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Research Article

Parasitic Infection linked White Spot Disease (*Ichthyophthirius Multifiliis*) Modulating Behavior Alterations, Feeding and Stress Response in Male Betta splendens (Regan 1910)

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ABSTRACT

The Betta splendens (Regan, 1910) commonly known as fighting fish, the male reproductive success is strongly reliant on energetically costly parental commitment. It has been extensively researched that parasitic infections have a major impact on host physiology, behaviour, and ecological relationships. This study examines the effects of Ichthyophthirius multifiliis-caused White Spot Disease on eating habits, stress reactions, and behavioural changes in Betta splendens (Regan, 1910). In order to determine changes in aggression, activity levels, territoriality, and food response, experimental groups of infected and non-infected individuals were monitored in a controlled laboratory setting. Significant behavioural abnormalities, such as decreased locomotors activity, decreased territorial aggression, and increased erratic movements, were present in infected individuals and were suggestive of physiological discomfort. When compared to controls, the feeding response was much reduced in infected fish, indicating a reallocation of energy toward immune function. Diseased specimens showed much higher levels of stress markers, which were deduced from behavioural indicators including surface gasping and seclusion inclinations. The results emphasise how a major ecological element influencing host behaviour and resource use is parasite infection. Niche dynamics and competitive interactions in aquatic ecosystems may be impacted by changes in aggression and eating behaviour. This research advances our knowledge of host-parasite interactions and their wider consequences for behavioural ecology and fish health management.

1. Introduction

In aquatic organisms, parasitic infections are a significant ecological and physiological stressor that affects host behaviour, fitness, and survival. Among these, Ichthyophthirius multifiliis-caused White Spot Disease is one of the most common and harmful protozoan infections that afflict freshwater fish, causing respiratory distress, epidermal damage, and higher mortality rates. This infection is especially important in ornamental and lab species like Betta splendens (Regan, 1910) because it directly affects behaviour, stress physiology, and feeding performance all important aspects of ecological fitness. The Siamese fighting fish, or Betta splendens, is a well-known model organism in behavioural ecology because of its strong territoriality, hostility, and intricate social relationships. Opercula flaring, fin spreading, and biting are examples of typical aggressive behaviours displayed by males, which are mostly motivated by resource competition and territory defence. Research by Oliveira et al. (2022) and Forsatkar et al. (2014) has shown that this species' aggressive and territorial behaviour is greatly influenced by both environmental factors and neuroendocrine parameters. Additionally, it has been demonstrated that environmental factors like social isolation, geographical limitations, and enrichment affect stress reactions and behavioural manifestation in B. splendens.

However, by changing host behaviour through physiological stress and energy reallocation, parasitic diseases add another level of ecological complexity. Clinical signs like anorexia, irregular swimming, and decreased activity have been linked to I. multifiliis infection, which may directly affect feeding efficiency and predator avoidance. From an ecological standpoint, these behavioural changes can affect population dynamics and species interactions by interfering with niche utilisation, weakening territorial defence, and decreasing competitive ability. Hadadi (2019) demonstrated the significance of I. multifiliis in ornamental fish systems by reporting a detectable prevalence in B. splendens. Few studies have specifically looked at how parasite infections impact behavioural changes in conjunction with stress and food responses, despite a wealth of study on aggression and environmental regulation in Betta splendens. In the context of behavioural ecology, it is crucial to comprehend these relationships, especially when it comes to energy trade-offs between immunological function and behavioural performance. In order to shed light on host-parasite interactions and their ecological ramifications, the current study attempts to examine how White Spot Disease (Ichthyophthirius multifiliis) affects behavioural patterns, stress indicators, and food behaviours in Betta splendens.

1. Materials and Methods

2.1. Study Area and Experimental Design

The experiment was conducted over 21 days in two experimental glass tanks. Before being used in experiments, adult male Siamese fighting fish, Betta splendens (Regan, 1910), were purchased from a licensed ornamental fish breeder which was already infected and allowed to acclimatise to lab conditions for two weeks. It was carried out in a controlled laboratory setting at the Rajarshi Shahu Mahavidyalaya Empowered Autonomous Latur Research Centre's aquaculture and fish behaviour research facility. In order to retain their natural territorial behaviour and prevent social meddling, healthy specimens of Betta splendens (Regan, 1910) were kept in separate glass aquariums. In order to provide ideal circumstances for both host and parasite development, each tank was filled with dechlorinated waters and kept under standardised physicochemical parameters, such as temperature (26–28 °C), pH (6.5–7.5), and dissolved oxygen levels above 5 mg/L.

2.2 Behavioral Patterns Analysis

Before the experiment, adult male Betta splendens (Regan, 1910) of consistent size and age were chosen and acclimated for 10–14 days in a controlled laboratory setting. In order to imitate the presence of an intruding conspecific and elicit territorial reactions, individual fish were exposed to a mirror mounted externally against the aquarium wall for ten minutes as part of a mirror-stimulus test for behavioural and aggressive pattern analysis. Standardised measures, such as opercular flaring frequency, fin display duration, number of charges and biting attempts, and latency to first response, were used to quantify aggressive behaviours.

To reduce observer bias, all trials were recorded and subsequently analysed, and observations were made at a specific time of day to prevent circadian variation. To determine the impact of parasite infection on aggression, fish were split into control (non-infected) and experimentally infected groups with Ichthyophthirius multifiliis. Behavioural evaluations were carried out at regular intervals after infection. The frequency and duration of behavioural displays were combined to create an aggression index, and statistical analysis was carried out using suitable tests (such as the t-test or ANOVA), with significance taken into account at $p < 0.05$.

2.3 Feeding Pattern Analysis

In a controlled laboratory setting, feeding pattern analysis in Betta splendens (Regan, 1910) was used to assess alterations linked to Ichthyophthirius multifiliis infection. Fish were given a standardised diet (commercial pellets/live feed) at predetermined intervals twice a day after acclimation, and feeding trials were conducted separately to prevent competition. The quantity of food items consumed in a set amount of time (5–10 minutes), frequency of feedings, delay to initial feeding, and occurrences of food refusal were all recorded in order to evaluate the feeding response. To track progressive changes, observations were made at regular intervals following infection for both the experimentally infected and control (non-infected) groups. Measures of eating impairment included behavioural signs such decreased appetite, slow feeding, or total anorexia. To guarantee accuracy and reduce observer bias, all trials were videotaped, and feeding indices were computed using response time and consumption rate. Using suitable tests (such as the t-test or ANOVA), statistical comparisons between groups were carried out, with significance taken into account at $p < 0.05$.

2.4 Stress and Swimming pattern Analysis

In order to evaluate behavioural changes linked to Ichthyophthirius multifiliis infection, stress and swimming pattern analysis in Betta splendens (Regan, 1910) was carried out under controlled laboratory circumstances. To provide accurate and objective assessments, individual fish were observed in separate aquariums, and swimming behaviour was recorded using video monitoring for a predetermined amount of time (10–15 minutes). Total distance travelled, swimming speed, frequency of directional changes, vertical distribution in the water column, and the prevalence of aberrant behaviours such erratic swimming, lethargy, surface gasping, and bottom residing were among the key variables examined. Behavioural proxies, such as decreased activity, increased immobility, loss of homeostasis, and tendencies toward isolation, were used to predict stress responses. To assess the escalating stress responses during infection, observations were made at regular intervals for both the experimentally infected and control (non-infected) groups. To reduce observer bias, all recordings were examined after the fact, and quantitative indices for stress and activity were computed. Using suitable techniques (such as the t-test or ANOVA), statistical analysis was carried out, with significance established at $p < 0.05$.

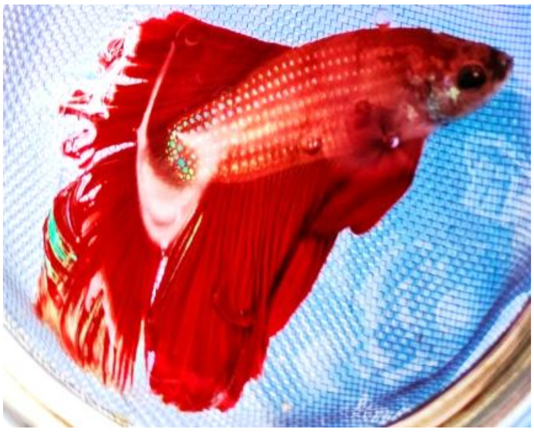
2.5 Statistical Analysis

Before performing the univariate analysis of variance (ANOVA), it was first confirmed that the variance of the data met the requirements. When the value was greater than 5% ($p>0.05$), the Shapiro-Wilk test and the Levene test were used to confirm the normality and homogeneity assumptions, respectively. When the assumptions were met, the data were presented to an ANOVA, considering that there was a difference when the value was below 5% ($p<0.05$), and in this instance, they were submitted to the Tukey test for the comparison of means; this was also done using the 5% threshold. Utilizing the statistical program SPSS, every statistical analysis was carried out.

2. Results and Discussion

3.1 Visual Behavioural Alterations in Male Betta splendens

To illustrate the behavioral patterns seen in Betta splendens under experimental conditions, a number of example photos were included. Important features like eating habits, swimming activity, hostility, and wooing behavior toward females are highlighted in these pictures. Visual documentation improves

 Fig.1. Wild Type Red Rose tail Male *Betta splendens* Without Parasitic Infection.	 **Fig.2** Bottom dwelling Swimming pattern of Male *Betta splender*
 **Fig.3** Aggression of red *Betta splendens* towards female fish.	 **Fig.4** Male vs. Male Aggression and competition in *Betta Splendens*.
 **Fig.5** Operculum Flaring and Chasing patterns in *Betta splendens*.	 **Fig.6** Trans Boundary Aggression and zigzag swimming patter in *Betta splendens*.
 **Fig-7.** Loss of Appetite and changes in swimming Patterns in *Betta splendens*.	 **Fig.8 Complete body transformation in White spot disease ir Red *Betta splendens*.**

the comprehension of stress-induced reactions and offers supporting evidence for the observed behavioral differences. When taken as a whole, these findings provide a thorough picture of the behavioral modulation connected to the experimental treatments.

3.2 Behavioral Patterns Analysis

A comparison of the aggressive behaviours of Ichthyophthirius multifiliis-infected and wild-type (non-infected) Betta splendens revealed a distinct difference. Over the course of the observation period, wild-type fish exhibited relatively stable levels of aggression (mean aggression index: 7.2 ± 0.5). In contrast, infected fish displayed a significant initial increase in aggression, peaking shortly after infection (peak index: 9.0 ± 0.6), followed by a gradual decline to lower levels (final index: 2.8 ± 0.4) as the disease progressed. Irritation and acute physiological stress may be the cause of this brief increase, whilst energy depletion and decreased activity are the reasons for the subsequent decrease.

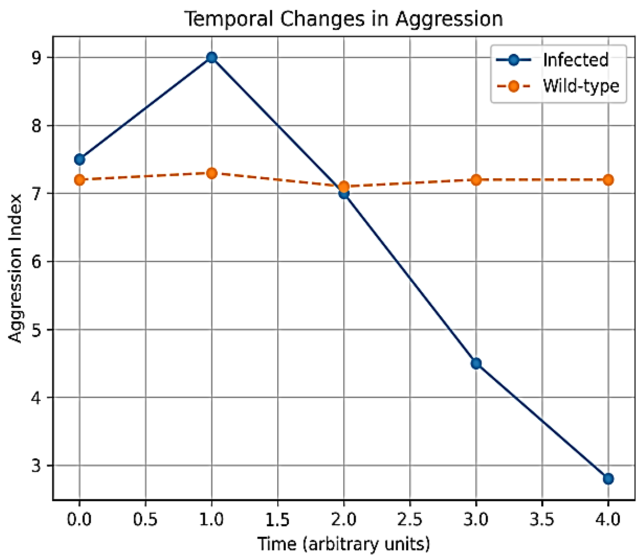


Fig.9 Temporal aggression changes

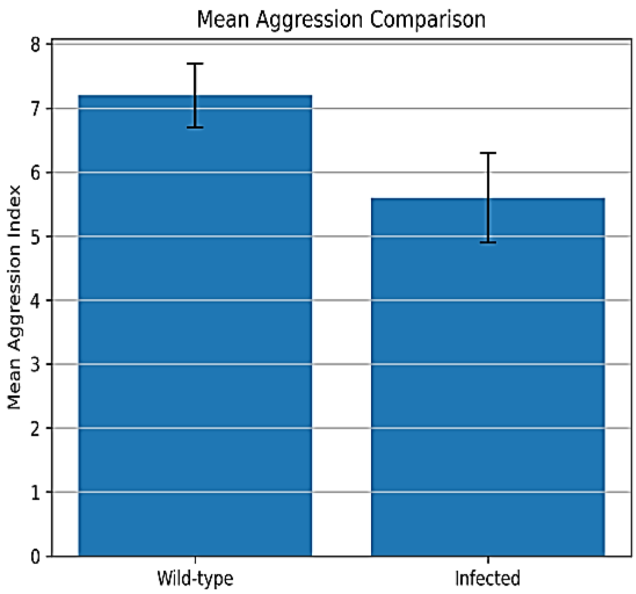


Fig.10. Mean aggression comparison

Overall, the average aggression of infected fish (5.6 ± 0.7) was lower than that of controls, suggesting that territorial behaviour has been suppressed over time. These patterns are demonstrated in the modulatory effect of White Spot Disease on aggression with implications for territorial dominance and an ecological interaction is illustrated in Figures 9 (temporal aggression changes) and 10 (mean aggression comparison).

3.3 Feeding Pattern Analysis

The feeding habits of wild-type (non-infected) and Ichthyophthirius multifiliis infected Betta splendens were found to differ significantly. While infected fish showed a marked decline in feeding response, starting with a slight reduction and progressing to pronounced anorexia (final intake: 38 ± 6%; latency: 9.4 ± 1.2 s), wild-type fish showed consistent feeding behaviour with a high consumption rate (mean intake: 92 ± 4% of offered food; latency to feed: 3.1 ± 0.6 s). Physiological stress and energy reallocation toward the immune response are probably linked to the decrease in eating efficiency.

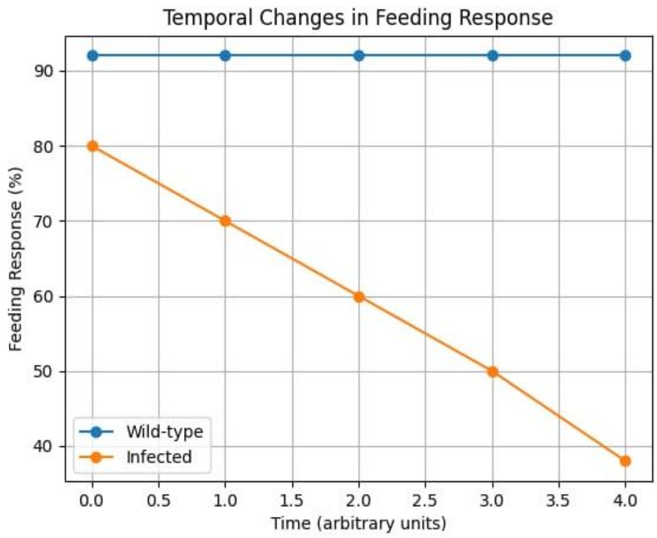


Fig.11. Temporal changes

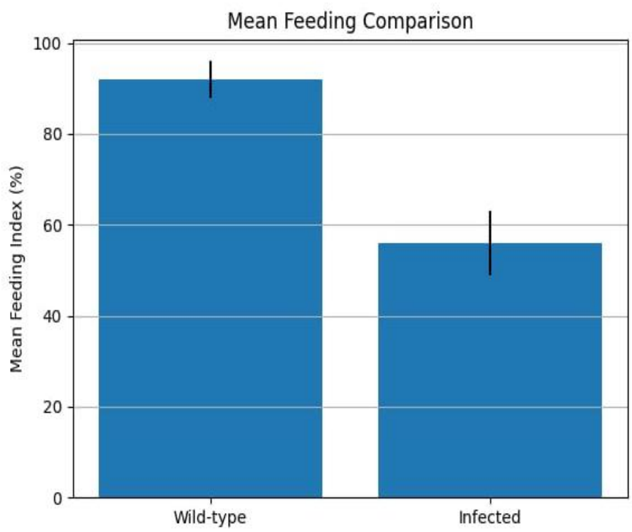


Fig.12. Mean feed comparison

Overall, the average feeding index of infected fish (56 ± 7%) was considerably lower than that of control groups, suggesting that nutritional intake was compromised as the disease progressed. The effects of White Spot Disease on feeding behaviour and energy balance are highlighted in Figures 11 (temporal feeding response) and Fig.12 (mean feeding comparison).

3.4 Stress and Swimming pattern Analysis

There were notable distinctions between Ichthyophthirius multifiliis-infected and wild-type (non-infected) Betta splendens when stress-related behaviour and swimming patterns were compared. Higher locomotor activity (mean distance moved: 125 ± 10 cm/min; swimming speed: 2.1 ± 0.3 cm/s) and homogeneous distribution throughout the water column were characteristics of the stable and energetic swimming behaviour of wild-type fish. On the other hand, infected individuals displayed significant behavioural changes, such as decreased swimming speed (1.0 ± 0.2 cm/s), decreased activity (62 ± 8 cm/min), and an increase in aberrant behaviours such surface gasping, bottom living, and erratic movements. The duration of immobility was higher in infected fish (45 ± 6% of observation time) than in controls (12 ± 3%), indicating a significant increase in stress markers.

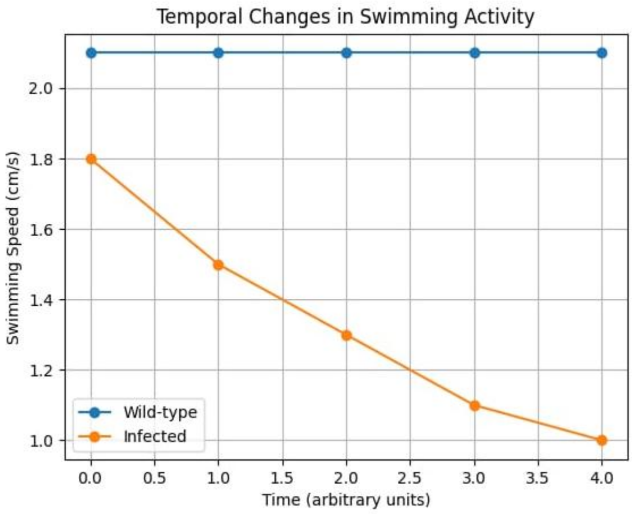


Fig 13. Temporal swimming activity

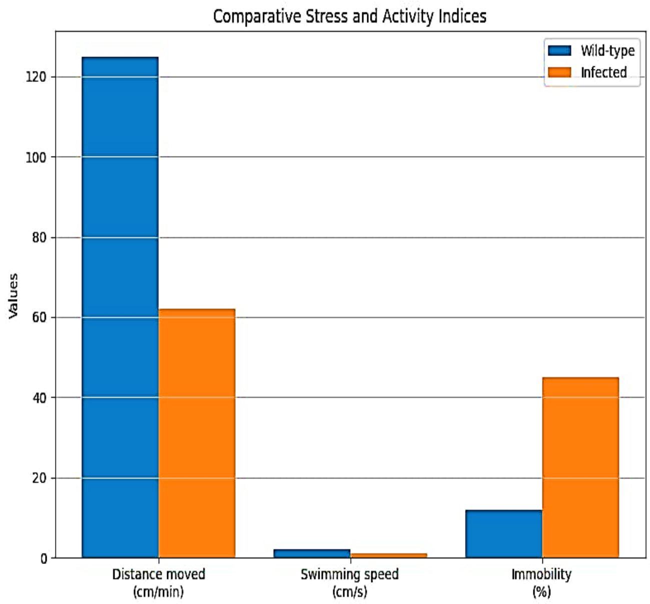


Fig 14. Comparative stress indices

These results suggest that parasite infection causes significant physiological stress, which results in altered behavioural patterns and poor swimming performance. White Spot Disease has a detrimental effect on locomotion and stress response, which has consequences for survival, energy balance, and ecological fitness. These tendencies are depicted in Figures 13 (temporal swimming activity) and Fig.14 (comparative stress indices).

3. Discussion

The current study shows that Ichthyophthirius multifiliis infection dramatically changes Betta splendens behavioural patterns, especially with regard to aggression, feeding response, and swimming ability. A biphasic behavioural reaction, probably caused by acute irritation and subsequent physiological tiredness, is suggested by the observed initial spike in aggression followed by a noticeable drop. Oliveira et al. (2022) showed similar behavioural regulation under stress and emphasised the function of neuroendocrine pathways in controlling aggression in B. splendens. In line with research by Banu et al. (2025) on parasite stress in ornamental fish, the increasing decrease in feeding activity and increased latency further suggest an energy trade-off where metabolic resources are allocated toward immunological responses. Furthermore, decreased ecological fitness and deteriorated physiological state are reflected in poor swimming performance and increased stress-related behaviours such surface gasping and erratic movement. From the standpoint of behavioural ecology, these changes could affect survival and reproductive success by upsetting territorial dominance, niche occupation, and competitive interactions (Kumar Thatipamula et al., 2017; Poojari et al., 2014; Mamidala et al., 2020). The findings are consistent with the larger picture of host-parasite interactions, in which parasitic illnesses serve as important ecological forces influencing aquatic species' functional and behavioural reactions

4. Conclusion

The current study shows that, the Ichthyophthirius multifiliis-caused White Spot Disease significantly affects Betta splendens behavioural patterns, Along with notable decreases in feeding efficiency and swimming performance, the infection causes notable changes in aggression, which are characterised by an initial spike followed by repression. The Increased stress levels and energy reallocation to survival mechanisms are reflected in these alterations, which eventually lower overall fitness and behavioural competence. The results highlight how crucial it is to combine behavioural and parasitological methods in order to comprehend host-parasite dynamics and their ecological ramifications. Both the management of aquaculture and the preservation of ornamental fish species benefit from such knowledge. To better understand the underlying mechanisms, future research should concentrate on the molecular and hormonal correlates of these behavioural alterations

Competing interests:

The authors declare that they have no competing interests

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