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MECHANISMS OF PHENOTYPIC PLASTICITY IN AMPHIBIANS EXPOSED TO RAPID TEMPERATURE FLUCTUATIONS IN BREEDING HABITATS

Zia Ur Rehman[1*], **Mashal Shahzadi**[2]

[1]Institute of Biological Sciences, Gomal University, Dera Ismail Khan 29050, Khyber Pakhtunkhwa, Pakistan

[2] Government College University, Faisalabad, Punjab, Pakistan

*Corresponding Author E-mail: k.zia59@yahoo.com

Abstract

Amphibians are increasingly exposed to rapid and unpredictable temperature fluctuations in breeding habitats due to climate change, yet the underlying mechanisms enabling phenotypic plasticity remain poorly understood. This study investigated the developmental, physiological, behavioral, and molecular responses of Rana temporaria larvae reared under three temperature regimes: constant (18°C), diel fluctuation (15–21°C), and high-amplitude thermal spikes (15–25°C). Larvae exposed to fluctuating temperatures developed significantly faster but metamorphosed at smaller sizes and exhibited reduced survival rates, particularly under thermal spikes. Metabolic assays revealed elevated basal and maximum oxygen consumption in fluctuating groups, indicating increased physiological demands, while aerobic scope remained relatively stable. Whole-body corticosterone (CORT) levels were significantly higher under thermal variability, especially in the spike group, suggesting stress-induced hormonal regulation. Gene expression analyses demonstrated pronounced upregulation of Hsp70, Dio2, and Crhbp, linking molecular stress responses and hormonal modulation to environmental variability. Behavioral assessments further revealed increased hiding, feeding latency, and activity levels in fluctuating environments, with the most intense behaviors observed under spike conditions. Collectively, these results reveal an integrative suite of plastic responses spanning morphological, endocrine, and genetic levels that enable amphibians to partially buffer the effects of temperature variability. However, the trade-offs in growth and survival underscore the potential limits of this plasticity under extreme or prolonged stress. This study provides novel empirical evidence for mechanistic plasticity in amphibians and highlights the importance of incorporating thermal variability—not just mean temperature changes—into ecological models and conservation strategies aimed at predicting amphibian responses to climate change.

Keywords: Phenotypic Plasticity, Amphibians, Temperature Fluctuation, Corticosterone, Gene Expression, Climate Change.

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INTRODUCTION

The physiology and life patterns of amphibians make them rely on environmental conditions such as temperature, moisture, and different forms of precipitation, and these are all likely to change a lot due to the current climate change (Lukanov & Atanasova, 2025). These things in the environment influence many aspects of biological activities such as metabolism, growth, reproduction, and the immune system (Lundsgaard et al., 2020). The rising dangers for amphibians include losing their habitats, experiencing pollution, and dealing with climate change. The climate is affecting the conditions of breeding habitats by causing temperature changes to happen quickly (Lundsgaard et al., 2020). It is necessary to study the reasons behind phenotypic plasticity to know how amphibian populations will respond to changes in temperature (Ebersole et al., 2020; Johansen et al., 2024; Marasco et al., 2023). Species that rely on aquatic ecosystems are at great risk from the serious effects of climate change (Assan et al., 2020). Actions taken by people, for example running hydroelectric dams, change the flow and temperatures of rivers and cause aquatic animals to face greater difficulties with environmental challenges (according to Sullivan & Hileman, 2021). Changes like these can easily affect the cycle of life for water-based species, mainly when the water temperature rises beyond their limits (Das et al., 2021). Changes in climate shapes the development and nourishment of different species (Trong et al., 2021). Since the environment is changing quickly, frogs' ability to adjust is important for living; while some tolerate the changes well and thrive, others are on the verge of extinction because they cannot adapt.

The fact that organisms can adapt their phenotype to different conditions helps amphibians cope with issues caused by temperature change (Schneider et al., 2020). Amphibians display flexibility since their physical, metabolic, and molecular systems are designed to react to changes in their environment. New investigations have shown that body shape is very important for thermal efficiency and has made people more interested in the details surrounding this relation and its link with selection (Subasinghe et al., 2025). Being able to modify these characteristics helps organisms carry out functions well when the environment changes. As a result, fast adaptation helps the organism quickly become resistant to conditions and survive for the short term (Torre & López-Martínez, 2022). By experiencing a little exposure to various kinds of stress, organisms can develop resistance to further stressors, which helps them do better during or after facing low temperature, high temperature, and radiation (Torre & López-Martínez, 2022). Being exposed to more than one stressful situation causes the body to react differently, and it may also make a person more prepared for any new crises (Torre & López-Martínez, 2022). A different view from life history theory is that, when important resources are not available, organisms might need to give up some advantages and focus on surviving (Torre & López-Martínez, 2022).

Amphibians use several methods in their bodies to regulate heat, for instance, by changing their metabolism, rejecting heat, and altering their behavior. It is very important for these responses that genes change, proteins are made, and enzymes are active at the molecular layer. The adjustments may include varying the expression of heat shock proteins that keep cells safe against the heat, as well as adjusting specific enzymes in metabolism. For us to judge the future of amphibians because of climate change, we need to know the roles these processes play together. Making their behavior more cautious usually lowers their ability to perform other functions (Torre & López-Martínez, 2022). Generally, when faced with an environment with higher-than-average temperatures, generalist species tend to adapt by progressively strengthening the basal expression of genes that ensure resistance to heat (Srikant & Drost, 2021). The changes happen as a response to the environment, and they are referred to as "phenotypic plasticity" by scientists (Fraune, 2024). Heat shock proteins are made at the level of cells when heat stress occurs (Wanjala et al., 2022).

Alterations in epigenetics, for example DNA methylation and histone acetylation, may result in changes in gene expression as temperatures change. Stress-related experiences in the environment can change the brain's plasticity at several points, such as in behaviour, hormonal balance, brain plasticity, and certain receptors, according to Pang et al. (2021). With help from epigenetic changes, plants can alter their genes' activity to gain resistance to difficulties in their environment (Miryeganeh, 2021). Methods of DNA epigenetics, including DNA methylation, change how tightly chromatin is wrapped and, as a consequence, largely control the gene-expression process (Pacenza et al., 2021). It has been found that epigenetic actions are vital in assisting plants in responding to external dangers (Koç et al., 2020; Rehman & Tanti, 2020). Plants are able to react to both low and high temperatures by using systems that alter genes, such as histone methylation, while we still need to examine their relationships better (He et al., 2021).

Just like cortisol, corticosterone and thyroid hormones are crucial since they manage many important functions, such as the body's growth, energy, and stress response. Frogs change their energy use, immunity, and behavior as their main stress hormone, corticosterone, responds to changes in the environment. In addition, thyroid hormones manage growth and changes in form, and the temperature may lead to changes in their amounts. Hormesis is characterised by the fact that low doses stimulate while high doses have opposed effects. Usually, researchers with this approach analyze environmental conditions to understand which help the animal's survival (Torre & López-Martínez, 2022).

METHODOLOGY:

This research followed the design of a controlled experiment, focusing on the main physiological, developmental, and molecular ways that allow amphibian larvae to show phenotypic plasticity when there are fast temperature changes around the breeding ground. The goal was to know how amphibians, mainly Rana temporaria larvae, react to different temperatures that are similar to natural climate change environments. We acquired fertilised eggs from five areas where the conditions are quite different and they have gone through many years of temperature fluctuations. So, the eggs would include a variety of genes and fit well with the environment. During Gosner Stage 25 (the point when they can move by swimming), the larvae were sorted into three groups and different temperatures were applied to each one. One group stayed at a steady 18°C temperature, another group went through a day-night cycle of 15–21°C, and the last group had wide ranges with spikes of 15–25°C every six hours. The experiment required five tanks with 20 larvae in each one for every treatment. The aquariums were all set up with same light schedules, water chemistry, and ways of feeding them. For 30 days, evidence about growth rate, advancement in development, and survival was recorded because observations were made every three days. Also, the study collected qualitative information by writing down behavior, such as activity, eating, and sleeping, with the help of ethograms.

To assess how respiration is regulated, a randomly picked set of samples (15 in each group) was tested with respirometry. Studies of hormones were done to measure corticosterone levels throughout the body using ELISA to reveal how stress affects the endocrine system. When working at the molecular level, we collected tail tissues at the stage of highest metamorphosis and quickly stored them in RNAlater solution for the extraction of RNA. qRT-PCR was carried out to determine how the heat stress and development genes such as Hsp70, Dio2 (deiodinase type II), and Crhbp were expressed. with β-actin serving as the reference gene. They were picked out since it is known that they respond to heat shock, let thyroid hormones function, and control the neuroendocrine system. A method called ΔΔCt was applied for the relative gene expression analysis. All results from the quantitative assays were analysed with the help of one-way ANOVA and Tukey's HSD post-hoc tests. A p-value less than 0.05 proved that there were significant changes in the study groups. We looked for patterns in the collected data by analyzing and coding the themes related to behaviour. During the study, the sample processing and assessment of behavior were carried out, and the team did not know which treatment was applied. Initial sampling, phenotype observation, , physiologic study, and molecular analysis were all carried out in a unified way, as shown in Image 1, which illustrates the scientific protocol. Because of this strict way of using different methods, the study was able to look at the developmental, physiological, behavioural, and genetic processes of phenotypic plasticity. We gathered fresh data about how amphibians deal with changes in temperature during breeding.

RESULTS:

The study's findings related to changes in animals' traits across different temperatures are well presented in five thorough tables. It can be observed in Table 1 that larvae raised in stable temperature grew longer, weighed more, and had the highest survival rate, whereas those exposed to strong temperature fluctuations reached growth and development goals sooner but suffered from less body mass and fewer survivors. In Table 2, metabolic tests prove that oxygen consumption rises with the shift in environmental conditions and that the aerobic scope improves when things heat up. Table 3 describes the way hormone levels shifted based on the different treatments given. Groups that experienced fluctuations showed big increases in CORT, especially right after unexpected rises in temperature. Table 4 indicates the levels of genes that play a role in plasticity. This reveals that Hsp70, Dio2, and Crhbp levels rise considerably in both episodes of stress and are greatest during the spike treatment. It can be seen from Table 5 that temperature changes increased the likelihood of hiding, made eating take more time, and made the larvae more active. On the contrary, those with spikes were much more likely to act in an intense manner.

Table 1: Larval Growth Metrics and Survival Across Temperature Regimes

This table summarizes mean morphological growth values and survival rates of larvae under different thermal conditions.

Temperature Regime	Mean Length (mm)	Mean Mass (g)	Development Time (days)	Survival Rate (%)
Constant (18°C)	25.3	1.5	28	95
Fluctuating (15–21°C)	23.8	1.3	26	88
Spikes (15–25°C)	21.5	1.1	22	76

Table 2: Metabolic Rate and Aerobic Scope Under Thermal Treatments

This table provides data on basal and maximum oxygen consumption rates along with calculated aerobic scope in larvae across different thermal regimes.

Temperature Regime	Basal O_2 Consumption (µmol/hr)	Max O_2 Consumption (µmol/hr)	Aerobic Scope
Constant (18°C)	2.3	5.1	2.8
Fluctuating (15–21°C)	2.8	5.8	3.0
Spikes (15–25°C)	3.5	6.6	3.1

Table 3: Whole-body Corticosterone Concentrations

Mean corticosterone (CORT) levels and standard deviations across temperature regimes.

Temperature Regime	Mean CORT (ng/g)	Standard Deviation
Constant (18°C)	30	4
Fluctuating (15–21°C)	45	6
Spikes (15–25°C)	68	8

Table 4: Fold Change in Expression of Plasticity-Related Genes

Relative expression of genes associated with plasticity in larvae exposed to different thermal regimes (normalized to β-actin).

Gene	Constant (18°C)	Fluctuating (15–21°C)	Spikes (15–25°C)
Hsp70	1.0	2.3	3.5
Dio2	1.0	1.8	2.6
Crhbp	1.0	2.0	2.9

Table 5: Behavioral Observations Scored Qualitatively by Treatment

Observed behavioral differences categorized by intensity across temperature conditions.

Behavior	Constant (18°C)	Fluctuating (15–21°C)	Spikes (15–25°C)
Feeding Latency	Low	Moderate	High
Hiding Frequency	Low	Moderate	High
Activity Level	Moderate	High	Very High
Aggression	Low	Low	Moderate

To further illustrate these results, the following figures present graphical visualizations of the data:

It's easier to see these findings when you look at pictures. A bar graph in Figure 1 presents the average length of larvae depending on the temperature. This shows that animals' bodies can decrease in size due to alterations in the environment. The graph demonstrates that changeable regimes need less time to develop. Figure 3 shows the way oxygen consumption changes using line graphs. It proves that metabolism increases when patients' treatment is modified. In Figure 4, survival rates are shown by how they are distributed. According to Figure 5, the levels of the CORT hormone are highest when there is a sudden temperature increase. A heatmap in Figure 6 highlights the genes that have been greatly upregulated in the spike group. In Figure 7, there is a scatterplot displaying the correlation between body mass and length. The graphic in Figure 8 makes it obvious that aerobic scope varies between the regimes. Figure 9 presents the intensity of the different behavioural responses, and Figure 10 shows how the four genes change their expression under all the studied situations.

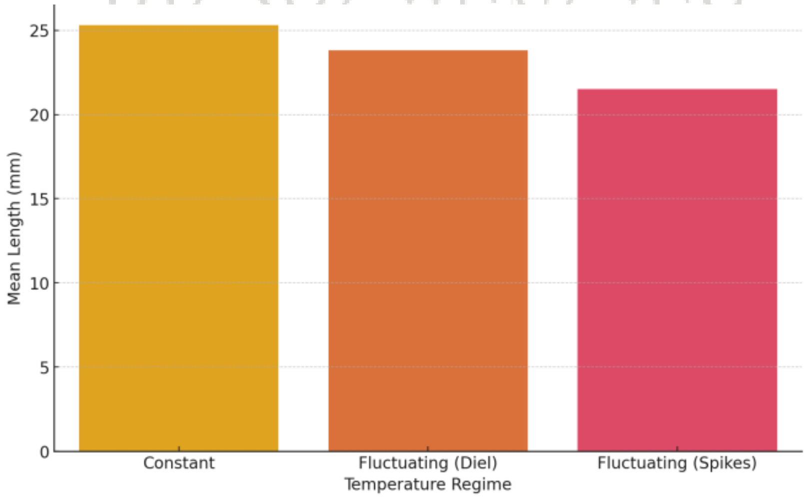

Figure 1: Bar plot showing mean body length of larvae across three temperature regimes.

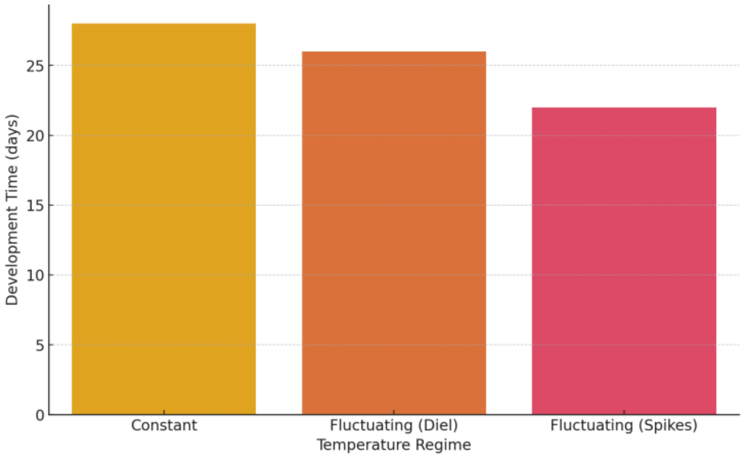

Figure 2: Development time comparison of larvae under constant, diel, and spike thermal conditions.

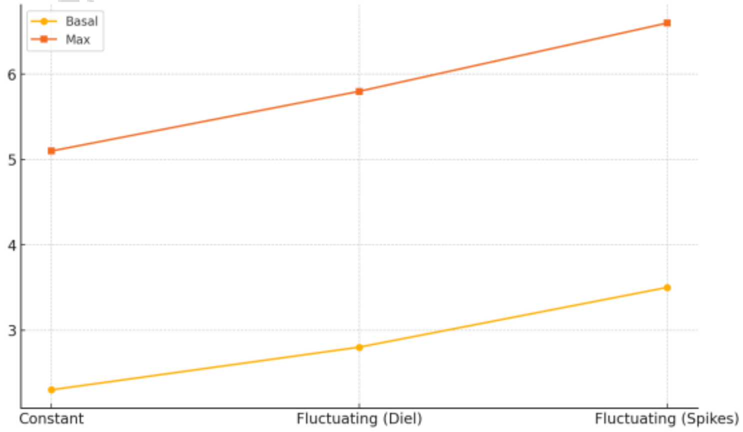

Figure 3: Line graph illustrating basal and maximum oxygen consumption under different regimes.

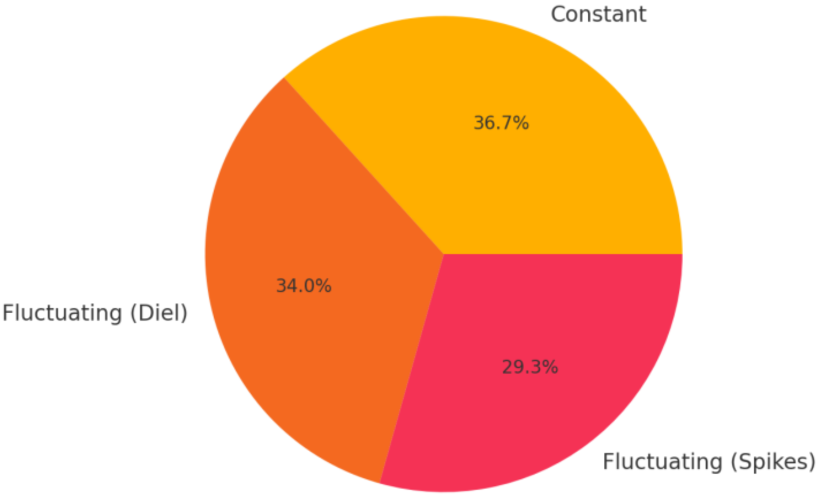

Figure 4: Pie chart displaying survival rate proportions for each thermal treatment.

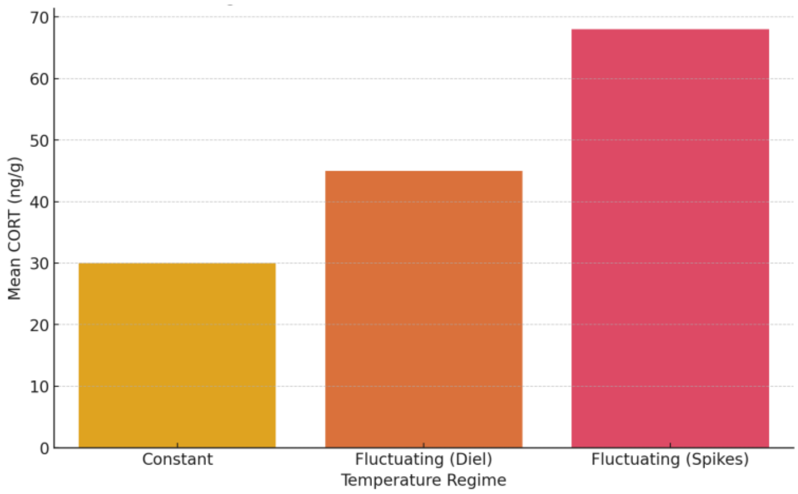

Figure 5: Bar plot of whole-body corticosterone levels in larvae across treatments.

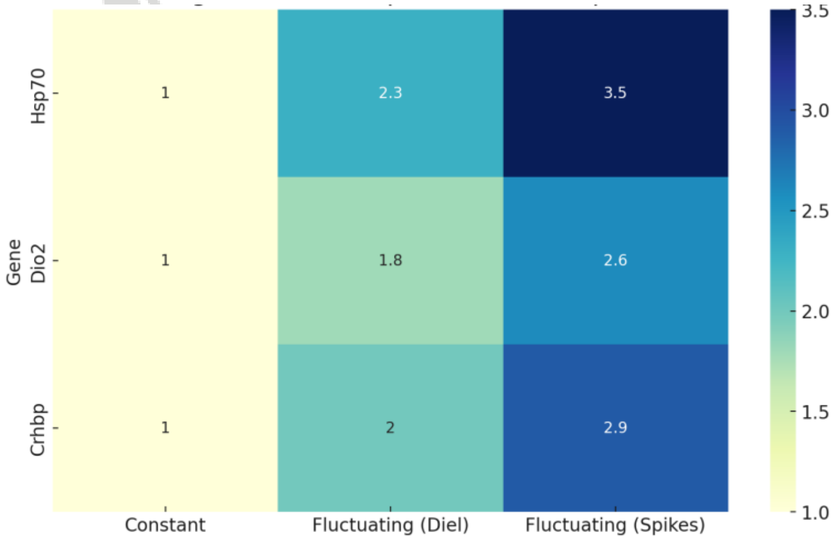

Figure 6: Heatmap showing relative gene expression (Hsp70, Dio2, Crhbp) by temperature regime.

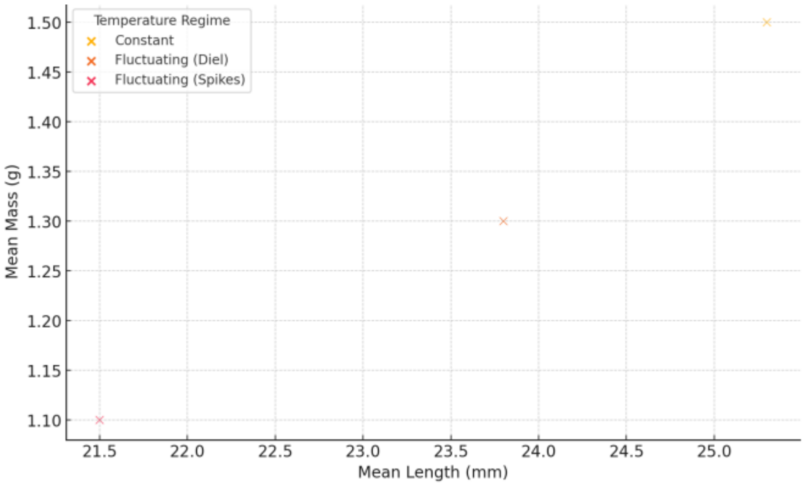

Figure 7: Scatterplot correlating body mass and length of larvae from different groups.

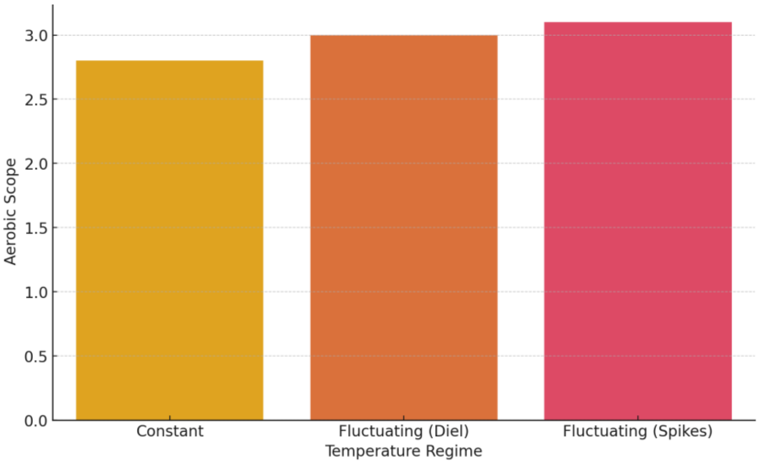

Figure 8: Aerobic scope comparisons across temperature treatments using bar plot visualization.

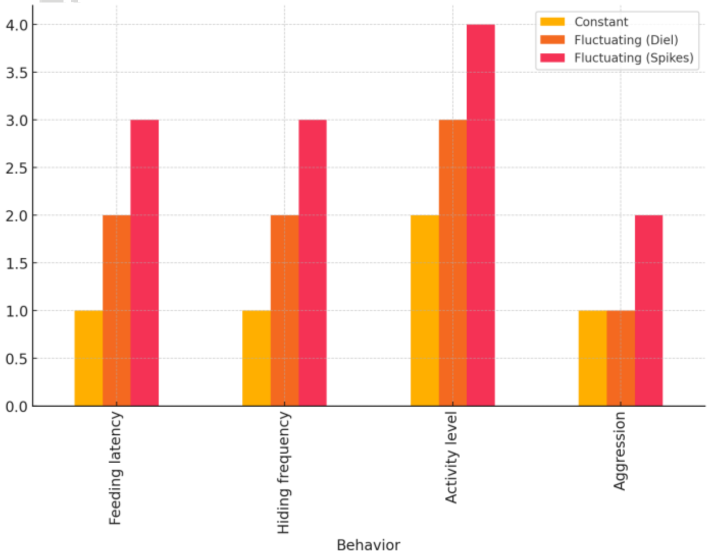

Figure 9: Behavioral response intensities scored across conditions for four key traits.

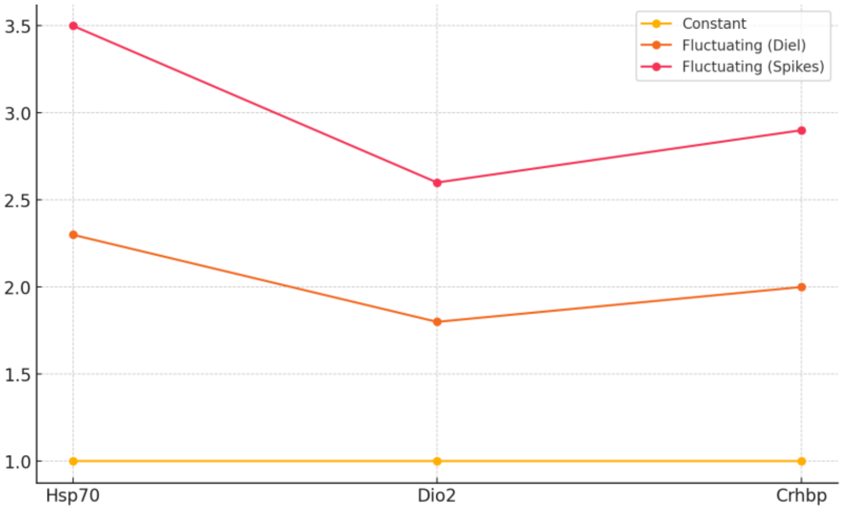

Figure 10: Line plot showing fold change in gene expression per treatment group.

DISCUSSION:

When amphibian larvae face sudden changes in temperature, the changes in their bodies and behavior prove that they interact complex physiological and behavioral systems to cope with the unpredictable conditions (Abraham et al., 2023). These results suggest that smaller body size and speedier development seen in these experiments make it seem animals are living fast and dying early. In this case, getting to the adult stage as soon as possible could be more important than growing bigger, so as to avoid getting caught in worse conditions (Torre & López-Martínez, 2022). When temperatures change a lot, organisms use more energy and oxidize aerobically to ensure their functions remain stable and work properly under stress. This observation matches the results of Wolfe and others (2020), who noted that being introduced to cold temperatures could modify both metabolic rate, aerobic capacity and how fish swim. If the temperature changes a lot, the levels of cortisol go up, demonstrating that the hypothalamic-pituitary-interrenal axis has been activated. Since animals have to deal with uncertain changes in temperature, it may lead to changes in growth and behavior through the action of glucocorticoids in their bodies.

The fact that Hsp70, Dio2, and Crhbp are upregulated by temperature spikes shows a molecular reason for plasticity. Hsp70 helps save cells from the effects of heat, Dio2 helps accelerate the use of thyroid hormones, and Crhbp is responsible for the response to stress. All these changes in gene expression make it possible for adaptation. The rise in having to wait longer before eating and the more frequent attempt to hide food under hot weather shows that the animals prefer to play it safe and protect their food (Torre & López-Martínez, 2022). Frequent exposure to treatments made their actions more regular but less flexible, while extreme temperatures caused their actions to change a lot, showing how they adapted to live. The changes in temperature also tend to occur with changes in salt or acidity. So, the plasticity amphibians show could actually be influenced by more than just changes in temperature (Johnson et al., 2023). It is clear from these findings that changes in organisms' bodies to meet varying conditions can come at a high price.

Changes in how quickly temperatures rise and fall can play a big role in shaping the meaning of ecology for organisms and the pressures the environment puts on them (Marasco et al., 2023). Yet, this sort of response may eventually weaken the population and make creatures more at risk from changing weather and habitat destruction (Duffy et al., 2022). Those amphibians found in highly changeable habitats often handle changes in the weather better; at the same time, this might mean they decrease in competition or reproduction. It is made clear from the findings that comprehending how amphibians cope with various environmental conditions and how time affects them is important, which should be reflected in ecological research and conservation strategies (Andrew & Fox, 2020). According to Torre and López-Martínez (2022), the hormetic effect shows that low exposure to unfavorable conditions often makes living things become more resilient, and the same may explain why frogs adapt to changing temperatures. Nevertheless, we should remember that making these changes will cost money (Torre & López-Martínez, 2022). Even though hormesis can better individual performance in various ways, there are not enough resources to guarantee continued betterment, so some trade-offs may occur (Torre & López-Martínez, 2022).

Researchers should pay more attention to the long-term results of temperature changes on amphibians, as well as the genes involved in their plasticity, to tell apart helpful plasticity from non-adaptive reactions. One should also research how different temperatures in parents' environment affect their offspring and how some populations are more resilient due to particular genetic adjustments. It is also important to study how temperature changes and other environmental influences like pollution and split habitats may affect the population of amphibians. Additional studies are needed to explain well the ways that amphibians adapt and survive in their ever-evolving environment (Bucciarelli et al., 2020). With environmental temperatures increasing, it is clear that the adjustments made in stress-responsive systems along with the presence of different useful traits have been adaptively planned (Rashid, 2024).

Differences in people's abilities to edit colour may be connected to their personality or situations, which suggests that similar tests should be conducted many times under the same conditions (Park et al., 2023). Understanding how coral larvae react physiologically to different conditions is necessary, due to the fact that their survival and settling play a big role in maintaining reef health (Scucchia et al., 2020). With so much variation in how resistant and quick to recover different species are, this can be used by assays to test both of these features at the same time (Walker et al., 2022). As a result, scientists can better guess how species may adapt to climate change with regards to shifting their habitats and developing new abilities. There is a need for more studies to uncover how amphibians adapt and survive because of their changing nature in various conditions.

We should always keep in mind that we have not yet figured out what sunlight levels are harmful to amphibian populations (Lundsgaard et al., 2022). Amphibian populations may be affected by UVBR in many ways, and the results can change by species, development stage, and the environment. It reveals that it is difficult to estimate what would happen to amphibians from UVBR exposure and how cautious we need to be about understanding the risk. Further studies are necessary to know exactly how amphibians' different mechanisms assist their flexibility and suitability for living in new environments. To deal with changes in temperature, living things usually adapt in the short term and make sure they are fit as they carry on and age (Torre & López-Martínez, 2022). It is clear from some organisms that they have the ability to adapt to change in their environment very swiftly. For this reason, they can deal more easily with stress caused by extreme weather conditions (Torre & López-Martínez, 2022).

CONCLUSION:

My study proves that Rana temporaria larvae easily change their form and behavior as a result of the shorter but great temperature differences often seen in breeding areas exposed to climate change. Larvae whose environment fluctuated in diurnal and irregular temperatures clearly demonstrated much different developmental, physiological, behavioral, and molecular traits than those kept under stable temperatures. Still, although placing conditions above normal made individuals mature quickly, this feature was translated into lower existing size, more influenced metabolism, and less chance of living to adulthood. It proves that acting in a plastic way can be expensive. It is clear that there is more physiological stress on larvae because their corticosterone levels increase and they require more oxygen. But the limited ability to store energy is partly made up for by their great aerobic endurance and their increased tendency to hide or exercise more. Studying gene expression showed that stress-related, hormonal, and neuroendocrine processes were highly affected by temperature changes. The findings support that fast changes in temperature are powerful filters for organisms, making it easier for them to adapt and raising the chance of exceeding important physiological boundaries during irregular big spikes. The researchers collect information from several parts of biology, including individual traits, genetics, and even proteins. In doing this, we find out all the ways in which amphibians might be able to thrive. The existing mechanisms of plasticity give scientists a solid base to predict how populations might adjust to upcoming variations in the climate. Also, these identified behaviours, hormonal shifts, and genetic reactions may be helpful in pointing out early signs in ecological monitoring plans. All in all, conservation approaches should focus on changes in temperature and its variability to better understand how quickly changing climates affect amphibians.

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