommendations for further research.

Total survival was 99.5%, 21% and 60% for the spring (only two days of data), summer and fall trials, respectively (Fig. 4). The analysis of the survival rates of Red shiner across different seasons, pool sizes, and predation control measures revealed significant variations, as indicated by a three-way ANOVA. The model showed that the season significantly affected fish survival ($F_{(2, 46)} = 44.569$, $p < 0.001$), while the pool size did not exhibit a significant influence ($F_{(1, 46)} = 0.905$, $p = 0.3465$; Table 1). The presence of predation control measures was also found to be significant ($F_{(2, 46)} = 3.813$, $p = 0.0294$). Most notably, the interaction between season and predation control significantly impacted fish survival ($F_{(4, 46)} = 22.998$, $p < 0.001$), suggesting that the effect of predation control varies across seasons (Table 1).

Further investigation through Tukey HSD post hoc tests for the significant interaction between season and predation control elucidated within-season differences. In spring, the survival rates between pools with and without predator control did not significantly differ ($p > 0.05$), indicating that predation control did not substantially affect fish survival during this season. Similarly, in summer, the presence of predator control measures did not lead to a significant difference in survival rates ($p > 0.05$). This lack of significant difference in summer suggests that factors other than predation (i.e., environmental filtering) play a more dominant role in influencing fish survival, as predicted.

Contrastingly, the fall season exhibited a different pattern. There was a significant difference in survival rates between pools with and without predator control ($p < 0.05$). This result highlights the increased importance of predation control in the fall, suggesting that predatory pressures may be more pronounced during this season, thereby affecting fish survival rates more substantially. The main predators spotted during the fall trials were herons and raccoons, with raccoons accounting for the majority of fish deaths. The biological significance of these findings lies in understanding the seasonal dynamics of predator-prey interactions in

riverine ecosystems. The increased effectiveness of predation control in the fall could be attributed to a combination of factors, such as changes in predator behavior, increased vulnerability of fish in smaller pools, or other ecological dynamics unique to this season.

The comprehensive study on the survival rates of Red shiner across different seasons, pool sizes, and predation control measures has elucidated key aspects of riverine ecosystem dynamics. The significant seasonal variation in survival rates, highlighted by the ANOVA results, underscores the critical role of temporal factors in such environments. Notably, the spring trial, despite being constrained to a two-day data collection period, indicated a potentially lower impact of stressors during this season, as reflected in the high survival rate. Conversely, the reduced survival rates observed in summer and fall trials point to heightened susceptibility to environmental and predatory pressures. The differential impact of predation control across seasons, particularly its pronounced significance in fall, suggests a shift in the predominant factors affecting fish survival, with predation emerging as a critical element during certain times of the year.

The study's findings emphasize the necessity of considering the seasonality in aquatic habitats, especially when developing conservation and management strategies. To build on these insights, further research should include a full-duration spring trial to confirm the preliminary observations and a more detailed exploration of environmental variables that may influence survival rates, especially in summer. Investigating predator and prey behaviors across seasons could shed light on the observed seasonal variance in predation control effectiveness. Additionally, conducting analogous studies in diverse riverine ecosystems could enhance the generalizability of these findings, while also considering the potential impacts of climate change on these delicate ecological dynamics. Such research endeavors would contribute significantly to our understanding of riverine ecosystems, informing more effective and seasonally tailored conservation approaches.

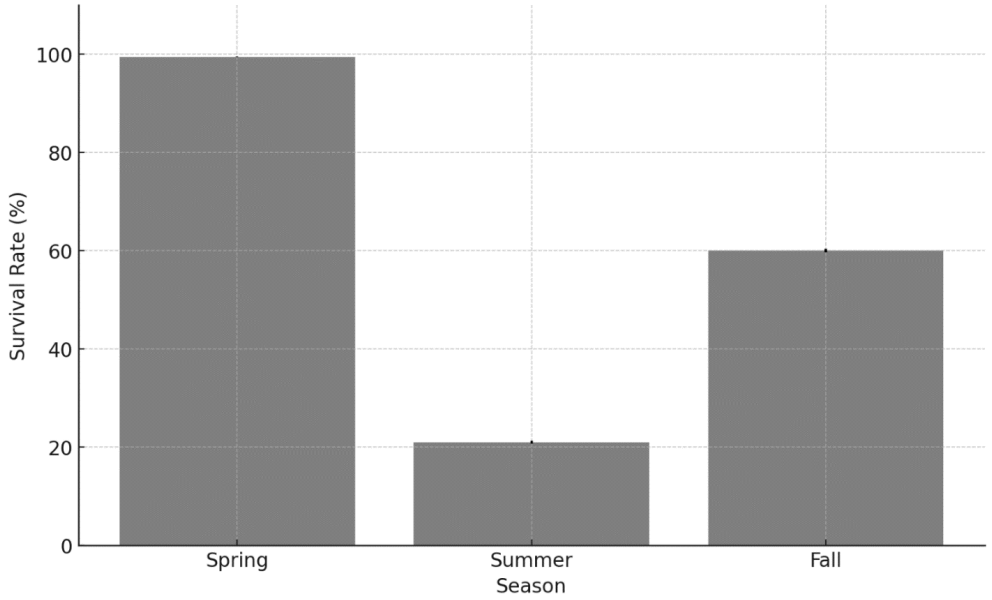


**Figure 4. Survival rates of red shiner fish across three seasons: Spring, Summer, and Fall.**

| Source | df | SS | MS | F-Value | p-Value |
|---|---|---|---|---|---|
| Season | 2 | 600.1 | 300.05 | 44.569 | < 0.001 *** |
| **Pool Size** | 1 | 6.1 | 6.09 | 0.905 | 0.3465 |
| **Predation Control** | 2 | 51.3 | 25.67 | 3.813 | 0.0294 * |
| **Season × Pool Size** | 2 | 18.3 | 9.16 | 1.361 | 0.2665 |
| **Season × Predation Control** | 2 | 309.7 | 154.83 | 22.998 | < 0.001 *** |
| **Pool Size × Predation Control** | 1 | 1.0 | 0.97 | 0.145 | 0.7054 |
| **Season × Pool Size × Predation Control** | 2 | 2.7 | 1.34 | 0.199 | 0.8201 |
| **Residuals** | 46 | 309.7 | 6.73 | | |

**Table 1. Analysis of Variance (ANOVA) Results for the Survival Rates of Red Shiner Fish. The table presents the degrees of freedom (df), sum of squares (SS), mean square (MS), F-values, and p-values for each factor and their interactions. Factors include Season, Pool Size, Predation Control, and their respective interactions. Significant p-values are indicated, highlighting the impact of these factors on fish survival rates in isolated pools.**

**6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.**

This study aimed to advance our ecological understanding of how species respond to heightened biotic and abiotic stressors during drought events. The findings of this research have significant implications for water managers, biologists, and conservationists not only in New Mexico but also in other arid and semi-arid regions facing similar challenges. A deeper ecological insight into fish responses in isolated pools during periods of reduced stream flows is invaluable for informing effective water management strategies. Furthermore, this knowledge is essential for enhancing conservation efforts aimed at protecting native and endangered fish populations during periods of low water flow. The outcomes of this study contribute to the broader goal of establishing informed environmental flow standards and sustainable water resource management practices in New Mexico and similar ecosystems.

**7. Describe how you have spent your grant funds. Also, provide your budget balance and how you will use any remaining funds.**

The grant funds were strategically allocated to support our research project. They were used to provide summer salary and fringe benefits for one graduate student who played a key role in fieldwork and data collection. Additionally, funds covered travel expenses for regular field site visits and the purchase of essential research supplies and equipment, including pools, game cameras, and various tools.

| Title | Amount Awarded | Available Balance |
|---|---|---|
| Student Salary | $2,700.00 | $6.00 |

| | | |
|---|---|---|
| Fringe Benefits | $270.00 | $70.48 |
| Travel | $985.00 | $10.00 |
| Supplies | $3,500.00 | $40.00 |

**8. List presentations you have made related to the project.**

To date, I have presented two posters at a university-level conference and state-level conference.
Pulcini B., Mitchell Z.A Influence of Seasonality on the Relative Importance of Abiotic and Biotic Factors in Determining *Cyprinella lutrensis* Survival in Isolated Pools in NM Rivers. 68th Annual New Mexico Water Conference. October 2023. **State** (Fig.7)

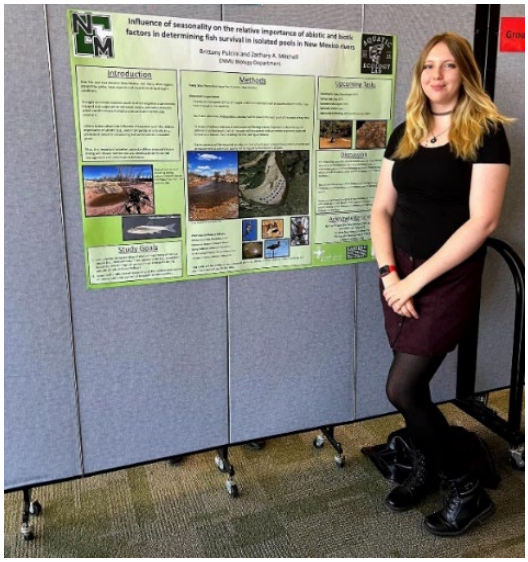


**Figure 7. Brittany Pulcini presenting her research at the Student Research and Creativity Conference at ENMU in April 2023.**

Pulcini B., Mitchell Z.A Influence of Seasonality on the Relative Importance of Abiotic and Biotic Factors in Determining *Cyprinella lutrensis* Survival in Isolated Pools in NM Rivers. ENMU Student Research and Creativity Conference. April 2023. **University**

**9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-FALL2022.**

No publications have been generated thus far but I plan to finish additional analyses and submit a manuscript for peer-review in April of 2024.

**10. List any other students or faculty members who have assisted you with your project.**

Jodie Montgomery and Li Zheng who are undergraduates at Eastern New Mexico University.

**11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.**

No special recognitions or notable achievements currently.

**12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.**

I'm currently on track to graduate with my master's in biology from Eastern New Mexico University in the Spring of 2024 and hope to apply my knowledge to the conservation of species that are or starting to see a population decline.

**13. Literature Cited:**


Cayan, D.R., Das, T., Pierce, D.W., Barnett, T.P., Tyree, M. and Gershunov, A., 2010. Future dryness in the southwest US and the hydrology of the early 21st century drought. *Proceedings of the National Academy of Sciences*, *107*(50), pp.21271-21276.

Dekar, M.P. and Magoulick, D.D., 2013. Effects of predators on fish and crayfish survival in intermittent streams. *Southeastern Naturalist*, *12*(1), pp.197-208.

Magoulick, D.D., 2004. Effects of predation risk on habitat selection by water column fish, benthic fish and crayfish in stream pools. *Hydrobiologia*, *527*, pp.209-221.

Mundahl, N.D., 1990. Heat death of fish in shrinking stream pools. *American Midland Naturalist*, pp.40-46.