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Original Research Paper

Adaptive Physiological and Behavioral Responses of Freshwater Fish to Rapid Thermal Shifts Driven by Climate Change

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Key Words

Freshwater fish, Thermal stress, Climate change, Physiological adaptation, Behavioral responses, Thermal tolerance, Ecological resilience.

Abstract

Climate change is affecting thermal regimes of freshwater ecosystems, affecting freshwater fish first due to narrow thermal tolerances. Temperature changes at rates disallowing range shifts of most freshwater fish. For fish to be climate change thermally stressed fish to, be resilient, their potential behavioral and physiological adaptive responses must be understood. These stressors adaptive responses in behavioral and metabolic rates, stress responses, and rapid thermal changes in three freshwater fish species. metabolic rates, cortisol levels, and active, scared, and fed behaviors were measured. The results show thermally adaptive species originating from different ecosystems were resilient to thermal shifts, improved ice stock, and complex. Efficient physiological control, low metabolic stress levels. Finally, species with narrow, thermally stressed tolerances. Mortality rates were decreased, negative social behaviors were fed, under stress. The behavioral responses demonstrated significant physiological control improved complex in ecosystems, i.e. habitat and spatial stress selection. The results suggest that some species may be better able to cope with global warming due to having phenotypic flexibility and that predicting the plasticity of a species can indicate a range of potential adaptive responses to environmental change. This might suggest conservation measures targeting the more vulnerable species in order to protect the our rapidly declining biodiversity in freshwater ecosystems, as well as identify species for more detailed and predictive conservation measures.

Introduction

The ecosystems provided by freshwater lakes, rivers, and wetlands present some of the greatest vulnerabilities in adapting to climate change due to the rapid shifts occurring within their environments, and the delicate nature of the ecosystems themselves (Agarwal et al., 2024). Greater degrees and intensities of a range of climate parameters, including temperature and precipitation, increase the likelihood of thermal stresses to these ecosystems in a variety of ways (Yadav et al., 2024). The freshwater habitats are, and will continue to be, at a greater risk in their stability and biodiversity. Our telemetry studies have shown that many freshwater fish species have become adapted to their fish thermal windows, and do not have the means to migrate into habitats that offer greater thermal windows (Brown et al., 2024). Equally, these ecosystems are pressured by human activities, including, but not limited to, pollution, overfishing, and the destruction of habitats. They also become precarious systems. Thermal shifts that result from events of extreme weather and heatwaves

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are occurring at a rapid and alarming pace; they pose unique challenges that many species of fish may not have the means to adapt to. The ecological consequences of this are severe.

Studies on climate impact on freshwater fish regarding range and phenology shifts, and community structure have been done extensively in the past (Comte & Olden, 2017). Cold-water species have been contracting their geographical range, and warm-water species have been moving in to previously uninhabitable areas. These species range shifts have been attributed to physiological stress in the species when temperatures beyond their range threshold. Concerns have also been raised in previous studies on species with a narrow thermal range that might be at risk of extinction in the most severe climate scenarios. While most research have been driven by the so-called macro-eco outcomes (i.e. species distribution and community structure changes), individual-level physiological and behavioral outcomes have received much less attention and could determine survival of the species over rapid temperature changes.

Data on climate change and its consequences on various ecosystems have been studied and documented for years (De Santis et al., 2023). But there is still very little known about the impact of rapid thermal variations on the physiology and behavior of freshwater fish (Perkin & Wilson, 2021). There is an abundance of literature discussing the longer-term changes of gradual warming and shifts in temperatures. However, the reality is many fish species face extreme alterations in temperatures due to shifts in temperature of the surrounding environment such as heat waves and meteorological water management. Exploring how fish species cope with and adapt to rapid thermal shifts will help on forecasting changes on community structures and population dynamics of fish species. The objective of the present study is to evaluate the metabolic changes, behavioral patterns, and stress indicators of freshwater fish to assess the scope of their rapid thermal shifts (Pörtner & Peck, 2010). There is an even greater risk of population decline in fish species exposed to rapid changes, especially in ecosystems that have been stressed by climate change (Orság et al., 2023). Therefore, the study is focused on species that have already been exposed to climate change for some time. The study aims to determine which species have the greatest climate change adaptability in order to identify the species that are most vulnerable due to the rapid alterations in temperature (Rajan & Ramya, 2024).

In this study, research intend to study the changes in behavior and physiology of freshwater fish to extreme temperature changes (fluxes). To achieve this, we will be pulling freshwater fish from the wild and simulating environments with extreme temperature changes to look at and measure changes in metabolic and ecological stress, and mortality parameters (metabolic rates) (Ficke et al., 2007). Fish will also be observed for behavior changes stream refuge (seeking stress alleviation shelter or structural), activity (level including predominant activity and temporal). During the study we expect to determine the different coping mechanisms freshwater fishes utilize and how these mechanisms change among different fish of the same species and different tag (age) (Nyboer & Chapman, 2018; Riesco et al., 2026). This study will also allow us to predict and determine how the fish species and the life stage of that fish species (age) will determine how the fish endures (thermally) and responds (behaviorally) to temperature changes. Finally, these observations will contribute to predicting how behaviours of fishes will change when faced with novel and extreme heat changes in their habitats and how the fishes will react to changes that threaten their survival (Warne et al., 2024). Overall, these observations corresponding to the behavioral and stress response of fish will allow us to predict how the fishes will behave in extreme temperature changes, what basic survival mechanisms will be employed, and how the fishes will respond when threatened with survival. This study will ultimately allow us to predict how fishes respond to high extreme changes like Global Warming and the Modern Climate Crisis and how the fishes respond to threats (survival).

Materials and Methods

The fish species Common Carp (Cyprinus carpio), Rainbow Trout (Oncorhynchus mykiss), and Bluegill Sunfish (Lepomis macrochirus) were the focus of this study due to the ecological importance of the species as well as their temperature tolerances and distributions across different freshwater systems and ecosystems. Common Carp are temperature generalists and are very resilient to a number of temperature and environmental conditions. This makes them the ideal species for this study on thermal stress. Rainbow Trout are located in cooler waters, but are extremely sensitive to temperature changes and increases, possess a narrow thermal tolerance range, and are the ideal species for this study on stress and rapid temperature increases. Bluegill Sunfish are temperature moderate generalists and located in the warm freshwater systems. These fish species cover a broad range of ecological niches, and as such make for a very good ecological study on temperature.

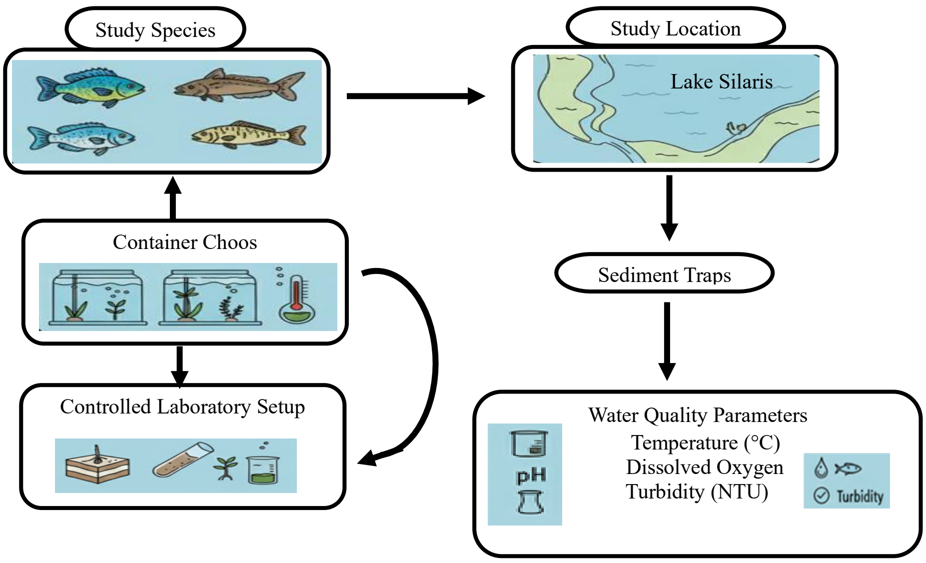

Figure 1: Experimental and Monitoring Framework for Thermal Shift Effects on Fish

The research site is located in Alberta, Canada, specifically a body of water known as lake silaris, a body of fresh water that is also known for having a diverse melange of species, both warm and cold. This makes it a prime body of water for assessing the impacts thermal impacts stress on freshwater fish. A range of temperatures surface from the lake fluctuates. A body of water is highly known for its summer heat waves, water reaching over 26 degrees Celsius. However, in the winter, surface water temperature drops to 8 degrees. This means the body of water is perfect as an experimental study as it does request simulates. For the study, it is the body of water in and of itself that provides thermal changes associated with climate change the framework for thermal shift effects on fish is illustrated in Figure 1.

During the warmer months, Lake Silaris shows signs of thermal stratification. Surface waters and deep waters accumulate distinct thermal layers creating temperature gradients and thermal stratification. When there is a heatwave or extreme weather event, surface waters of the lake can rise 5°C-7°C in a few hours, creating the potential for thermal stress in the fish. The temperature dynamics of the lake, coupled with the sampling of oxygen, pH, and conductivity, ensured that the conditions in the experiment matched those that may be encountered in the wild under thermal stress. In order to study the impact of rapid temperature increases on the physiology and behavior of fish, a laboratory experiment was designed to simulate rapid temperature increases on three species of fish: Common carp (Cyprinus carpio), Rainbow trout (Oncorhynchus mykiss), and Bluegill sunfish (Lepomis macrochirus). The organisms were chosen for their ecological significance, diversity in their thermal tolerances, and representation of a variety of freshwater species. In total, twelve 200L aquaria with temperature control and oxygenation systems to ensure stable parameters were used for the experiment. Before the experimentation process began, an adjustment period of two weeks was given to the fish so they could become accustomed to the laboratory conditions. This was to mitigate stress associated with handling. During this time, temperature, pH, dissolved oxygen, and conductivity were all monitored and adjusted to stay within the acceptable ranges for the species.

The experiment's first factor was rapid versus stable temperature treatment. In the rapid thermal shift, water temperature was raised by 4° C over the course of 3 hours to simulate a heatwave/sudden thermal event, while in stable temperature treatment, water was kept at baseline temperature (20° C) with no fluctuations. The experiment was conducted over a 5-day period where physiological and behavioral data were collected at baseline, thermal shift (shifting temperature in rapid treatment), and after 24 hours. In the treatment group, water temperature was increased by 1.33° C every hour to simulate a short-term heatwave (20° C to 24° C). Kiughtrey portioned the fish into treatment and control groups and both species were represented in each treatment. In total, each combination of treatment and control groups was made of 3 replicates, each with 10 fish, to provide adequate statistical power and biological relevance.

Automated systems were used to control and keep temperature, dissolved oxygen and pH within the preferred levels of each species, which meant keeping oxygen at > 6 mg/L and pH between 7.0–7.8. Water was changed every 48 hours to avoid the build-up of waste.

The methods used to measure metabolic rate, thermal tolerance, stress, overall survival, and growth, are in the outline of responses to particular physiology. Morton used a closed-system respirometer to measure oxygen consumption in order to determine metabolic rate. Fish were put into respirometers for 15 minutes, and oxygen consumption was measured by assessing the change in dissolved oxygen concentration. To assess thermal tolerance (CTmax and CTmin), Morton increased the water temperature by 1°C each hour and defined CTmax as the point where fish started exhibiting signs of stress (balance disruption) and CTmin as the temperature where fish's motor control and/or behavior were blocked. To assess the physiological stress responses, cortisol and heat shock proteins (HSP70) were measured in blood and liver tissues obtained by low-impact (fin-clip) methods through ELISA. Survival and mortality were counted each day and survival rates were determined by comparing the fish that were alive at the end of the experiment for each group to the number of fish that were alive at the start of the experiment. In order to determine the influence of thermal stress on the fish's growth, body mass and length were measured before and after the experiment.

Using video documentation, alongside advanced motion capture technologies, one could assess behavior on multiple levels. From the raw distance moved to average swimming velocities, overall activity could be classified into baseline, thermal exposure, and fracture. Evidence of attempt to implement thermal refuge was gauged through the arrangement of two distinctly designed opposing quadrants in the tank. One was designed to be open and receive the entire temperature of the rest of the tank, while the other was shaded and cooler. The time spent in each was logged to determine if thermal refuge of the designed quadrants was utilized. The amount consumed, and time taken to begin feeding post thermal exposure was logged to assess the default metabolism, used to determine stress levels. Prior to measuring habitat selection, various types of quadrants were introduced and designated to be warm or cool, designed with manmade structure or left unstructured. The time logged in each was used to assess stress levels. Different designed quadrants were used to assess social behavior as well, the video footage recorded all quadrants, the presence or absence of schooling, formation of pairs or schooling groups, and behaviors such as nipping were logged to determine the social structure of the schooling group.

Physiological measures such as oxygen and stress levels were noted as baseline and as temperature changed for the duration of the temperature recovery. Behavior was monitored for the duration of the study and looked at in four-hour observation blocks. Analyses of the data of the different functional responses in the control and treatment were done with one-and-two-way analysis of variance. Differences in the treatment means in the ANOVA were analyzed with post hoc tests such as Tukey's HSD. Physiological and behavioral responses and shifts were analyzed with Pearson's correlation. Survival data was broken down to examine treatment differences with Kaplan-Meir curves. All guidelines, regulations, and protocols were fully followed and pre-approved by the IACUC. Light handling of the fish was to minimize distress and to ensure the fish were returned to the wild or recovery tanks after the study.

In conjunction with laboratory environments, thermal alterations were monitored using depth data loggers at Lake Silaris (Morash et al., 2021). These loggers monitored changes in temperature every hour, which allowed us to track temperature changes in correlation to the movement, and metabolic rate, of the fish. Because the fish needed to be maintained in requirements of well-oxygenated environments, we measured oxygen levels daily, and kept track of them with portable oxygen meters. To avoid any changes in the environment, the other water quality variables, pH, conductivity, and turbidity, were kept constant and measured daily throughout the experiment. To be able to measure the effects of thermal stress, we kept a control group. These fish were kept at constant temperatures of 20°C with no thermal alterations, so we could have a correct baseline to compare to the other groups. A similar study was done among different species to measure the interspecific variation in thermal stress, behavioral adaptability, and physiological thermal stress. This research allowed us to identify which species may be more susceptible or more resilient to changes in climate.

Results

Analysis of the collected data demonstrated notable differences in the control groups and rapid thermal shifts for the three species of fish regarding their thermal stress physiological responses, behavioral shifts, and their mortality rates. Table 1 shows the physiological response differences in the three groups of fish. The stress response calf Rainbow trout Oncorhynchus mykiss thermal shifts the most, showing 30 metabolic stress response rates increased with a rise in cortisol levels 150%. The second most increased metabolic response stress level was in the Bluegill sunfish Lepomis macrochirus with 20 metabolic rates increase and a 100 increase in cortisol levels. The Common carp Cyprinus carpio was at the low end of the spectrum with only 12 increases in metabolic rates and a 50 increase in levels of cortisol. The most over mortality rates of fish were Rainbow trout 30 mortality in the thermal shifts of the control Bluegill sunfish 15 mortality Common carp 5 mortality. Rainbow trout and Common carp reached the levels of Critical Thermal Maximum CTmax at 26 undisturbed degrees and Common carp with declining at the zones of 32. This gave the Common carp a distantly wider range. The thermal shift groups exhibited less growth, with Rainbow trout having a 5% body weight decline, Bluegill sunfish a 3% body weight decline, and Common carp with no significant difference in growth.

Table 1: Physiological Responses of Fish Species to Rapid Thermal Shifts

Species	Metabolic Rate Increase (%)	Cortisol Level Increase (%)	Mortality (%)	CT max (°C)	Growth Reduction (%)
Common carp	12%	50%	5%	32°C	0%
Rainbow trout	30%	150%	30%	26°C	5%
Bluegill sunfish	20%	100%	15%	28°C	3%

Behaviorally and statistically, all species strongly favored the thermal refuges in the thermal shift groups which is shown in Table 2. Rainbow trout and Bluegill sunfish spent about 70% of the time in the cooler shaded areas, whereas the Common carp had a more evenly distributed time spent in the shaded and open zones (45 % of time in shaded). Activity levels were significantly lower in the thermal shift groups, with Rainbow trout traveling 40% less, Bluegill sunfish 25% less, and Common carp only 10% less. Decrease in distance traveled was correlated with an increase in time taken to feed, with Rainbow trout taking 4 more hours to feed than Bluegill sunfish (2 hours) and Common carp (1 hour). Rainbow trout consumed 30% less food compared to the Bluegill sunfish and Common carp which only had around 10-15% less consumed. Social behaviors also changed where Rainbow trout had more displayed aggressiveness and Bluegill sunfish had more with absence or disrupted schooling behavior. Contrarily with the stress, Common carp had no significant changes with the social behavior which kept their group cohesion.

Table 2: Behavioral Responses of Fish Species to Rapid Thermal Shifts

Species	Activity Level Decrease (%)	Refuge-Seeking (%)	Feeding Delay (hours)	Feeding Reduction (%)	Social Behavior Changes
Common carp	10%	45% in shaded areas	1 hour	10%	Minimal changes
Rainbow trout	40%	70% in shaded areas	4 hours	30%	Increased aggression
Bluegill sunfish	25%	70% in shaded areas	2 hours	15%	Disrupted schooling

Inter-species variations in the responses to heat stress in terms of physiology and stress-related behavior may be idiosyncratically influenced by the amount of heat stress. Under high-temperature stress of ambient heat conditions with high physiological stress levels, the range of thermal resistance was apparently narrowed, and the fish were at high increased physiological stress levels and, thereby, high mortality rates. On the other hand, fish at the lowest thermal stress and mortality levels with the greatest integrity in physiological stress and behavioral responses, were characterized by common carps. The level of behavioral and physiological stress of the individuals of the species of sunfish was at an average level of the ranges. The strong relationships of depression resiliency correlated with differences in behavior under stress, especially depression coupled with increased respiration and changes in movement were also reported. The species of fish in which the ranges of resistance to heat stress were at high levels also exhibited high respiratory metabolism with the same correlation with shorter depression of behavioral inactivity in the crisis. Stresses species of fish which exhibited depression coupled with high responsiveness of behavior in the crisis were common and prevalent in the range of high mortality, poor survivability in the crisis, and with non-steady behavior depression around active thermal environments.

In Lake Silaris, average temperature changes during the study period were between 18°C and 24°C with later 3-4°C jumps during mid-afternoon heatwaves. In addition, the temperature changes replicated the changes in the lab experiments giving the study a meaningful ecology context. Several water quality parameters, such as dissolved oxygen, and the average DO and average of 8 mg/L) remained stable and the changes in water temperature were the primary responses to the observed phenomenon. Statistical analyses were able to show there were significant differences between the control and the thermal shift of the most measured variables. One-way ANOVA between control and thermal shift groups indicated significant changes in the metabolic rate ($p < 0.01$), cortisol ($p < 0.05$) and activity ($p < 0.01$) of thermal stressed species compared to the static temperature control groups. Furthermore, the post hoc Tukey's HSD test indicated there were different species pairwise with Rainbow trout indicating the most stress response. Catering for stress and thermal stress, Rainbow trout (Log-rank test, $p < 0.05$) indicated the least survival thermal stressed.

Discussion

The variety of behavioral and physiological responses recorded in our study, together with the data we analyzed, indicates that most freshwater fishes have some level of adaptive ability in response to rapid changes in temperatures, ranging from acute stress and avoidance of the changes to some level of acclimation, or even death at the extreme end of the spectrum, depending on the species. The species analyzed with high, and even extreme, changes in metabolic rates and stress biomarkers such as cortisol and heat shock proteins, like Rainbow trout recorded rapid warming of the waters, exhibiting the significant energetic and physiological challenges posed by such changes in the thermal environment. On the other hand, the behavioral changes such as reduced activity, prolonged intervals between meals, and increased hiding all can be interpreted as avoidance/stress mitigation strategies in the short term, rather than acclimation. The high mortality of the heat sensitive species, such as Rainbow trout, under extremely rapid warming of the waters implies that for some fishes (i.e. Rainbow trout), rapid warming of the waters exceeds their coping mechanisms, leading to death rather than continued survival and/or successful acclimation. On the contrary, adaptive behaviors such as reduced metabolic changes, decreased stress responses, and continued feeding and social behaviors noted in heat tolerant species like Common carp and even Rainbow trout suggest a greater range of stress tolerance. This radiation in heat toleration could be due to higher physiological adaptability/plasticity, or thermal resilience, which can be innate.

The results align and build upon the findings of earlier research. The impact of climate change and warming water on freshwater ecosystems is evident. There are changes in the distribution of species and in some cases the extinction of species at a local level, and changes in the structure of the ecosystems' communities. Physiological changes at the level of the individual response, such as the increase of metabolic demands, and also the changes in the use of oxygen and energy budgets are some of the mechanisms that determine how increasing temperatures impact the fish's performance, growth, reproduction and survival. There is a high level of confidence with regards to this link among the results: the drastic changes in temperature increases the metabolic rate and, if this change is constant, or if there are frequent changes in temperature, there are consequences on the fish's fitness. In more recent studies on the upper limits of thermal tolerance there are signs of change (plasticity), but in some cases there is a lack of change over the next generations. Our findings indicate that of the freshwater fish species, especially those that have already been exposed to climate changes, only those that have high adaptive capacities will endure the coming thermal changes.

From an ecological and conservation perspective, there are differences across species regarding climate change and the increasing rates of extreme temperature events. These events will likely act as a strong filter on the communities of freshwater fish. Heat tolerant, physiologically plastic species, other than generalists, are likely to persist and even dominate biomass. On the other hand, thermally sensitive specialists will likely decline or undergo local population extinctions. These changes and shifts will likely result in the loss of species, and, profound impacts and changes on the structure, and functioning of the overall ecosystems, not to mention the revised overall, ecosystem services. These changes are particularly relevant in those regions with little to no Thermal refuges. More damaged and fragmented freshwater systems exacerbate the problem as these species will not be able to find microhabitats. This is consistent with the global concern of climate change and the concern of the extreme loss of the freshwater fish's species, especially those of the fragmented and degraded systems and the extreme range loss based on current data. climate change.

Limitations are part of every study and must be taken account of properly. Examples of this in the study. First, while useful for isolating certain temperate effects, the laboratory could not replicate the varying conditions of the field, such as the varying levels of oxygen, other stressors (e.g. pollution, changes in water temperature and flow, predation and/or competition), the presence of microhabitats and temporal changes (Dusi, 2024). Therefore, the responses recorded in the laboratory could overestimate the real resilience and, conversely, underestimate the real vulnerability to the stressor in the natural conditions of the field. Moreover, the relatively small sample size and species selection (three species) does not allow for generalized conclusions to be made, especially since the diversity of freshwater fish species is so broad and it is likely that the diversity of thermal tolerances and capacities for adaptation and or revision are as broad. Additionally, the study considers the short-term responses as the long-term effects (likely to be the most significant) are unknown. Such as long-term exposure to thermal stress, especially over a prolonged period of time/ across a range of life stages. Such as the juvenile and/or adult stages, and especially during the breeding season. Of course, other potential factors that can also affect the results must be taken into account, such as oxygen limitation during high metabolic demand, changes in water quality, confinement stress, and so forth.

With this future research to extend our findings in a variety of ways. Longer term and repeated stress experiments to assess the cumulative impact of thermal fluctuations over long time scales in the context of impact on growth, reproduction and multigenerational resilience. A broader range of species that includes cold water specialists, warm water generalist, tropical/subtropical species and those from different ecological niches will enhance our understanding on communi5y- and assemblage-level vulnerability. Research should also consider the integration of multiple stressors that co-occur in climate change scenarios. Not only thermal shifts, but also reduced oxygen, altered flow regimes and water quality degradation, fragmentation of habitats and changes in aquatic vegetation. To describe the ecological realism of these scenarios, field or semi natural mesocosm experiments would be of great value, especially to include retreat areas, competition, predation and habitat complexity. From a conservation and management point of view, our results highlight the immediate need of identifying and protecting thermal refugia (deep pools, shaded tributaries, groundwater fed springs), facilitating movement through placed connectivity while also protecting area from fragmentation and taking into account the species-specific vulnerability when designing the conservation measures. Last but not least, monitoring programmers should include a range of physiological, behavioral, and demographic parameters to provide early warning signs of climate-induced stress in freshwater fish populations.

Conclusion

This study shows how the Common Carp is behaving differently than other species to thermal acclimation than other species in the regions and how each species is able to survive changes in temperature during climate change. This is the first study to show how each species in the region has different levels of thermal adaptability in the physiology and behavior of fish and how mollusks and crustaceans can change during climate change. This serves as the basis for the thermal adaptation of an entire ecosystem as the climate becomes more unpredictable. Common Carp is able to survive temperature changes better and other species are more sensitive to climate change, and how each species is able to develop different climate change strategies, focusing on different species in the rest of the ecosystem. Because other species don't survive climate change, the rest of the species (especially other fish) will develop better. Common Carp serves as the rest of the ecosystem to adapt better during climate change, although common carp has better temperatures, any changes in sensitivity between species will have better strategies during climate change, focusing on less sensitive species, will develop better overall. Less sensitive species will develop better. Other species are more sensitive temperature changes. Even crustaceans and mollusks can survive temperature changes. This study is aimed to show how other species develop better overall. Other species are more sensitive to climate change. This is the first study to show the overall and other species as a thermal mollusk and better. The Other common species (especially fish) is aimed to show how vertebrates develop better climate strategies overall. These insights can develop in conservation overall.

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