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Ocean Acidification's Effects on the Growth Rate and Haematological Markers of Asian Seabass *(Lates calcarifer)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Anthropogenic carbon dioxide (CO_2) absorption in the seas is changing the chemistry of saltwater globally, which has an impact on marine biota. The increasing partial pressure of CO_2 (pCO_2) is shoaling the calcium carbonate saturation horizon in a number of regions, particularly high latitudes and those that connect with notable hypoxic zones. Early calcareous skeleton formation in marine organisms, especially fish, is directly impacted by the CO_2 chemistry of seawater.
Furthermore, CO_2 alters the physiology of marine animals by reducing their ability to transfer oxygen and creating an acid-base imbalance. There is not enough study done at relevant pCO_2

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levels to help us predict future effects on food-web dynamics and other ecological processes. The seabass fingerlings are exposed to various pH levels over a duration of nine weeks. Ocean acidification (OA) levels were measured at pH 7.8, pH 7.5, and pH 8.1, which served as the control. Results revealed that fingerling of seabass had a greater impact on growth rate and haematological markers. The results also revealed that long term effect of OA leads to changes the red blood cells (RBCs) and white blood cells (WBCs) count, which indirectly affect the other haematological indices. We conclude that there is a tremendous deal of potential for broad alterations to marine ecosystems as a result of OA and the combined effects of other human stressors.

Keywords: Ocean acidification; RBC count; WBC count; growth rate; seabass.

1. INTRODUCTION

Ocean acidification (OA) is a gradual reduction in ocean pH mostly brought on by carbon dioxide (CO_2) emissions from the atmosphere. Climate change imperils life as we know it directly because of its consequences on the marine ecology [1]. Since the start of the industrial revolution, the ocean has absorbed around 30% of the carbon dioxide released by human activity. Reduced $CaCO_3$ saturation and pH in seawater are brought on by the increased partial pressure of carbon dioxide (pCO_2). According to Rhein et al. [2], the pH may drop by 0.13 to 0.42 units when the pCO_2 value, which is currently at 375 atm, rises to 420 to 940 atm by 2100. According to Rhein et al. [2], there has been a 34.91% fall in the pH of sea water from 8.17 units in pre-industrial times [3] to 8.06 units now. Certain coastal areas may observe fluctuations in H+ that are at least two to three times the global average due to eutrophication and the amplification of natural CO_2 [4]. Such changes in ocean chemistry are expected to affect the physiological activities of many marine organisms, which could have significant effects on marine biodiversity and ecological processes [5-9].

In recent years there has been discussion over the acidity of the oceans brought on by anthropogenic pollution. The majority of the atmospheric CO_2 generated by burning fossil fuels is absorbed by the ocean, lowering the pH of the water. The scientific community is generally concerned owing to reports of the acidity of the water affecting biological activities [10-13]. Fish are particularly important to humans as a food source among all marine species [14,15]. As a result, several perspectives on ocean acidification's consequences on marine fish have been explored, and physiological imbalances have been noted [16,17,18]. Additionally, some species showed affects on behaviour and reproductive performance whereas others did not [19-21]. The species-specific heterogeneity in these responses highlights the importance of carrying out additional study on the effects of ocean acidification on fish physiology.

Because OA and pH variations alter oxygen availability and delivery capacities, fish are susceptible to these conditions and are predicted to negatively impact marine fish performance [7]. Because acidic pH requires less energy for development, reproduction, and digestion, it may be detrimental to an individual's ability to survive [11,13]. Ecological communities' composition and the results of processes that are significant to the environment can both be altered by the OA [7]. Acidified seawater may have an impact on the development of aquatic species, as abiotic variables such as temperature, salinity, and oxygen availability are known to have the greatest impact on these species during their early life stages [22]. Few studies have been conducted on the effects of OA on aquatic organisms during their early developmental stages, and those that have focused on invertebrates [23-27] suggest that there is little information available regarding the effects of OA on vertebrates during their early life histories.

Blood plays a crucial role in all physiological systems as the medium of intercellular transmission and point of contact with numerous organs and tissues of the body; hence, an animal's physiological status is reflected in its blood at any given time. A healthy organism experiences oxidative stress from any form of environmental stress, which can be used as a biological monitoring method and shows up as changes in blood proteins and the hygiene system [28]. Even though reactive oxygen species do not directly cause oxidative stress in organisms, extended exposure to these agents can nonetheless result in oxidative damage, including changes in enzyme activity that impair compensatory mechanisms [29,30]. The

utilisation of haematological markers in environmental monitoring and aquatic biota health has numerous applications, as adverse alterations in these markers signify toxicity [31,30].

Recently, a few studies on the effects of hypercapnia on different phases of the life cycle of marine teleosts have been reported [32,11,13]. Kikkawa et al. [33] used elevated pCO_2 levels to investigate the acutely fatal impact of pCO2 on marine fish embryos. Ocean acidification (OA) is affecting all of the world's seas, particularly coastal estuaries and streams. Fish and shellfish are important to many economies, and seafood is a key source of nourishment for people all around the world. An experiment was created to examine the effects of seawater acidity on the growth rate of fingerling Indian seabass, taking into account the urgency of the situation and the significance of the fisheries industry. We looked into how an acidic medium changes blood's haematological markers and how that impacts growth. The long-term impacts of ocean acidification on Indian seabass growth will thus be better understood thanks to this study, which may have ramifications for creatures found in coastal and marine ecosystems.

2. MATERIALS AND METHODS

2.1 Collection and Maintenance of Seabass Fingerlings

Lates calcarifer fingerlings were collected from the Rajiv Gandhi Centre for Aquaculture (RGCA) located in Thirumullaivasal, Tamil Nadu, India. Fish that weighed 6.78±0.9 g and had an average length of 7.58±1.2 cm were acclimated for a week in a fiberglass tank before they were used in the experiment. Throughout the acclimatization phase, the water's temperature, salinity, and pH were maintained at 28±1°C, 20 PSU, and 8.1, respectively, and the tank received constant oxygenation. Throughout the conditioning period, the fish were routinely fed commercial feed and finely diced fresh prawn meat. The average weight of 180 fingerlings was determined before the exposure began, and they were divided into nine rectangular glass aquariums at random.

By reducing the pH of seawater and exposing different size groups of fish to three different pH conditionsa control pH of 8.1 and two lower pH levels anticipated under various climate change scenarios (pH 7.8 and pH 7.5)—the impacts of OA were studied. A lower pH was achieved by rapidly bubbling CO_2-enriched air at the appropriate CO_2 content. A dual variable area flow controller was used to regulate the CO_2 level in the bubbled air and to periodically evaluate the pH in the experimental tank. During the experiment, the fish were fed fresh flesh from prawns. In order to minimize fluctuations, the temperature and salinity were regularly monitored using a probe, and the pH of the seawater was measured three times a day using a pH meter that was properly calibrated.

2.2 Survival, Length and Weight Measurement

The mean survival, mean length and mean weight increased in the test species during the experiment was calculated by using the formulae given below:

Mean survival (%) = No. of fish survived after 60 d/initial number of fish stocked × 100.
Mean weight gain (%) = Average (Final weight (g) - initial weight (g)/ initial weight (g) × 100.
Mean length gain (%) = Average (Final length- initial length / initial length) × 100
SGR (%) = Average (Final body weight - initial body weight / Day) × 100
Cannibalism (%) = 100 X (Initial loading (IL)– Mortality (M)- Count Final (CF) / IL).

2.3 Surgical Procedures and Blood Haematology Analysis

Healthy fish in both control and exposure group were sampled after three, six, and nine weeks respectively. Six fish from each tank in triplicate for each time point were dissected. Fish were placed in ice-cold aquarium water for 30 seconds, after which they were removed and their weight and length measured. To keep it quiet, the fish was gently wrapped in a paper towel dipped in ice-cold aquarium water. The tail was incised 1-2 mm from the rostral end to the caudal fin. To prevent clotting, blood was collected from the cut end using a micropipette with heparinized tips, and it was quickly diluted with Tris EDTA solution. Three fish in the same tank provided one replication of their blood samples for fish fingerlings.

2.4 Haematological Indices Analysis

A Neubauer hemocytometer was used to count the white blood cells (WBC) and red blood cells

(RBC) after the samples had been diluted with Grower's and Dacie's solutions, respectively (Voigt, 2000). The haemoglobin (Hb) content was measured using the photometrical cyanohaemoglobin technique. The following standard formulae were used to compute the haematocrit (HCt), mean cellular volume (MCV), mean cell haemoglobin (MCH), and mean cellular haemoglobin concentration (MCHC) [34]. By multiplying the hemoglobin concentration by 1.25 oxygen and combining the power of Hb/g, the oxygen carrying capacity was calculated [35,36].

2.5 Statistical Analysis

To perform the statistical analysis, SPSS software was used. The normality and homogeneity of the data were checked before using a two-way analysis of variance (ANOVA) to look at differences between the groups. When analyzing the statistical difference between the different treatment groups using Tukey's multiple comparison post hoc tests, a p-value of less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Haematological analysis is a widely used method for evaluating fish physiological status. The characteristics of red blood cells, white blood cells, and thrombocytes per unit of blood volume are included. One of the fundamental methods for determining how environmental stress affects fish is haematological study, which is frequently combined with biochemical and histological investigation [37].

Physicochemical parameters did not substantially differ ($p > 0.05$) across the exposed groups during different exposure periods. The growth of the fish substantially reduced after 9 weeks of exposure to OA, both the OA concentration reduced the growth rate significantly compared to the respective control group (Fig. 1). When we calculated the growth rate, the growth rate was inhibited much in the fish exposed to OA. The results of the present study shows that the hepato-somatic index was not altered when the fish exposed to OA. The study also checked the cannibalistic activity in the fish exposed to OA.

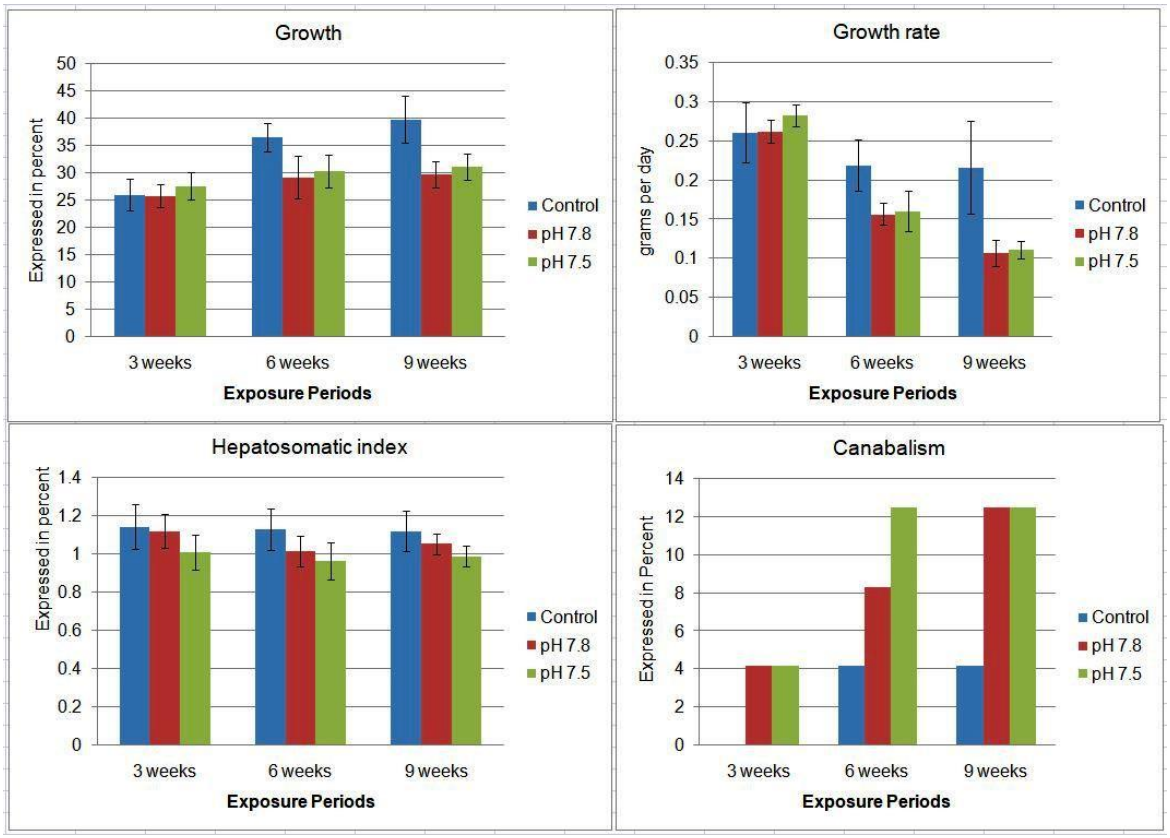

Fig. 1. Impact of OA on fish fingerling growth, growth rate, hepatosomatic index and cannibalisms. Fish fingerling exposed to different pH for prolonged period 9 weeks. Values are expressed mean ±S.D

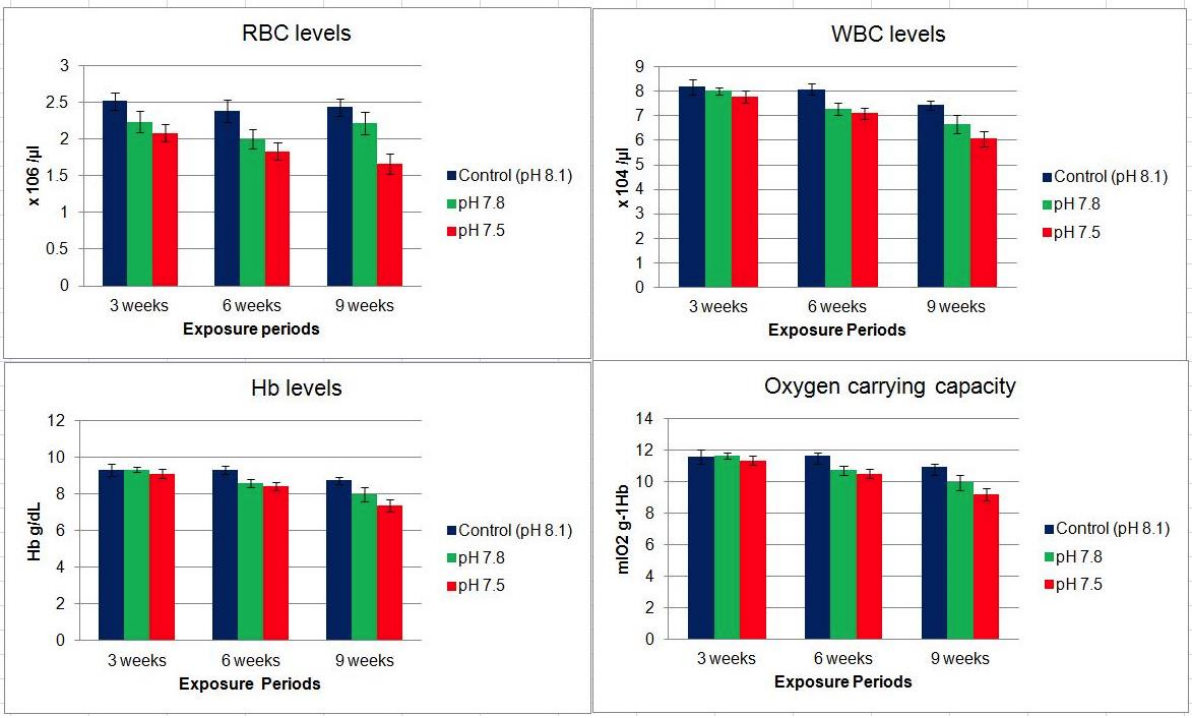

Fig. 2. Impact of OA on haematological indices in fish fingerling: RBC levels, WBC levels, Hb levels and Oxygen carrying capacity. Fish fingerling exposed to different pH for prolonged period 9 weeks. Values are expressed mean ±S.D

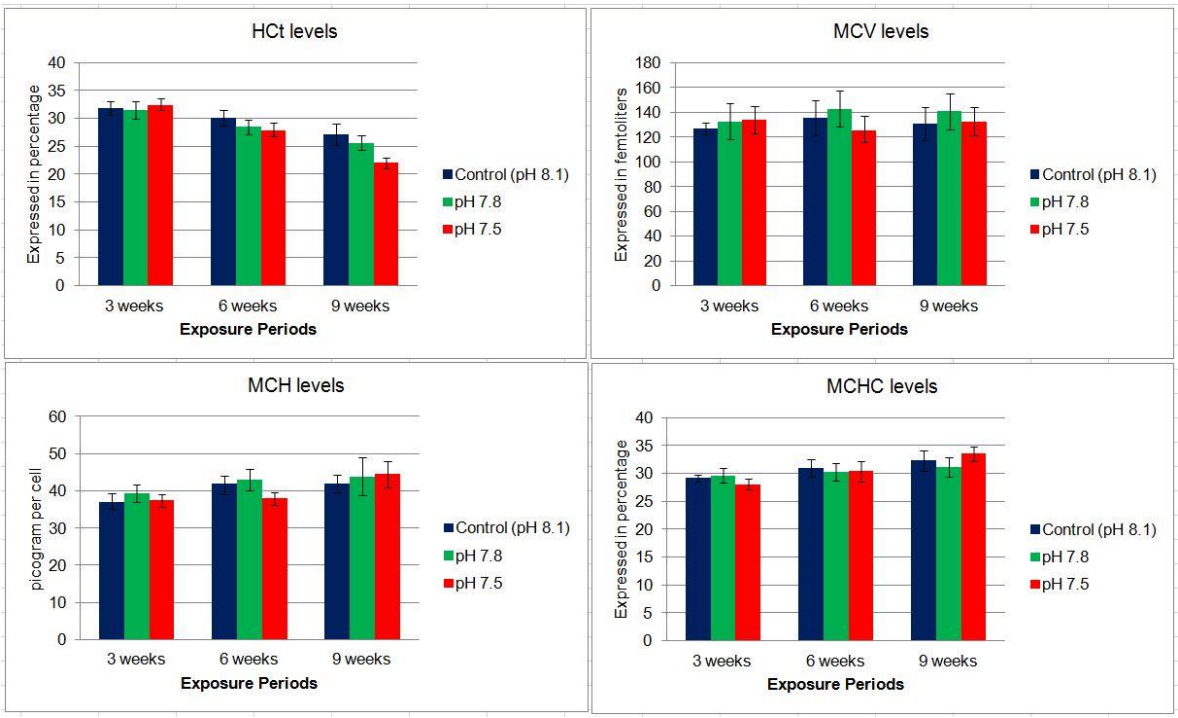

Fig. 3. Effect of OA on fish fingerlings' haematological indices: levels of Haematocrit (HCt), Mean Cellular Volume (MCV), Mean Cellular Hemoglobin Concentration (MCHC), and Mean Cell Hemoglobin (MCH) in the fish fingerlings exposed to varying pH over a duration of nine weeks. The values are given as mean ±S.D

Results revealed that both OA concentrations increase the cannibalisms in the fish after 9 weeks of exposure (Fig. 1).

The results demonstrate the alterations in haematological parameters in sea bass fingerlings exposed to different pH groups, including red blood cell (10^6/µL), white blood cell (10^3/µL), and Hb (g/dl) level, as well as HCt, MCV, MCH, MCHC, and O_2 carrying capacity (Figs. 2 and 3). The mean values for the majority of blood parameters revealed significant differences ($p < 0.05$) between the experimental and control groups. While MCV and MCHC significantly increased in fingerlings, WBCs, RBCs, Hb, HCt, and O_2 carrying capacity all shown a pH-sensitive reduction. Nevertheless, after 9 weeks of exposure at pH 7.5, fish MCV dramatically decreased. The MCH values of fish fingerlings exposed to OA do not significantly differ from those of the corresponding control groups, with the exception of a 9-week exposure period at pH 7.5. This indicates that fish may be more vulnerable to OA-induced changes in their MCH levels after prolonged exposure.

Long-term exposure to OA may change the haematological indices, in accordance to a related study by Srinivasan et al. [38]. In the current study, when the fingerlings were exposed to varying pH settings (8.1, 7.8, and 7.5), the overall RBC count drastically reduced. As indicated by Table 1 and represented by variations in red blood indices such as Hb, HCt, MCV, MCH, and MCHC, hemolysis, or disruption of blood cells, may be the reason of this drop in RBC count. The composition and function of blood may become aberrant as a result of these RBC changes. Moreover, increased MCH and MCHC levels in sea bass blood are connected with decreased Hb concentration in each cell and changes in erythrocyte count caused by red blood cell disintegration.

The results of RBC indices are in fact used to distinguish between different types and pinpoint the cause of anaemia. The macrocytic type of anaemia was revealed by the elevated MCV and MCH values, as well as the stable MCHC values. The MCHC value measures variations in erythrocyte size, shape, and haemoglobin concentration in addition to the severity and cause of anaemia. Despite the fact that seabass fingerling regarded to pH 7.8 did not significantly differ from the control groups, the MCHC contents of seabass treated to acidified environments for 9 weeks at pH 7.5 significantly decreased. For fingerlings data on fish life expectancies and changes in measuring the length compared to starting values. Growth was inhibited when seabass were maintained in seawater that had a pH of 7.8 or 7.5, which is acidified seawater. When seabass were raised in untreated control (pH 8.1), their length rose by 80% in comparison to starting values. In pH 7.8 and pH 7.5, the length rose by 60% and 40%, respectively. Corresponding to this, the growth rate of fingerlings exposed to two conditions with lower pH was substantially slower than that of the control groups. At pH 7.8 and 7.5, the bodyweight of the fingerling reduced to 75% and 66%, respectively, after increasing by 112% over the course of 9 weeks to 5 g. An analogous pattern was followed by fingerlings. These findings conclusively show that exposure to acidified seawater causes fish to develop less rapidly.

Through lowest component modulation, such as pH-dependent nutrient and metal diversification, ocean acidification may have an impact on food webs and carbon sequestration [6]. This might lead to variations in the variety of species and photosynthesis rates. The interrelationships and reciprocal impacts of increasing eutrophication, alien species, overfishing, changing seawater CO_2 chemistry, and these stressors alone may modify the response of an ecosystem [39,40]. More empirical data, especially at local levels, and new modelling efforts are needed to assess these complex ecological processes.

Moreover, food-web discussions and responses to open access are very unpredictable. However, they will have an influence on marine populations and, in the event that a major predator is eliminated, may tip the scales from being generally detrimental to being beneficial for a particular species. The current state of our understanding of how OA impacts ecosystems is very lacking. Similarly, early life stages, especially the fingerling, sometimes show increased susceptibility to OA; nevertheless, the effects of exposing a life stage to low pH settings on survival life stages have only seldom been investigated. Comparably, not all life phases have been taken into account, particularly when looking at comparatively well-studied species and most definitely not when looking at the same.

Haematological variables, which include hazardous substances, are sensitive and trustworthy markers of the effects of the environment on fish. They could exhibit either the protective (increased blood oxygen transport

capacity or inflammation) or the destructive (anaemia and immunosuppression) effects of poisoning. Fish exposed to toxicity frequently exhibit haematological alterations that are indicative of a generalised stress response that is difficult to pinpoint exactly what causes it. Haematopoietic tissue's cellular makeup and activity may reveal more details regarding the physiological impacts of toxicity, and a thorough haematological and haematopoietic examination may paint a fuller picture. Therefore, in addition to the standard basic haematological tests, alterations in the cellular composition and activity of haematopoietic tissue can be employed as an essential supplementary biomarker and this biomarker could easily predict the environmental impact on the marine organisms.

4. CONCLUSION

In summary, the present study examines the haematological and growth effects due to prolonged exposure of *L. calcarifer to* OA. Results of this study indicate that fish exposed to OA experience alterations in most of the haematological components. The study also revealed that OA was pH dependant and affected physiological processes, such as growth rate. We suggest that the growing focus on fish farming and the increasing levels of pollution in coastal and marine environments have made fish haematological research increasingly significant. A long-term management strategy to lessen ocean acidification may benefit from its impact on fish growth.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

For the care and use of animals, all applicable international, national, and/or institutional guidelines were followed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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