The Impact of Temperature Change on the Aggressive Behaviours of Siamese Fighting Fish

Research Proposal

INTRODUCTION

            Multiple studies have demonstrated that temperature, as one of the major abiotic factors in aquatic ecosystems, has a significant effect on respiration, energy budgets, locomotion performance, growth, and behavior of aquatic ectothermic species.1The researchers further point out that the investigation also shows that fish, as a dominant group of the ectothermic animals, characteristically experiences a broad range of temperatures across days and nights. Since the temperature has a considerable direct influence on the metabolic rates of fish, including the rest of the animals that have been categorized as ectotherms, there is a high chance for it to have large effects on their physiology and behavior.2 For instance, annual increases in temperature may result in rises in both activity and risk-taking in fish, which in turn has a correlation with elevated predation mortality.1The existing pieces of empirical evidence on the effects of temperature on the behavioral traits have not been explored sufficiently in fish in contrast to the physiological endpoints.3,4 In this respect, most of the studies on the temperature dependence of the behavior of ectotherms, in particular fish species, have placed significant emphasis on foraging and swimming,5which are closely linked to survival and growth indices. In the recent years, nonetheless, there has been a rapid increase in scholarly interest in the fitness consequences of temperature changes in several aquatic organisms, particularly fish.1

            Subsequently, the male Siamese fighting fish (*Betta splendens*) are typically known to be highly territorial and aggressive towards any intruder conspecifics.1, 6Castro et al. explain that in aggressive interactions, the male Siamese fighting fish competitors often exhibit a ritualized order of signal behaviors, also referred to as displays.6 The researchers add that during this ritualized sequence of signal behaviors, these male competitors exchange information concerning their readiness and ability to engage in a fight. It is argued that both competitors are thought to benefit from exhibiting such ritualized sequence of signal behaviors by resolving the conflict in question without costly escalated fighting. Accordingly, these sequential typical behaviors of the male Siamese fighting fish explain the evolution of communication technique amongst the ectotherms. In particular, the male aggressively defend their territory; while doing this they utilize several highly conspicuous and stereotyped visual displays.6 It is interesting to point out that by using displays of their readiness and ability to fight to reduce the costs associated with escalated fights, it is possible for those animals that are not prone to cheat by displaying higher fighting capability than they can sustain.6 Moreover, studies that have evaluated the costs of fights between the male Siamese fighting fish have shown increased metabolic rates, the depletion of the energy reserves, and damage in ectotherms. Similarly, multiple pieces of empirical evidence have also demonstrated that there is a close correlation between temperature and metabolic rates.7, 8, 9

Incidentally, water temperature beyond the optimal range has been found to possess profound influence on the growth and formation of fish.7Thus, it can be inferred that this, in turn, has an effect on the behavior of fish because the growth and formation of fish also affects how an organism acts in a particular setting.10For example, in a study that involved seven-band grouper larvae *Epinephelus septemfasciatus*, it was established that there was a tendency for those animals that were reared at elevated temperatures to grow faster as opposed to the cohorts in cooler water.11Nevertheless, Tsuji et al. observed that the survival rates of these species were highest in the optimal temperature range of 25 °C and 26 °C. A separate research has shown that the growth of brown *Salmo trutta* is considerably lower at fluctuating temperatures.12Furthermore, the researchers also established that when the fluctuating water temperatures are coupled with low water levels, the *Salmo trutta*exhibit negative growth.  The implication of this empirical evidence is that the growth of ectotherms, including the male Siamese fighting fish, is influenced by the temperature of the surrounding water in which they inhabit. According to Flodmark et al., these findings suggest that both water temperature and stability are essential during teleost growth and development.12 Therefore, this effect on growth and, therefore, on energy requirements, also has an effect on behavior. In fact, it is worthwhile observing that with the increased metabolic rates arising from increased growth rate, organisms such as the male Siamese fighting fish are more predisposed to taking risks during foraging, a behavior they would otherwise avoid in optimal or low metabolic rates.13

Parenthetically, research has demonstrated that temperature is the most important environmental parameter that has an effect on the reproductive biology of fish.14The researchers add that this potent environmental factor is important in maintaining the testes of fish when it is reproductively active. In their research, Srikrishnan et al. observed that in order for reproduction to be successful in the male Siamese fighting fish, the water temperature should range between 25 °C and 28 °C. The rationale for this water temperature range is that it provides the requisite conditions for the male Siamese fighting fish to form their bubble nest for purposes of mating.14, 15 It is important to note that research has indicated that although the male Siamese fighting fish is able to construct a bubble nest in a broad range of temperatures, from 25°C and 29°C, while an ideal temperature for the construction of a bubble nest is 28°C.14

In contrast, there is no contention in the scholarly community that temperature has significant effects on the metabolic rate as well as some of the important physiological processes, which in turn can alter behavioral responses.1There is sufficient empirical evidence that exposure to elevated temperature has significant effects on the daily activity rate of the male Siamese fighting fish.5,16In particular, various studies show that temperature has pronounced effects on the locomotion activities of fish, for example, the swimming performance and the opercula movements of the fish. Furthermore, research has also revealed that temperature has the ability to alter the risk assessment and energy demands of the ectotherms1, thereby increasing the levels of activity of individuals which in turn improves their predation risk. Forsatkar et al. point out that exclusively in male Siamese fighting fish, it has been demonstrated that the opercula display rates often decline under conditions of reduced dissolved oxygen, for example, hypoxia. The researchers argue that since the opercula rate is recognized as an important predictor of success in the male-male interactions, and that the frequency of hypoxic conditions is appreciated as one of the most evident outcomes of increasing temperatures, it means that it is correct to conclude that elevated temperature can decrease the possibility of success in the contacts between the male Siamese fighting fish. However, the Siamese fighting fish is equipped with an air-breathing organ whose work is to eliminate the requirement for oxygen during display behavior.

Successively, research has also shown that the latency to move is one of the primary behaviors measured when examining for boldness variations between individuals.17 In particular, the research has revealed that there is a tendency for the bolder individuals not only to react faster but also move sooner than their wary counterparts after being released into a novel environment. In their study, Forsatkar et al. found out that fish that has been held in 4°C warmer water exhibit a significant decrease in latency so as to leave the release area for the boldness assessment proves.1It is important to note that the mechanistic influence of temperature on fish has in the recent years received considerable attention from researchers.13The researchers add that these pieces of empirical evidence on the mechanistic impact of temperature on fishes has shown that changes in the thermal environments have a direct impact on their physiology. The overall conclusion that Forsatkar et al. draw from their research is that the Siamese fighting fish that have been exposed to increased temperatures tend to reduce their latency to leave the point of release, spend more time in an area that is farthest from the point of release, and are generally active.1The particular inference of Forsatkar’s et al. finding is that the behaviors of the Siamese fighting fish reflect that they are “more bold” when the water temperature increases and vice-versa.

In the preceding discourse, it was pointed out that multiple studies recognized temperature as one of the major abiotic factors in aquatic ecosystems, and one with a significant effect on respiration, energy budgets, locomotion performance, growth, and behavior of aquatic ectothermic species. The implication of this empirical position is that there are other abiotic parameters that have, in one way or another, an effect on the physiology of ectothermic species. In this respect, pH has been identified as one of the core parameters that influence the physiological processes in both male and female Siamese fighting fish.18,19Interestingly, the impact of pH on the physiology of organisms has a correlation with temperature. In other words, these two factors, in combination, affect the metabolic rates of organisms by interfering with the rate at which enzymes activate various physiological processes.19In their study, Thongprajukaew et al. sought to determine the influence of temperature and pH profiles of lipase and amylase at various developmental stages in Siamese fighting fish. The researchers observed at least nine amylase activities during development: pH 7 at 40°C, and 50° C, pH 8, and pH 11 at 50°C in females, and pH 8 and pH 11 at 40°C and 60°C in males. Thongprajukaew et al. concluded that the ideal pH to determine lipase and amylase activity in the Siamese fighting fish is pH 8.19 On the other hand, the temperature of 50°C is suitable for amylase and 40°C is appropriate for lipase, regardless of the age and sex of the Siamese fighting fish. Accordingly, the findings by Thongprajukaew et al. provide a fundamental basis for inferring that pH, alongside temperature, has an indirect impact on the metabolic rates of the Siamese fighting fish. The observed suitable temperatures for the action of the enzymes lipase and amylase definitely affect the rate at which metabolism takes place.

Research shows agonistic interactions that take place between individuals that are within the same category of species accentuate phenotypic traits over evolution time.20In fact, these traits are usually most evidently expressed among the males, although it cannot be argued that this is always the case. Reagan et al. contend that although the external morphological features can often be the most obvious, the underlying phenotypes of the animals in question are essential for supplying the energy that is needed to support the agonistic interactions.20As mentioned earlier, the Siamese fighting fish, in particular, the males, are highly territorial and aggressive towards any intruder conspecifics. Therefore, this can only mean that their underlying phenotypes have proper adaptive mechanisms to enable them to engage in fierce fights with any intruder conspecifics.

Although it is not yet confirmed, it may be argued that the traits of the underlying phenotype of the Siamese fighting fish are largely drawn from their genotype and the environment in which they inhabit. In this respect, the environment of this fish includes all the abiotic conditions that surround them in their habitat, for example, water pH and temperature. Essentially, the temperature has a direct impact on the biochemical activities within the body of the Siamese fighting fish and, consequently, affects the metabolism rate. It is important to point out that biochemical capacities tend to have significant implications for predator avoidance, physiological performance, resource acquisition, social behavior, and aggression.1,12,20

Agonistic interactions entail a substantial metabolic cost, as exhibited by the increases in whole-animal aerobic metabolism as well as oxygen consumption rate.6,20Moreover, the metabolic demand created by the rapid and exhaustive sessions of activity during aggressive behavior of the Siamese fighting fish can outstrip the capacity for both oxygen supply and aerobic metabolism. Consequently, this contributes towards increased reliance on the anaerobic metabolism supported by the substrate-level phosphorylation through anaerobic glycolysis and creatine phosphate (CrP).19,20Therefore, it can be concluded that the capacities for energy metabolism in the Siamese fighting fish can restrict the success of agonistic interactions and also result in potentially adverse consequences for fitness. It is worth pointing out that despite the significance of energy supply, only very few pieces of empirical evidence have examined the relationship between the metabolic capacity and aggressive behaviors, particularly with respect to the cellular biochemical machinery.21Since temperature is a critical abiotic factor in both aerobic and anaerobic processes, it is argued that it plays a pivotal role in determining the metabolic rates which in turn affects the aggressive behavior of the Siamese fighting fish.

According to Reagan et al., more aggressive male Siamese fighting fish exhibit considerably higher aerobic capabilities in the locomotory muscle than the less aggressive male fighting fish.20Meanwhile, the more aggressive male Siamese fighting fish possess only a modestly considerable use of the anaerobic ATP production. Therefore, the oxidative phosphorylation that is evident in these aggressive males can fuel a significant fraction of the muscle activity during the aggressive encounters and at the same time, can speed up the recovery from the muscle bursts linked to each striking episode. In the same breadth, studies have confirmed that the more aggressive individuals often tend to surface more frequently during fights, presumably to have access to the aerial source of oxygen for purposes of helping them meet the increased demand for oxygen when striking their opponents.7,16,20The striking characteristic of all these events relating to the aerobic activities is that they are somewhat dependent on water temperature because this important abiotic factor is also responsible for determining the amount of dissolved oxygen in the water.22 Water temperature is, without doubt, one of the most important characteristics of any aquatic system.18,20The basic principle as to the dissolved oxygen levels is that the solubility of oxygen reduces as water temperature increases. In other words, an extremely high water temperature means that the aquatic life would not have sufficient oxygen for metabolic utilization.

Moreover, the enzymes responsible for catalyzing various metabolic reactions within the cells of the aquatic organisms are likely to be denatured. The overall implication of this is that the aquatic living beings, for example, the Siamese fighting fish, would in the long-run die. In contrast, extremely low water temperatures mean that it would be impossible for the enzymes responsible for metabolism to activate the requisite metabolic reactions in the Siamese fighting fish. Therefore, a fish would in the long-term die due to the absence of the different metabolic reactions that are responsible for supporting and maintaining its life. Subsequently, Reagan’s et al. findings suggest that the biochemical capacity for the aerobic metabolism in Siamese fighting fish muscle is, in fact, a crucial determinant of the inter-individual distinction in aggressive behavior, presumably more significant than the utilization of and the capacity for anaerobic metabolism.20

Overall, it is evident from the preceding discussion and background information that water temperature is one of the most critical abiotic factors that have a direct impact on metabolic rates of ectotherms, including the Siamese fighting fish, which in turn influences the aggressive behavior that is often displayed by the same animals. In particular, this explicit observation drawn from the existing pieces of empirical evidence confirms the hypothesis of the present research: temperature directly impacts metabolic rates of Siamese fighting fish which in turn impacts aggressive behavior. In order to widen the existing scientific evidence on how water temperature indirectly influences the aggressive behavior of the Siamese fighting fish, the present research seeks to develop a concise methodological approach that can be utilized to measure the extent to which temperature affects the metabolic rates of the Siamese fighting fish subsequently affecting the aggressive behavior they display in their habitat. In a nutshell, the following section provides a concise discourse on how the impact of temperature on metabolic rates of Siamese fighting fish can evaluate from both a quantitative and quantitative perspectives and the relationship of the obtained results with the aggressive behavior displayed by the fish.

MATERIALS AND METHODS

The initial step in developing the methodology for the current study involves the identification of the suitable research species. In this respect, the researcher recommends that a total of forty mature Siamese fighting fish should be acquired from a reputable distributor. Specifically, twenty of the purchased fish should be male and the rest female and more importantly, their phenotypic characteristics should explicitly reveal that they are healthy. The fish should be transported to the laboratory and kept separately in opaque 0.5 L plastic containers so as to limit visual contact between them. It is important to reiterate that the Siamese fighting fish are naturally territorial and can become highly aggressive when they feel that their space is threatened by other animals or fish. If transparent plastic containers are used in this experiment, there is a considerable predisposition that the fish in these containers may become aggressive to defend their territory from being invaded by the fish in the “other” container. The temperature of the room in which the experimental Siamese fighting fish should be kept shall be maintained continuously at between 20°C and 24°C.1 In order to establish conditions with higher as well as constant temperatures, the fish containers shall be placed into a large water-bath. It is suggested that the water bath should be made of a wide, short-wall rectangular plastic container with water filled to a depth of about 7-8 cm.1 It is worth pointing out that the water that is used in the water-bath in which the opaque 0.5 L plastic containers with the fish is to be heated using the available aquarium heaters until the desired water temperature is achieved. Research has shown that the Siamese fighting fish prefer water that is between 25°C and 31.5°C.1 Nonetheless, a broad range of temperature, between 21°C and 30°C, have in many cases been used to maintain the fish in a laboratory setting.3,5,16 The researcher should feed the fish with libitum once every day with formulated pellet diet and frozen blood worms. The water in each of the holding containers should be completely changed every three days. Meanwhile, the photoperiod in the laboratory shall be adjusted to between 14 hours’ light to 10 hours’ dark, with lights being turned on at 08.00.1 The fish shall be kept in these conditions for fourteen days so as to ensure that they have acclimatized to the new environment before the temperature is manipulated.

Subsequently, the experimental design for the research shall entail the selection of thirty size-matched fish being drawn from the stock population before the temperature is manipulated. The selected fish are to be allocated into one of the two groups of 15 using a random approach, and their behavior is to be observed with a goal of obtaining the baseline for boldness. On completion of this phase, the researcher is to randomly designate one of the groups and control whether the trial is maintained at the normal temperature of 26°C.1 On the other hand, the other group shall be assigned a high-temperature treatment in which the subjects are kept at 30°C. In this proposed research approach, the high temperature of 30°C shall be used to stimulate the average temperature during their breeding season in their natural habitat. The other rationale for using this high temperature of 30°C is that previous studies have used it to investigate the behavior of the Siamese fighting fish.1 The stated temperature shall be attained by transferring the fish containers to the water baths. The light regime used during the acclimatization period shall be maintained throughout the trials. The temperature manipulation for the behavioral observations shall be carried out for 10 days. There are at least two reasons that justify the selection of 10 days as the period for manipulating the temperature conditions: (i) it is within the acclimation period that previous studies have employed when investigating the Siamese fighting fish, and (ii) the majority of the previous experiments that have previously quantified the behavioral responses of the Siamese fighting fish, which were carried out in a timeline of one to two weeks.1

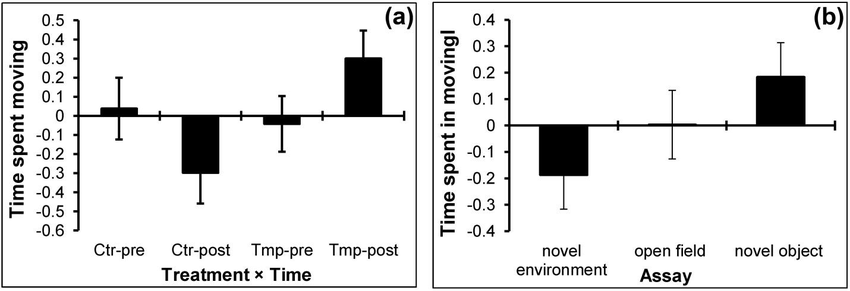
Incidentally, two identical rectangular glass tanks shall be used as the experiment apparatus when assessing the boldness of the Siamese fighting fish. The researcher shall cover the back and the side walls of the tanks by white opaque objects to eliminate any potential disturbances to the behavior of the fish. The front of the tanks shall be left uncovered, and a video camera should be set at 1 meter to record the fish behavior. The experimental apparatus shall be filled to a depth of 20cm with treated tap water and the bottom left empty. A 30-watt fluorescent lamp shall be used to light the tank from above and the water temperature in the test tanks kept at 26°C and 30°C for the control and the high-temperature treatment respectively.

In the present study, the researcher shall draw lines on the outside of the test tanks with the objective of dividing them into three equal sections. The area where the fish were first placed shall be designated as an area I, the middle identified as area II, and the opposite end designated as area III.  The fish in this experiment apparatus shall be acclimatized for 10 days after which the individuals are to be tested over three subsequent days. The temperature is then to be manipulated in the treatment group only and the behavior observations recorded. The fish in all the tanks are then tested again after 10 days’ exposure to varied water temperature. The general linear mixed-effects models shall be used to analyze the collected data, assuming the normal errors.

CONCLUSION

Multiple studies have identified temperature as one of the most important factors that regulate the metabolic rates of the Siamese fighting fish. Fundamentally, this abiotic factor achieves this by providing the pre-requisite conditions that are necessary for biochemical reactions within the cell structure of the fish, including both the aerobic and anaerobic processes, and activation of the enzymes responsible for catalytic actions. The metabolic rates in the Siamese fish require optimum temperature, which is projected as 28°C. Apart from influencing the metabolic rates of the Siamese fighting fish, temperature also impacts on their aggressive behavior. The overall conclusion is that temperature has a direct impact on the metabolic rates of the Siamese fighting fish, which in turn influences aggressive behavior.

Tables and Figures



Appendix A: Fish Latency in the various conditions

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