# “EFFECT OF CAFFEINE IN RESPIRATORY RATE AND SWIMMING BEHAVIOUR OF THREE DIFFERENT FISH SPECIES”


**Project Work By**

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# EFFECT OF CAFFEINE IN RESPIRATORY RATE AND SWIMMING BEHAVIOUR OF THREE DIFFERENT FISH SPECIES

# CERTIFICATE

This is to certify that the project report entitled "**EFFECT OF CAFFEINE IN RESPIRATORY RATE AND SWIMMING BEHAVIOUR OF THREE DIFFERENT FISH SPECIES"** submitted by Ms. U. Nilachandana, Register No. : AB21ZOO028 in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.


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**EXAMINERS**

**1)**

**2)**

# DECLARATION

I, Hereby declare that this project work entitled "**EFFECT OF CAFFEINE IN RESPIRATORY RATE AND SWIMMING BEHAVIOUR OF THREE DIFFERENT FISH SPECIES"** is submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirements of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

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# ABSTRACT

The project 'Caffeine Effect in Respiratory Rate and Swimming Behaviour of Three Different Fish Species" delved into the intricate relationship between caffeine exposure and its influence on the respiratory rate and swimming behaviour of fish. Through a meticulously designed experimental approach, various concentrations of caffeine were administered to a diverse range of fish species in a controlled environment.

The methodology involved continuous monitoring of respiratory rates and swimming behaviours post-caffeine exposure, allowing for a comprehensive analysis of any discernible changes. The fish species *Carassius auratus* (Goldfish), *Pangasius hypophthalmus* (Albino shark fish) and *Pterophyllum sp.* (Angelfish) were introduced into an experimental aquarium tank at normal water temperature. Three fishes of each species were used for the study and results were obtained. The frequency of opercula beats, which indicates the respiratory rate, was determined by observing and recording the number of opercula movements in fish over a specific time period.

The experiment studied the influence of caffeine on fish respiration using a 19L aquarium by housing a single fish in 5L of water at a particular time. After acclimation, the fish's respiratory rate was measured for 1 minute, followed by adding 0.5ml of caffeine and revaluating the rate after 20 minutes. This process was repeated with increasing caffeine concentrations (1ml, 2ml, 5ml and 10ml) for various fish species, ensuring precision through direct observation and video recordings. This systematic approach provided a thorough understanding of caffeine's effect on fish respiratory, demonstrating how it impacts different species.

The swimming behaviour of fish was investigated by introducing caffeine at different concentrations into the aquarium tank. Following the addition of each incremental concentration, the fish was allowed a 20-minute period for the caffeine to take effect. The fish's swimming behaviour was meticulously observed through direct observation and video recordings using a mobile camera for duration of 1 minute per observation. This comprehensive approach facilitated a detailed analysis of the fish's behaviour and locomotor activities in response to varying caffeine concentrations.

The results unveiled a nuanced interplay between caffeine concentrations and the observed alterations in both respiratory rates and swimming behaviours among the fish subjects.

Goldfish exposed to caffeine exhibited a decrease in respiratory rate, coupled with disturbed swimming behaviour, ultimately leading to their death after 7 days. Similarly, Albino shark fish displayed increased breathing rates, sluggish swimming movements, and difficulty in opening their operculum and mouth. Angelfish, when exposed to caffeine, showcased varying respiratory rates, slow swimming behaviour, and eventually perished on the 4th day post-exposure. These observations highlight the detrimental effects of caffeine on aquatic organisms, emphasizing the importance of understanding its ecological impact. These findings suggest that caffeine has stimulatory and anxiogenic properties in fish, influencing their physiological responses.

This study contributes valuable insights into the potential repercussions of caffeine on aquatic organisms, underscoring the necessity for further investigations to unravel the intricate dynamics at play. By unravelling how caffeine modulates fish behaviour and respiratory functions, this research paves the way for a deeper understanding of its ecological implications, thereby informing conservation strategies and ecosystem management practices.

# CONTENT

# INTRODUCTION

Caffeine, a naturally occurring stimulant found in various plants, is one of the most widely consumed psychoactive substances in the world. Known for its ability to enhance alertness, concentration, and energy levels, caffeine is commonly found in coffee, tea, chocolate, and energy drinks. Its effects on the central nervous system make it a popular choice for combating fatigue and improving cognitive function. However, moderation is key, as excessive consumption can lead to negative side effects such as insomnia, jitteriness, and increased heart rate. Understanding the impact of caffeine on the body can help individuals make informed choices about their consumption habits**.**

Caffeine affects humans and fish differently. In humans, caffeine metabolism varies by age and gender, with the enzyme CYP1A2 playing a key role. It has a half-life of around 80 hours in humans. In fish, chronic caffeine exposure can lead to behavioural changes like increased avoidance behaviour and anxiogenic effects. Studies show negative impacts on marine life's reproduction, growth, and metabolic activity due to caffeine pollution in oceans.

Caffeine, with the chemical formula C8H10N4O2, is a methylxanthine alkaloid that acts as a central nervous system stimulant. Chemically related to adenine and guanine bases, it is a bitter, white crystalline purine. Caffeine's structure includes a pyrimidinedione and imidazole ring system, making it an achiral molecule with ten pi electrons, classified as aromatic. Its synthesis involves reactions like dimethylurea and malonic acid, and it can be extracted from various plants like coffee, tea, and cocoa. Caffeine's chemical properties enable it to block adenosine receptors, enhancing alertness through increased neurotransmitter release.

Caffeine's impact on aquatic environments, particularly on fish, has been a subject of study. Research has shown that caffeine exposure can affect fish behaviour, with studies indicating changes in activity levels, feeding behaviour, growth rates, and social interactions. The presence of caffeine in water can lead to alterations in fish behaviour, inducing aggressive tendencies, reducing exploratory behaviour, and affecting social interactions. These findings highlight the importance of monitoring caffeine contamination in aquatic systems to safeguard fish populations and ecosystem health.

Fish respire through gills, not lungs, extracting oxygen from water via countercurrent exchange. Gills, rich in blood vessels, facilitate gas exchange as water flows over them, absorbing oxygen and releasing carbon dioxide. Fish have a hydrostatic organ, the swim bladder, aiding buoyancy control. Some fish, like lungfish, have evolved to breathe air, showcasing diverse respiratory adaptations. Overall, fish respiration involves intricate mechanisms for oxygen uptake and waste removal, crucial for their survival in aquatic environments.

The swimming behaviour of fish is a complex interplay of stability and maneuverability, influenced by various locomotion mechanisms like anguilliform, carangiform, thunniform, and more. Fish adapt their swimming styles based on habitat conditions, with faster-swimming species favoring wave-swept areas for efficiency. Different fish species exhibit diverse locomotor strategies, combining behaviors for optimal movement and survival in their environments. Additionally, the morphology of fish fins and body shape directly impact swimming performance and energy consumption.

*Carassius auratus* (Goldfish), are commonly used in studies investigating the effects of substances like caffeine on fish physiology due to their availability, ease of maintenance, and well-documented physiological responses. Goldfish have been extensively studied, providing a baseline for comparison with other fish species. Their respiratory and swimming behaviors are well-documented, making them a suitable model for assessing the impact of caffeine on respiratory rate and swimming behavior in fish. Additionally, the use of goldfish allows for comparative analysis with existing research, enhancing the understanding of caffeine's effects on aquatic organisms.

The benefits of using *Pangasius hypophthalmus* (Albino sharks) in respiratory rate studies lie in their unique physiological adaptations, such as the presence of UCP1-containing fat deposits near their gonads. These adaptations provide insights into localized endothermy, supporting reproductive functions and offering a molecular explanation for increased heat production in the nervous system. Studying albino sharks helps unravel the complex mechanisms of respiratory and thermal regulation in these animals, contributing to a deeper understanding of vertebrate evolution and the diverse strategies marine organisms employ to thrive in their environments.

The respiratory rate of *Pterophyllum sp*. (Angelfish) has been studied to understand the effects of water temperature and other environmental factors on their respiratory. Angelfish, like other aquatic ectotherms, adapt to their environments by regulating their metabolism through various means, including respiratory. A study found that goldfish, which are also aquatic ectotherms, regulate their respiratory in response to changes in water temperature, with a decrease in respiratory rate observed in colder temperatures. Similarly, angelfish may also regulate their respiratory rate in response to changes in water temperature, although specific studies on angelfish are not mentioned in the search results. Understanding the respiratory rate of angelfish and other aquatic ectotherms is important for conservation efforts, as changes in environmental conditions can impact their survival and distribution.

As our understanding of environmental pollutants continues to evolve, the impact of caffeine on aquatic ecosystems has emerged as a significant concern. Caffeine finds its way into water bodies through various sources, posing a potential threat to aquatic life. Fish, as vital components of aquatic ecosystems, are particularly vulnerable to the effects of caffeine exposure. By investigating the influence of caffeine on the respiratory rate and swimming behavior of fishes, this study aims to shed light on the intricate interactions between pollutants and marine organisms. Understanding how caffeine alters these fundamental physiological and behavioral aspects in fish is crucial for assessing the broader implications on population dynamics and ecosystem health. Through this research, we strive to contribute valuable insights that can inform conservation efforts and management strategies aimed at safeguarding aquatic environments from the detrimental effects of caffeine contamination.

"Caffeine Effect in Respiratory Rate and Swimming Behavior of Three Different Fish Species" aims to investigate the impact of caffeine on two vital aspects of fish physiology: respiratory rate and swimming behavior. In a carefully controlled aquarium environment, each fish species was introduced to caffeine in varying concentrations. The respiratory rates was monitored and recorded, shedding light on the subtle yet profound changes induced by caffeine exposure. Simultaneously, the swimming behavior of the fish was analyzed and video recordings were employed, providing a source of data for further analysis.

This study will delve into the intricate relationship between caffeine exposure and fish biology, exploring how changes in respiratory rate may alter oxygen uptake and metabolic functions. Additionally, the project will investigate how caffeine influences swimming behavior, considering factors such as speed, agility, and overall locomotor performance in different fish species such as ; *Carassius auratus* (Goldfish), *Pangasius hypophthalmus* (Albino shark fish) and *Pterophyllum sp.* (Angelfish). By examining the effects of caffeine, this aims to contribute valuable insights into the physiological responses of fish to environmental stressors. The findings from this study have the potential to enhance our understanding of how caffeine pollution may impact aquatic organisms and ecosystems, ultimately guiding conservation efforts and environmental management strategies.

# OBJECTIVES

## 1. To study the effect of caffeine in three different species of fishes

The objective of this study is to evaluate the behavioral and physiological responses of three ecologically relevant fish species to environmentally realistic concentrations of caffeine. The study will examine three different fish species, representing diverse taxonomic groups and ecological roles, such as *Carassius auratus*, *Pterophyllum sp*. and *Pangasius hypophthalmus.* Using caffeine concentrations that have been detected in natural aquatic environments, rather than unrealistically high levels, the findings of this study will contribute valuable insights into the potential impacts of caffeine pollution on aquatic ecosystems.

## 2. Quantify Respiratory Changes

The study involves a detailed examination of the alterations in respiratory rates of fish species when exposed to different concentrations of caffeine. This entails a systematic measurement and analysis of how caffeine exposure directly influences breathing patterns in these aquatic organisms. By meticulously quantifying the changes in respiratory rates, the study aims to provide a comprehensive understanding of how caffeine affects the respiratory dynamics of fish, shedding light on the specific physiological responses triggered by this common environmental contaminant. This objective seeks to elucidate the direct impact of caffeine on fish respiratory, contributing valuable insights to ecotoxicological research and enhancing our knowledge of the effects of caffeine contamination on aquatic ecosystems. It also helps in understanding their metabolic activity and energy expenditure. The respiratory rate of a fish can be measured by counting the number of times the operculum flaps or the number of times the fish opens and closes its mouth. The effect of caffeine on the respiratory rate of fish varies depending on the concentration. At low concentrations, caffeine can increase the respiratory rate, while at higher concentrations, it can decrease it. This is done in a controlled aquarium environment.

## 3. Evaluate Swimming Behavior

It involves a comprehensive assessment and documentation of the alterations in swimming behavior exhibited by fish species when exposed to caffeine. This evaluation encompasses analyzing changes in swimming speed, agility, and overall movement patterns in response to varying concentrations of caffeine to gain insights into how this stimulant impacts fish locomotion. By meticulously observing and recording these behavioral changes, the study aims to elucidate the effects of caffeine on fish movement dynamics, providing a detailed understanding of how caffeine exposure influences the swimming behavior of aquatic organisms. This objective seeks to contribute valuable data to the field of ecotoxicology, enhancing our knowledge of the behavioral responses of fish to caffeine contamination in aquatic environments.

## 4. Correlate Respiratory and Behavioral Changes

The study aims to establish connections between the changes in respiratory rates and alterations in swimming behavior of fish species exposed to caffeine. By analyzing these two sets of data, the objective is to identify potential relationships, dependencies, and patterns influenced by caffeine exposure. This correlation seeks to unveil how changes in breathing patterns may be linked to shifts in swimming behavior, providing insights into the integrated effects of caffeine on both physiological and behavioral aspects of aquatic organisms. Through this analysis, the study aims to elucidate the interplay between respiratory and behavioral responses to caffeine exposure, contributing to a deeper understanding of how this stimulant impacts fish health and locomotion.

## 5. Comparative study of caffeine exposure

The objective of this comparative study is to investigate the effects of varying concentrations of caffeine on the respiratory rate and swimming behavior of fishes. By analyzing the changes in these physiological and behavioral parameters, the study aims to gain a better understanding of how caffeine interacts with and impacts the aquatic organisms. It identifies respiratory rate and swimming patterns of the fishes exposed to different concentrations of caffeine, with the goal of elucidating the dose-dependent responses and the mechanisms by which caffeine affects the overall health and functioning of these aquatic creatures. The findings from this study could provide valuable insights into the potential ecological implications of caffeine contamination in aquatic environments and inform future conservation and management strategies.

## 6. Contribute to Ecotoxicological Knowledge

The provision of valuable data and insights into the physiological and behavioral effects of caffeine on aquatic organisms provide an idea about ecotoxicology. By conducting research that delves into how caffeine exposure impacts fish at both physiological and behavioral levels, this objective aims to enhance the broader understanding of ecotoxicity. Through the analysis of the effects of caffeine on aquatic organisms, particularly fish species, the study seeks to contribute essential information to the field of ecotoxicology. By shedding light on how caffeine influences both the physiological functions and behavior of aquatic organisms, this research aids in assessing the impact of caffeine contamination on aquatic ecosystems. Ultimately, this objective aims to advance our knowledge of ecotoxicity and its implications for aquatic environments by focusing on the specific effects of caffeine on fish health and behavior.

# REVIEW OF LITERATURE

The study is based on the effect of caffeine in respiratory rate and swimming behaviour of fishes. The primary objective of the review is to examine the available evidence on the effect of caffeine in fishes. By synthesizing the current understanding of this topic, the review aims to identify any gaps in knowledge and provide a foundation for future research on the potential impacts of caffeine on aquatic ecosystems.

A study conducted by Yuki Harada *et al.*,(2022) aimed to assess the pharmacological effects of caffeine on adult zebrafish (*Danio rerio*) under free-swimming conditions. Ventilation rates were measured in zebrafish exposed to varying caffeine concentrations, comparing results under restraint and free-swimming conditions. The study utilized linear mixed model analysis to evaluate caffeine's effects. Electrocardiogram data and swimming speeds were cross-referenced with previous studies to determine appropriate caffeine dosages. Notably, caffeine decreased heart rate and increased ventilation under restraint, while ventilation rates rose with higher caffeine concentrations during free-swimming, demonstrating the feasibility of assessing chemical effects on zebrafish ventilation.

Joyce Andreia Dos Santos *et al*., (2021) exposed endemic Neotropical catfish *(Rhamdia quelen)* larvae to varying caffeine concentrations for 30 days and it revealed that the highest dose (16 mg L−1) led to skeletal deformities and reduced growth. A global assessment of environmental caffeine levels indicated low risk to *R. quelen* and similar fish species in about 90% of freshwater ecosystems. However, South and Central American ecosystems, where *R. quelen* is native, face higher risk. While current ecotoxicological risks are generally low, rising caffeine consumption and inadequate sanitation may elevate concentrations globally, endangering local fish with sublethal morphological impacts.

Marcela Munoz Penuela *et al.*, (2021) investigated the physiological reproductive responses of male *A. altiparanae* exposed to DCF, CAF, and their mixture at nominal concentrations for 96 hours. The study measured the levels of three steroids (17β-Estradiol, Testosterone, and 11-Ketotestosterone), the expression of the estrogenic biomarker vitellogenin (vtgA), the morphology of testes and liver, and mortality rates. The results showed that DCF and CAF degraded by only 5% after 24 hours, with LC50 values of 30.8 mg L−1 and 95.9 mg L−1, respectively. Males exposed to DCF and CAF separately exhibited a reduction in 17β-Estradiol levels compared to the control group. However, males exposed to the DCF and CAF mixture did not show any differences in Testosterone, 17β-Estradiol, and 11-Ketotestosterone levels. The vtgA gene expression and sperm concentration did not vary among the treatments. Acute exposure to DCF and its mixture with CAF resulted in hepatocyte hypertrophy, indicating potential liver damage. The study concluded that DCF and CAF, when isolated, exhibit endocrine disruptive activity in *A. altiparanae* males, while their mixture abolishes these endocrine disruptive effects. Despite the low concentrations used in the study, these pharmaceuticals can cause significant harm to aquatic organisms.

Cooper callings and Marianne Robertson (2021) in a study investigated the impact of caffeine and sertraline, commonly found in nature due to pharmaceutical dumping and inadequate water treatment, on spatial learning in goldfish (*Carassius auratus*). A control group of 15 goldfish without drug influence was compared to experimental groups exposed to 8.5 µg/L of caffeine and 164 ng/L of sertraline. The goldfish were tested in an X-shaped maze, and their time and number of incorrect choices were recorded for 10 trials. Statistical analysis revealed that the control group exhibited spatial learning, while the caffeine group did not, and the sertraline group showed only partial signs of spatial learning. These findings suggest that exposure to these substances could negatively affect fish populations' learning abilities, highlighting the need for improved water treatment and pharmaceutical disposal methods.

A study conducted by N. B Goodson *et al*., (2015) examined the impact of chronic caffeine exposure on the recovery of the dorsal light reflex (DLR) in goldfish (*Carassius auratus*) with unilateral vestibular organ removal. A comparison between functional recovery of goldfish in caffeine solutions of 2.5 mg/L, 5.0 mg/L, 10.0 mg/L, and a control group (0.0 mg/L) over 24 days was done. All caffeine-treated groups showed significant changes in DLR on day 1, while the control group recovered and approached sham surgery DLR by the end of the study. However, caffeine groups exhibited a deterioration of postural control from day 10 onwards, with all three caffeine groups significantly deficient compared to the control on days 10-24. These findings suggest that high dosage or prolonged caffeine exposure can hinder functional recovery.

Stavros Chatzifotis *et al.,* (2008) examined the effect of caffeine on sea bream (*Sparus aurata)* growth, feed efficiency, and consumption during winter rearing, with no significant impact on diet palatability. However, growth was hindered at concentrations above 1 g kg−1 diet, and feed conversion ratio increased in the 2 and 5 g kg−1 treatments ($P < 0.05$). Caffeine did not alter cerebral acetyl-cholinesterase (AchE) or nitric oxide synthase (NOS) histochemistry, suggesting no central action in the brain. In aquaculture, algae play crucial roles, including water quality management and nutrient removal, while a caffeine-supplemented diet in sea bream showed no significant effect on growth and feed efficiency. Coffee silverskin dietary administration promoted the growth rate and feed efficiency of *Nile tilapia*, and a 50%-substituted RM feed was investigated for its effect on red sea bream growth and immune response, with promising results for sustainable aquaculture feeds. These findings highlight the potential of plant-based feed additives and waste valorization for sustainable aquaculture practices.

Luana. C. Santos *et al.,* (2017) investigated delves into the time-course and dose-dependent alterations in zebrafish (*Danio rerio*) behavior upon caffeine exposure. Findings revealed an inverted U-shaped trajectory for locomotor parameters and a crescent path for anxiety-like parameters. High doses transition from stimulating to anxiogenic effects, indicating harm.

A study by V. Pellouch and M. Vornanen (1996) showed effects of caffeine (5mM) on the contractile properties of ventricular myocardium in fishes and revealed that thermal acclimation alters calcium activation of contraction. Specifically, caffeine increases the force of contraction more in the hearts of warm-acclimated fish (110%) than in cold-acclimated fish (40%). Additionally, the relaxation rate and time-course of contraction are differently affected by caffeine in cold- and warm-acclimated fish, suggesting that thermal acclimation changes the function of proteins responsible for excitation-contraction coupling. The role of caffeine in depleting SR Ca2+ stores has been studied in isolated cells, and it has been found that caffeine causes a transient increase in contraction amplitude followed by a sustained decrease in contraction amplitude, indicating that caffeine primarily inhibits calcium sequestration. Its action suggests that a portion of calcium influx associated with contraction coupling will accumulate in the cytosol, leading to an increase in the force of contraction.

A study by Bethany Brockhoff (2015) examined the impact of chronic caffeine exposure on the functional recovery of the dorsal light reflex (DLR) in hemilabyrinthectomized goldfish (*Carassius auratus*). The unilateral removal of vestibular organs causes temporary loss of postural control, which is restored over time through vestibular compensation (VC). She compared the functional recovery of goldfish exposed to caffeine solutions of 2.5mg/L, 5.0mg/L, and 10.0mg/L with a control group (0.0mg/L) over 24 days post-surgery. All caffeine-exposed groups showed significant changes in DLR compared to the sham surgery group on day 1. While the control group recovered over the study period, the caffeine groups initially recovered similarly but then regressed, deviating significantly from the control group on days 15-24. These findings suggest that early caffeine exposure may be harmless, but chronic exposure may hinder the functional recovery process in goldfish.

Dr. Michelle Gibson (2014) studied the effect of caffeine on the heart rate of the common goldfish, *Carassius gibelio* and found that caffeine decreased heart rate and increased ventilation in a concentration-dependent manner under restraint conditions. The data was collected by placing the fish in 100 g of caffeine diluted in 1000 mL of distilled water and recording the number of opercular movements at a consistent rate. The analysis of the data involved comparing the opercular movements before and after exposure to caffeine to determine the effects of caffeine on the fish's heart rate. However, the study did not find a significant change in average swimming speed under low caffeine exposure, and a high concentration of caffeine exposure significantly decreased swimming speed. Therefore, it can be inferred that appropriate caffeine stimuli were also applied under free-swimming conditions. However, there was a difference in the threshold concentration at which swimming speed was affected.

A study conducted by Andrew Lee *et al.*, (2022) investigated the impact of varying caffeine concentrations on the aggressive behavior of Siamese fighting fish *(Betta splendens)* when an intruder is introduced. Four experimental groups were used: a control group with no caffeine, a low-dose group (120 µg/L), a medium-dose group (200 µg/L), and a high-dose group (280 µg/L). A T-test was conducted to determine behavioral differences between the control group and the caffeine-dosed groups, aiming to evaluate caffeine's effects on *Betta splendens*. However, the results do not strongly support a correlation between caffeine concentrations and increased aggression in male territory-holding *Betta splendens*.

Julia Ruiz *et al.,* (2019) conducted an experiment in which zebrafish (*Danio rerio*) were trained to associate specific visual cues with rewards amidst distractors, testing the effects of caffeine on their performance. Fish exposed to 0 and 10 mg/L caffeine spent more time near the target and had quicker responses, with the 10 mg/L group showing the shortest latency. Both caffeine doses increased speed and distance traveled. This study affirms zebrafish's conditioned learning ability, highlighting that low-dose caffeine enhances visual cue discrimination and performance in complex tasks requiring focus and attention to secure rewards.

Daniel Cerveny *et al.,* (2022) investigated the ecological consequences of caffeine contamination on wild perch (*Perca flavescens*) behavior by monitoring fish swimming performance during light and dark conditions after waterborne exposure to 10 µg L−1 of caffeine. The research utilized a three-dimensional tracking system, which enabled positioning even in complete darkness, and conducted behavioral trials before exposure, after 24 hours of exposure, and after 5 days of exposure. The findings revealed that caffeine exposure had no significant effect on fish activity under light or dark conditions. However, fish swimming performance was significantly affected by both light-dark conditions and the repeating of behavioral trials, with individuals swimming significantly more during the light condition and their activity increasing over time. The study also confirmed the effectiveness of the three-dimensional automated tracking system based on infrared sensors for conducting behavioral experiments under completely dark conditions.

Bikker and Jacqueline (2023), exposed fathead minnow (*Pimephales promelas*), a common freshwater fish in North America, to environmentally relevant concentrations of caffeine (0 ng/L; 1,000 ng/L; 10,000 ng/l) for 35 days. Caffeine exposure did not affect morphology (e.g., length, mass, growth) or metabolism (maximum metabolic rate, resting metabolic rate, and aerobic scope), but decreased their hepatosomatic index (liver investment). While caffeine did not affect the number of trials taken to associative or reversal learn, or the latency of fish to avoid an aversive trawl, three weeks of exposure to low caffeine concentrations decreased anxiety.

A study conducted by Thais G. Santos-Silva *et al.*,(2018) analyzed the effects of caffeine on biochemical and genotoxic biomarkers in juveniles of *Prochilodus lineatus.* Fish were exposed to caffeine concentrations of 0.3, 3, and 30 μg/L for either 24 or 168 hours. The results showed that longer exposure to caffeine led to a significant reduction in the activity of the phase I biotransformation enzyme *ethoxyresorufin-O-deethylase* (EROD) in the brain but a significant increase in the liver. However, no changes were found in glutathione content, glutathione S-transferase activity, or lipid peroxidation in the liver and brain of fish exposed to caffeine. Additionally, no DNA damage in erythrocytes was detected. These findings suggest that caffeine may interfere with the biotransformation mechanism of *P. lineatus* after 168 hours of exposure, but it does not generate sufficient changes to trigger a state of oxidative stress.

Adrienne. M. Gibson *et al.,* (2009) investigated on *Pseudomonas* which exhibited enhanced growth in the presence of caffeine, resulting in altered spectrophotometric absorbance, increased bacterial colony counts, and the appearance of a bio-film like sheen on the glass of the experimental aquarium. This heightened bacterial growth was accompanied by a significant increase in ammonia concentrations, a known toxin for fish. The correlation between the metabolic activity of *Pseudomonas bacteria* and ammonia levels suggests that caffeine-rich environments could potentially harm aquatic life, making it a critical area for further investigation.

L. R Vieira *et al.,* (2022) evaluated the occurrence, concentration, and impacts of caffeine in coastal ecosystems, focusing on its potential adverse effects on marine biota and human health. It contributed to the development of effective strategies for managing caffeine pollution in coastal ecosystems, ensuring the ecological safety and human health.

Hayley Gill (2010) investigated the impact of varying caffeine concentrations (1.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, and 0 mg/mL) on 40 zebrafish (*Danio rerio)* embryos.The research aimed to understand how caffeine affects zebrafish embryos by observing deformities. Higher caffeine concentrations resulted in more defects and higher mortality rates. Given the similarities between zebrafish and humans, this study has implications for human health. Over time, zebrafish exposed to caffeine developed deformities, suggesting potential risks for human embryos exposed to caffeine in utero.

A study conducted by Gabriela Vasquez (2019) showed zebrafish (*Danio rerio)* embryos treated with high levels of caffeine, looking mainly for growth retardation. Results demonstrated that the higher the caffeine's concentration, the higher the levels of growth retardation by diminishing the zebrafish's body length. Results also showed that the higher the caffeine's level of concentration, the higher the mortality rate.

Luiz Vinicius Rosa *et al,*. (2008) acutely exposed fishes to caffeine (25, 50, 100 and 200 mg/L) for 15 min and further tested in the novel tank. Endpoint data and 3D reconstruction plots revealed that caffeine was anxiogenic in both WT and leo populations by altering vertical swimming, freezing, and erratic movements depending on the concentration. Prominent anxiogenic effects during habituation to novelty were observed in WT, suggesting a fundamental role of the phenotype in caffeine-mediated neurobehavioral responses. Although untreated leo showed higher baseline cortisol levels than control WT, caffeine increased whole-body cortisol in both populations. Moreover, caffeine induced aberrant swimming profiles in WT and leo following 200 mg/L exposure, which could reflect nonspecific toxicity and/or seizure-like behaviors. Collectively, the findings showed that caffeine effects in zebrafish (*Danio rerio)* differ in a population-dependent manner.

A study was conducted by Neha Rana *et al*., (2010) who studied the effect of caffeine on heart rate (HR) and other phenotypes of zebrafish embryos (*Danio rerio*) using visual microscopy and simple imaging. Acute treatment with millimolar concentrations of caffeine in embryo medium caused a dose-dependent decrease in HR in 2-3-day-old zebrafish embryos, ultimately resulting in complete HR cessation. The effects of caffeine were not reversed by cotreatment with ruthenium red and adenosine, agents known to be antagonistic to caffeine, or by changes in calcium concentration in embryo medium. Apparent cardiac arrhythmia and a typical kinking effect in the trunk/tail region were also observed because of caffeine treatment. These findings suggest that caffeine exerts its effects on embryonic HR of zebrafish by inhibition of ether-a-go-go potassium channels, although further experimentation is required to dissect the molecular basis of caffeine action.

A study conducted by Carlie Close (2019) used zebrafish (*Danio rerio*) as a model to study the effects of caffeine on embryonic development, with concentrations of 1.00 mg/mL causing severe deformities and a 100% mortality rate by 96 hours post fertilization. This research highlights the potential hazards of caffeine consumption, particularly for pregnant women, as it can inhibit proper development of the fetus. Additionally, studies have shown that low doses of caffeine can promote attention and focus in zebrafish, while high doses can create a stress response and impair learning. The pharmacological effects of caffeine on ventilation in adult zebrafish have also been investigated, with results indicating that caffeine can significantly increase ventilation rate under free-swimming conditions. Furthermore, environmental concentrations of caffeine have been shown to induce behavioral effects in zebrafish, including reduced exploratory behavior, increased feeding latency time, decreased growth rate and fish weight, and induced aggressive behavior

# METHODOLOGY

## MATERIALS USED IN THE EXPERIMENT

### 1. Aquarium tank

A rectangular glass tank of 19L was utilized. The tank was filled with 5L of water and equipped with an aerator used as a filter, under normal temperature. Plant substrates and small live plants were incorporated into the tank's setup. The experiment featured *Carassius auratus* (Goldfish), *Pangasius hypophthalmus* (Albino shark fish) and *Pterophyllum sp.* (Angelfish) as the chosen fish species. Maintenance procedures included feeding the fish every 3 days, regular water changes, and maintaining a water temperature of 22°C with a normal pH level. Throughout the project, diligent monitoring ensured that no traces of ammonia or nitrate were present in the tank, maintaining optimal water quality for the aquatic inhabitants.

### 2. Fish bowls

Fish bowls were used for the maintenance of three different species of fishes. Proper maintenance practices like regular water changes, monitoring water quality parameters, and selecting suitable fish species are highlighted to ensure a healthy environment for fish in bowls.

### 3. Fish net

Fish nets were used to take proper care of the fishes and their safe transfer from one water medium to the other ( aquarium & bowls )

### 4. Caffeine

In the study, 100% pure caffeine sourced from caffeine tablets purchased on Amazon was utilized. Each tablet contained 200mg of caffeine, which was crushed, diluted with warm water, and mixed to create a specific concentration of the caffeine solution measured in milliliters. To ensure accuracy, a measuring cylinder was employed to precisely measure the amount of caffeine used. The prepared caffeine solution was stored in a dry, uncontaminated environment to prevent any degradation or alteration in the concentration. Careful attention was given to avoid overdosing the fish with caffeine. Administration of the caffeine solution into the tank was facilitated using a dropper, allowing for controlled introduction. Subsequently, the effects of caffeine on the fish were observed and studied to evaluate its impact under the experimental conditions


**Fig 1.**


**Fig 2.**

### 5. Measuring cylinder

In the experiment involving the measurement of specific concentrations of caffeine, the use of a measuring cylinder played a crucial role in ensuring precise and accurate readings. Proper measuring techniques are essential to obtain reliable data and maintain the integrity of the experiment. When using a measuring cylinder, it is imperative to follow correct procedures such as eye-level reading, ensuring the meniscus aligns with the measurement mark, and avoiding parallax errors. Accurate measurements are vital for determining the exact concentration of caffeine and for achieving consistent and reproducible results.

### 6. Dropper

In the experiment, a dropper was employed to introduce caffeine into the tank in a controlled and gradual manner. This method allowed the caffeine to be added drop by drop into the water, ensuring that the fish were not overwhelmed by a sudden influx of caffeine. It aimed to prevent any adverse effects on the fish due to rapid exposure to caffeine.

### 7. Mobile camera

In the research protocol, a mobile camera is strategically utilized to observe fish behavior from diverse angles and accurately document their activities. Given the challenges posed by the rapid swimming movements of fish, which can obscure the visibility of opercula flapping and mouth actions, the camera is positioned in landscape mode to capture clear footage. Videos are recorded continuously for one-minute intervals to assess the respiratory rate of each fish systematically. This method allows for a detailed examination of operculum movements and mouth actions, ensuring precise data collection. We can pause and slow down recorded videos to meticulously analyze fish behavior and respiratory patterns. The swimming behavior of each fish is documented using this approach, providing comprehensive insights into their physiological responses and behaviors under observation.

## SPECIES OF FISHES USED IN THE EXPERIMENT

To study the effects of caffeine on the respiratory rate and swimming behavior of fishes, three distinct species were selected: *Carassius auratus* (Goldfish) *Pterophyllum sp.* (Angelfish) and *Pangasius hypophthalmus* (Albino shark fish). By incorporating these diverse species with unique physiological and behavioral characteristics, the research aims to provide a comprehensive understanding of how caffeine exposure influences respiratory patterns and swimming dynamics across fish species.

1. ***Carassius auratus* (Goldfish)**

Goldfish, scientifically known as *Carassius auratus*, are renowned for their captivating respiratory and swimming behaviors that reflect their adaptability to diverse aquatic environments. One key aspect of goldfish physiology is their efficient gill system, which enables them to extract oxygen from water for respiratory. Studies have shown that goldfish possess a remarkable ability to adjust their respiratory rate in response to environmental cues, such as changes in water temperature. As cold-blooded organisms, goldfish exhibit a decrease in respiratory rate as water temperature decreases, showcasing their physiological flexibility in adapting to varying conditions.

In terms of swimming behavior, goldfish are known for their agile and graceful movements, displaying a wide range of locomotor activities. These fish are capable of swift bursts of speed when needed, allowing them to evade predators or capture prey efficiently. Goldfish also exhibit schooling behavior, where they form groups for protection and social interaction. This schooling behavior not only enhances their safety in numbers but also facilitates communication and social bonding among individuals within the group.

Furthermore, goldfish demonstrate remarkable cognitive abilities and learning behaviors that contribute to their survival and success in aquatic habitats. Studies have shown that goldfish possess associative learning skills, enabling them to remember and respond to various stimuli in their environment. Their capacity for associative learning allows them to navigate complex environments, recognize feeding cues, and adapt their behaviors based on past experiences. This combination of physiological adaptability, diverse swimming behaviors, and cognitive capabilities makes goldfish a fascinating subject for research into the intricate interplay between biology and behavior in aquatic organisms.

**Fig 3.**

2. ***Pangasius hypophthalmus*** **(Albino shark fish)**

Aquarium sharks, such as the Albino sharks and other freshwater shark species, offer captivating additions to aquariums with their unique behaviors and striking appearances. These sharks, despite not being true sharks but rather freshwater fish resembling their marine counterparts, exhibit diverse behaviors that add vibrancy to aquatic environments. From the graceful movements of Albino fish scavenging through substrates to the peaceful shoaling behavior these species bring its own charm and dynamics to aquarium settings. The care requirements for these aquarium sharks vary, with factors like tank size, water conditions, and compatible tank mates playing crucial roles in ensuring their well-being and optimal growth. Understanding the distinct characteristics and behaviors of different freshwater shark species is essential for creating a harmonious and thriving aquatic ecosystem within an aquarium setting

**Fig 4.**

3. ***Pterophyllum sp.*** **(Angelfish)**

Angelfish, scientifically known as *Pterophyllum sp,* are captivating freshwater fish known for their unique body shape and striking appearance. These fish have a laterally compressed body that gives them a tall and slender profile, resembling a diamond. Angelfish typically grow to about six inches in length and exhibit distinctive fin configurations that contribute to their graceful swimming movements. In terms of respiratory rate, angelfish, like other fish species, rely on efficient gill respiratory to extract oxygen from water. Their respiratory rate is influenced by factors such as water quality and temperature, highlighting the importance of maintaining optimal aquatic conditions for their well-being.

When it comes to swimming behavior, angelfish are known for their agile movements and somewhat aggressive nature typical of the *Cichlidae* family. While they can coexist with fish of similar size, angelfish may display territorial behaviors and can be aggressive towards smaller tank mates. Their swimming patterns vary from cruising at moderate speeds to sudden bursts of acceleration when hunting prey or defending their territory. Angelfish also exhibit monogamous breeding behavior, forming pairs that diligently care for their eggs. This species' swimming behavior reflects its adaptability to different environments and its ability to navigate complex habitats efficiently.

In their natural habitat in South America's Amazon River Basin, angelfish prefer slow-moving waters with dense vegetation for hunting and hiding. Their omnivorous diet includes small invertebrates and plant matter, contributing to their versatile feeding habits. Angelfish are popular choices for home aquariums due to their striking appearance and relatively peaceful demeanor compared to other cichlid species. Understanding the respiratory rate and swimming behavior of angelfish provides insights into their unique adaptations for survival in freshwater ecosystems and enhances the care provided to these fascinating aquatic pets.


**Fig 5.**

## PROCEDURE

The experiment was conducted in a 19L aquarium that was equipped with small live plants, stones, pebbles, and an aerator to create a natural aquatic environment. 5L of water was filled in the tank at normal temperature to provide a suitable habitat for the fish species. Three different fish species were selected for the experiment, namely *Carassius auratus* (Goldfish), *Pterophyllum sp*. (Angelfish), and *Pangasius hypophthalmus* (Albino shark fish). Each species was introduced into the tank sequentially.

The first fish introduced into the tank was *Carassius auratus* (Goldfish). The fish was introduced into the tank filled with water and allowed to acclimate for ten minutes. Caffeine is then introduced into the tank using a dropper, starting with a low concentration of 0.5ml. To prevent the sudden exposure of fish to caffeine, which may affect their metabolic activities, a dropper was used to gradually introduce the caffeine into the tank. The fish is given 20 minutes to absorb the caffeine, during which the camera is set up at an angle in landscape mode to observe the fish's movement and behavior. After the 20-minute period, the fish's respiratory rate is measured by counting the number of times the operculum flaps or the number of times the fish opens and closes its mouth in 1 minute (60 seconds). This value is recorded, and the process is repeated with 1ml of caffeine, allowing fish to acclimate and absorb the caffeine for 20 minutes before measuring the respiratory rate. The same procedure is followed for 2ml, 5ml and 10ml concentrations of caffeine by counting the operculum flaps for 60 seconds, each.

The respiratory rate is recorded after each concentration, providing a dataset of respiratory rates at different caffeine concentrations. This dataset can be analyzed statistically to determine the impact of caffeine on the fish's respiratory rate.

The study was replicated with the other two fish species *Pterophyllum sp. (*Angelfish) and *Pangasius hypophthalmus* (Albino shark fish) to compare the effects of caffeine. The swimming behavior of the fish was closely observed and recorded through videography to provide detailed insights into the effects of caffeine on their behavior. The fishes were observed 5 days prior to the caffeine exposure to understand their swimming pattern and activities. The variation in their speed, locomotory activities and other behavioural patterns were observed, studied and noted down. The entire process was repeated for each of the three fish species to ensure consistency and reliability of the results. The results can contribute to understanding the environmental toxicology of caffeine and its impact on aquatic life.

Respiratory rates and behavioral observations were noted down for each fish at different caffeine concentrations to create a comprehensive dataset. The collected data was analyzed using appropriate statistical methods to determine the impact of caffeine on respiratory rates and swimming behavior across the different fish species. The results of the experiment were interpreted to understand the effects of caffeine on the fish species and to draw conclusions regarding its impact on their respiratory rates and behavior.

The experiment provided valuable insights into the environmental toxicology of caffeine and its potential lethal effects on aquatic organisms, contributing to a better understanding of the ecological consequences of caffeine contamination in aquatic environments.

# OBSERVATION AND RESULT

The study was conducted on 'Effect of Caffeine In Respiratory Rate and Swimming Behavior of Three Different Fish Species'. The methodology involved monitoring the respiratory rate and swimming behavior of fish exposed to varying concentrations of caffeine in the tank. Three fish species (*Carassius auratus*, *Pterophyllum sp., and Pangasius hypophthalmus*) were introduced sequentially, with caffeine concentrations (0.5ml, 1ml, 2ml, 5ml & 10ml) added at 20-minute intervals to observe the effects on respiratory rates and behavior. A dropper was used to gradually introduce caffeine, allowing fish to absorb it and acclimate. Respiratory rates were measured by counting the number of operculum flaps or mouth openings in 60 seconds after each concentration. The study was replicated for each species, with behavior recorded through videography. Data was analyzed using statistical methods to determine the impact of caffeine on respiratory rates and behavior across species. Results provided insights into environmental toxicology and caffeine's impact on aquatic life.

Concentrations of caffeine used in the experiment :

**0.5ml conc. of caffeine**
**Fig 6.**

**1ml conc. of caffeine**
**Fig 7.**

**2ml conc. of caffeine**
**Fig 8.**

**5ml conc. of caffeine**
**Fig 9.**

**10ml conc. of caffeine**
**Fig 10.**

# RESULT

## Estimation of respiratory rate

The impact of caffeine on the respiratory rate and behaviour of *Carassius auratus, Pterophyullun sp.,* and *Pangasius hypophthalmus* was studied. The respiratory rate of each fish was assessed by tallying frequency of operculum flapping or mouth opening and closing within a 60-second interval. The results obtained were as follows :

**Table 1. Showing respiratory rate of *Carrasius auratus* (Goldfish) in varying concentration of caffeine**

| Sl no. | Concentration of caffeine (ml) | Respiratory rate of Fish 1 (flaps/min) | Respiratory rate of Fish 2 (flaps/min) | Respiratory rate of Fish 3 (flaps/min) | Average respiratory rate (/min) |
|---|---|---|---|---|---|
| 1 | 0.5ml | 68 | 132 | 85 | 95/min |
| 2 | 1ml | 64 | 116 | 72 | 84/min |
| 3 | 2ml | 72 | 129 | 68 | 89.6/min |
| 4 | 5ml | 60 | 129 | 70 | 86.3/min |
| 5 | 10ml | 68 | 117 | 66 | 83.6/min |

The normal respiratory rate of *Carrasius auratus* under no influence of caffeine, vary from 108 flaps/min, 181 flaps/min and 129 flaps/min for Fish 1, Fish 2 and Fish 3, respectively. From the results obtained it is concluded that the respiratory rate of the fish first decreases from the normal rate, then increases, further decreases and finally undergoes a rapid decrease again. Hence the caffeine decreases the respiratory rate in *Carassius auratus.*

The above data can be expressed in the form of a line graph.

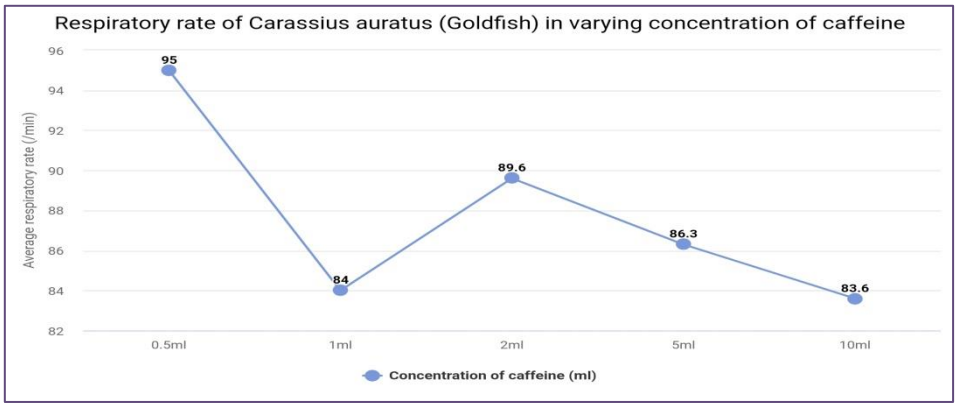



**Fig 11.**

**Table 2. Showing respiratory rate of *Pterophyllun sp.* (Angelfish) in varying concentration of caffeine**

| Sl no. | Concentration of caffeine (ml) | Respiratory rate of Fish 1 (flaps/min) | Respiratory rate of Fish 2 (flaps/min) | Respiratory rate of Fish 3 (flaps/min) | Average respiratory rate (/min) |
|---|---|---|---|---|---|
| 1 | 0.5ml | 166 | 161 | 171 | 166/min |
| 2 | 1ml | 167 | 162 | 176 | 168.3/min |
| 3 | 2ml | 167 | 160 | 166 | 164.3/min |
| 4 | 5ml | 170 | 167 | 171 | 169.3/min |
| 5 | 10ml | 175 | 165 | 164 | 168/min |

The normal respiratory rate of *Pterophyllum sp.* under no influence of caffeine, vary from 172 flaps/min, 165 flaps/min and 174 flaps/min for Fish 1, Fish 2 and Fish 3, respectively. From the results obtained it is concluded that there is a decrease in respiratory rate from the normal respiratory rate once caffeine is introduced. Then the respiratory rate increases, decreases, increases and finally decreases again. Hence in *Pterophyllum sp.* the respiratory rate varies due to the effect of caffeine.

The above data can be expressed in the form of a line graph.

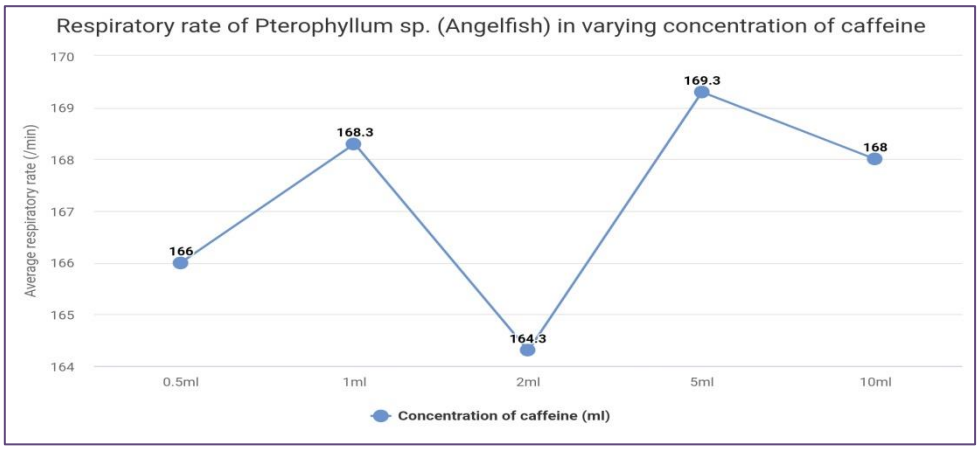



**Fig 12.**

**Table 3. Showing respiratory rate of *Pangasius hypophthalmus* (Albino shark) in varying concentration of caffeine**

| Sl no. | Concentration of caffeine (ml) | Respiratory rate of Fish (flaps/min) | Respiratory rate of Fish 2 (flaps/min) | Respiratory rate of Fish 3 (flaps/min) | Average respiratory rate (/min) |
|---|---|---|---|---|---|
| 1 | 0.5ml | 162 | 157 | 189 | 169.3/min |
| 2 | 1ml | 160 | 153 | 189 | 167.3/min |
| 3 | 2ml | 153 | 157 | 187 | 165.6/min |
| 4 | 5ml | 151 | 152 | 179 | 160.6/min |
| 5 | 10ml | 190 | 149 | 175 | 171.3/min |

The normal respiratory rate of *Pangasius hypophthalmus* under no influence of caffeine, vary from 183 flaps/min, 160 flaps/min and 193 flaps/min for Fish 1, Fish 2 and Fish 3, respectively. From the results obtained it is concluded that the respiratory rate initially decreased from the normal level, but subsequently experienced a sudden increase. Hence in *Pangasius hypophthalmus* the respiratory rate increases under the effect of caffeine.

The above data can be expressed in the form of a line graph.

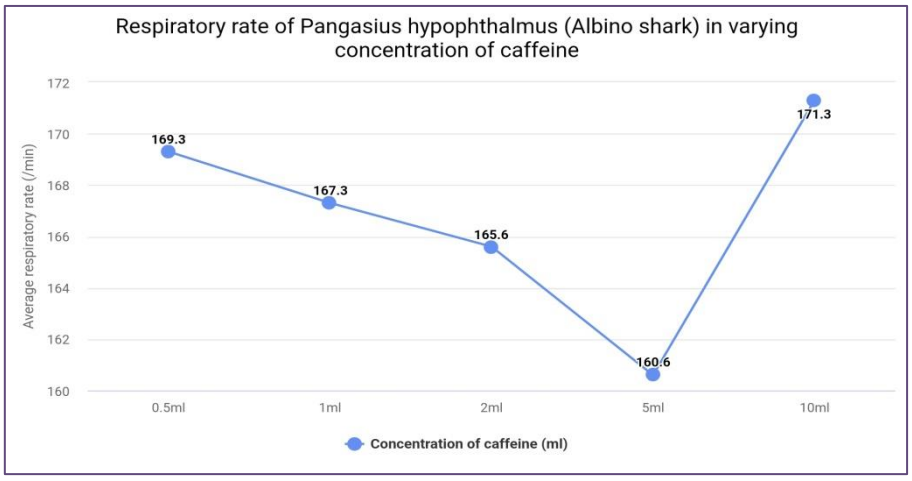



**Fig 13.**

## Estimation of swimming behaviour

The impact of caffeine on the swimming behaviour of *Carassius auratus*, *Pterophyullun sp.*, and *Pangasius hypophthalmus* was studied. The fish was exposed to a range of varying caffeine concentrations (0.5ml, 1ml, 2ml, 5ml, 10ml). The fishes were observed 5 days prior to the caffeine exposure to understand their swimming pattern and activities. On caffeine exposure their activities were closely observed for 14 days (2 weeks) and videos were recorded (in landscape mode) for proper understanding. The results obtained were as follows:

**Table 4. Showing swimming behaviour of *Carrasius auratus* (Goldfish) after caffeine exposure**

| Day | Fish 1 | Fish 2 | Fish 3 |
|---|---|---|---|
| 1-2 | No observable changes | No observable changes | No observable changes |
| 3-4 | Increased activity | No observable changes | Rapid swimming |
| 5-6 | Erratic swimming | Erratic swimming | Increased activity |
| 7-8 | Fish died | Fish died | Gasping for air |
| 9-10 | | | Fish died |
| 11-12 | | | |
| 13-14 | | | |

**Table 5. Showing swimming behaviour of *Pterophyllum sp.* (Angelfish) after caffeine exposure**

| Day | Fish 1 | Fish 2 | Fish 3 |
|---|---|---|---|
| 1-2 | Fish died | No observable changes | Slow swimming |
| 3-4 | | Slow swimming | Fish died |
| 5-6 | | Fish died | |
| 7-8 | | | |
| 9-10 | | | |
| 11-12 | | | |
| 13-14 | | | |

**Table 6. Showing swimming behaviour of *Pangasius hypophthalmus* (Albino shark) after caffeine exposure.**

| Day | Fish 1 | Fish 2 | Fish 3 |
|---|---|---|---|
| 1-2 | No observable changes | No observable changes | No observable changes |
| 3-4 | Increased activity | Rapid gill movement | Increased activity |
| 5-6 | Sluggish movements | Increased activity | Sluggish movements |
| 7-8 | Rapid gill movement | Gasping for air | Erratic swimming |
| 9-10 | Erratic swimming | Sluggish movements | Gasping for air |
| 11-12 | Gasping for air | Rapid gill movement | Fish died |
| 13-14 | Erratic swimming | Gasping for air | |

The study results indicate that *Carassius auratus* (Goldfish) displayed rapid and erratic swimming behavior when exposed to varying caffeine concentrations, with fatalities occurring around the 7-8th day post-exposure. *Pterophyllum sp.* (Angelfish) were notably affected, experiencing mortality within three days post-caffeine exposure, alongside observed slow swimming. In contrast, *Pangasius hypophthalmus* (Albino shark) exhibited increased activity, erratic swimming, rapid movements, and sluggish behavior when exposed to caffeine.

**Pictures taken during the experiment :**

**Fig 14.**

**Fig 15.**

**Fig 16.**

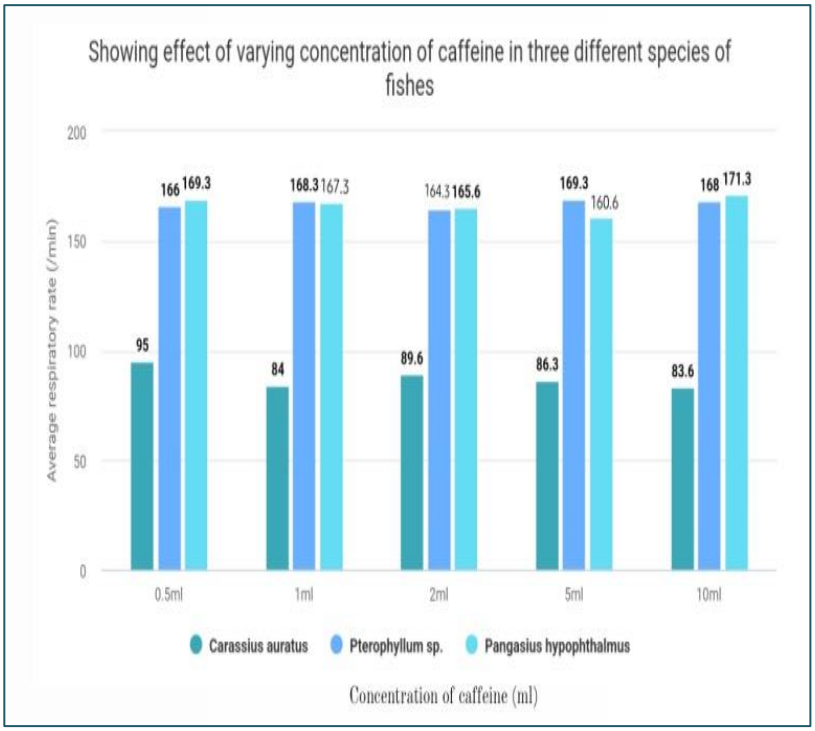
## Comparative study

**Table 7. Showing the effect of varying concentration of caffeine in three different species of fishes**

| Conc. of caffeine | Average respiratory rate of *Carassius auratus* (/min) | Average respiratory rate of *Pterophyllum sp.*(/min) | Average respiratory rate of *Pangasius hypophthalmus* (/min) |
|---|---|---|---|
| 0.5ml | 95/min | 166/min | 169.3/min |
| 1ml | 84/min | 168.3/min | 167.3/min |
| 2ml | 89.6/min | 164.3/min | 165.6/min |
| 5ml | 86.3/min | 169.3/min | 160.6/min |
| 10ml | 83.6/min | 168/min | 171.3/min |

Based on the respiratory rate responses observed, it is evident that *Carassius auratus* (Goldfish) exhibit a notable decrease in respiratory rate when exposed to caffeine. In contrast, *Pterophyllum sp*. (Angelfish) display fluctuating respiratory rates, showing both increases and decreases. *Pangasius hypophthalmus* (Albino shark), on the other hand, experience an increase in respiratory rate. These findings suggest that Goldfish are more susceptible to caffeine contamination compared to Angelfish and Albino Sharks, as evidenced by the distinct decrease in their respiratory rate following exposure.

The above data is expressed in the form of a bar diagram



# DISCUSSION

The study on caffeine's effects on respiratory rates and swimming behaviors of fishes showed that caffeine can decrease respiratory rates and alter swimming behaviors, including erratic swimming, rapid gill movement, and slow swimming behavior. This suggests that caffeine can have significant physiological effects on fish, which could have cascading impacts on fish populations and ecosystems. The study on sertraline's and caffeine's effects on spatial learning in *Carassius auratus* (goldfish) by Cooper callings and Marianne Robertson (2021), revealed that exposure to these chemicals can negatively affect spatial learning abilities, highlighting the need for improved water treatment and pharmaceutical disposal methods. This study suggests that sertraline and caffeine, can have significant impacts on fish behavior and cognition, which could have important implications for fish populations and ecosystems. A comparison of the two studies reveals that both caffeine and sertraline can have significant impacts on fish behavior and physiology. However, the mechanisms by which these substances exert their effects are likely to be different. Caffeine's effects on fish appear to be related to its stimulant properties, which can alter swimming behaviors and respiratory rates. In contrast, sertraline's effects on spatial learning in goldfish suggest that it may be affecting the brain's ability to process spatial information, which could have important implications for fish navigation and behavior. Overall, these studies highlight the need for further research into the impacts of pharmaceuticals and other substances on aquatic ecosystems.

The study by N. B Goodson *et al,*. (2015) examined the effect of chronic caffeine exposure on the recovery of the DLR in *Carassius auratus* (goldfish) with unilateral vestibular organ removal. The results showed that all caffeine-treated groups exhibited significant changes in DLR on day 1, while the control group recovered and approached sham surgery DLR by the end of the study. However, caffeine groups exhibited a deterioration of postural control from day 10 onwards, with all three caffeine groups significantly deficient compared to the control on days 10-24. These findings suggest that high dosage or prolonged caffeine exposure can hinder functional recovery. The study based effect of caffeine in Respiratory Rate and Swimming Behavior of Three Different Fish Species revealed that caffeine has a detrimental effect on both the respiratory physiology and swimming behaviors of the fish studied. The decrease in respiratory rate and the abnormal swimming patterns observed are likely due to the physiological impacts of the caffeine exposure. Both studies observed changes in fishes in response to caffeine exposure.

Swimming behavior of the study can be compared with the effect of caffeine on the heart rate of the common goldfish, *Carassius gibelio* by Dr. Michelle Gibson (2014) and found that caffeine decreased heart rate and increased ventilation in a concentration-dependent manner under restraint conditions. The data was collected by placing the fish in 100g of caffeine diluted in 1000mL of distilled water and recording the number of opercular movements at a consistent rate. The analysis of the data involved comparing the opercular movements before and after exposure to caffeine to determine the effects of caffeine on the fish's heart rate. However, the study did not find a significant change in average swimming speed under low caffeine exposure, and a high concentration of caffeine exposure significantly decreased swimming speed. Both studies demonstrate that caffeine has complex, concentration-dependent effects on fish physiology and behavior, including impacts on respiratory, heart rate, and locomotion.

The study by Yuki Harada et al (2022) examined the ventilation and heart rate changes in adult zebrafish (*Danio rerio*) exposed to varying caffeine concentrations under both restrained and free-swimming conditions. They found that under restraint, caffeine significantly decreased heart rate and increased ventilation in a concentration-dependent manner. However, during free-swimming, ventilation rates rose with higher caffeine concentrations, demonstrating the importance of assessing chemical effects in unrestrained conditions. In contrast, the study on respiratory rate and swimming behavior of fish under influence of caffeine focused on the behavioral effects of caffeine on fishes, reporting that increased caffeine concentrations led to decreased respiratory rates and altered swimming behaviors, such as erratic swimming, rapid gill movement, and slow swimming. This suggests that caffeine has a biphasic effect, initially stimulating ventilation but then depressing it at higher doses. The combination of physiological and behavioral endpoints provides a more holistic view of how chemicals can affect aquatic organisms.

# CONCLUSION

A study was conducted to investigate the impact of caffeine on the respiratory rate and swimming behavior of various species of fish. The research aimed to explore how caffeine exposure influenced these physiological and behavioral aspects in fish. By examining multiple species, the study sought to understand if there were consistent patterns or species-specific responses to caffeine.

The study was conducted to investigate the effects of varying caffeine concentrations on the respiratory rates and swimming behaviors of three different fish species: *Carassius auratus* (Goldfish), *Pterophyllum sp*. (Angelfish), and *Pangasius hypophthalmus* (Albino shark). The experiment was carried out in a 19L aquarium, which was filled with small live plants, stones, pebbles, and an aerator to create a complete aquarium setup. 5L of water at normal temperature was added to the tank, and three fish of each of the three species were used for the experiment.

The first fish introduced into the tank was *Carassius auratus* (Goldfish 1). The fish was kept in the water for 10 minutes, and then caffeine was introduced into the tank using a dropper, starting with 0.5 ml. The fish was given 20 minutes for the caffeine to take effect, and its respiratory rate was determined by counting the number of times the operculum (gill cover) flapped or the number of times the fish opened and closed its mouth in 1 minute (60 seconds). The process was repeated with increasing concentrations of caffeine (1 ml, 2 ml, 5 ml, and 10 ml), and the respiratory rate was noted down. The same procedure was then repeated with the other two Goldfish (Goldfish 2 and Goldfish 3), and the entire process was then repeated with the *Pterophyllum sp*. (Angelfish) and *Pangasius hypophthalmus* (Albino shark) fish. The swimming behavior of the fishes was observed closely and recorded through videography.

The results showed that *Carassius auratus* (Goldfish) exhibited a notable decrease in respiratory rate when exposed to caffeine. In contrast, *Pterophyllum sp*. (Angelfish) displayed fluctuating respiratory rates, with both increases and decreases observed. *Pangasius hypophthalmus* (Albino shark), on the other hand, experienced an increase in respiratory rate when exposed to caffeine. In terms of swimming behavior, *Carassius auratus* (Goldfish) displayed rapid and erratic swimming, with fatalities occurring around the 7-8th day post-exposure. *Pterophyllum sp.* (Angelfish) were notably affected, experiencing mortality within three days post-caffeine exposure, alongside observed slow swimming. *Pangasius hypophthalmus (*Albino shark) exhibited increased activity, erratic swimming, rapid movements, and sluggish behavior when exposed to caffeine.

The comparative analysis suggests that *Carassius auratus* (Goldfish) are more susceptible to caffeine contamination compared to *Pterophyllum sp*. (Angelfish) and *Pangasius hypophthalmus* (Albino shark), as evidenced by the distinct decrease in their respiratory rate following exposure. These findings highlight the importance of understanding the differential responses of various fish species to environmental contaminants, which can inform conservation efforts and environmental management strategies.

In summary, the findings of this study emphasize the significance of considering the impact of caffeine on fish respiratory rates and swimming behavior across various species. This research highlights the importance of continued investigation into the effects of environmental contaminants on aquatic life to support informed conservation practices and ensure the sustainability of our marine ecosystems.

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